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# Isolation and Identification of Spoilage Organisms in Bakery Products: Comparative Study of the Effects of Formulation Factors and Preservatives

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**Abstract**

Bakery products are highly susceptible to microbial spoilage, which compromises shelf life, safety, and overall quality. This study evaluated the effects of chemical preservatives and formulation-based interventions on the microbial stability, physicochemical properties, and sensory quality of bread and cake stored at ambient temperature for 12 days. Four samples were assessed: preserved bread (PB) containing calcium propionate, unpreserved bread (UB), preserved cake (PC) formulated with calcium propionate and vinegar, and reformulated cake (RC) produced using hot milk without chemical preservatives. Microbiological analyses, pH and moisture determination, and sensory evaluation were conducted at regular intervals during storage. Results indicated that bread samples exhibited higher microbial loads than cakes. Total bacterial counts ranged from 0–8.15 $\log_{10}$ CFU/g in PB and 0–7.18 $\log_{10}$ CFU/g in UB, while lower values were recorded for cakes, particularly RC (0–4.0 $\log_{10}$ CFU/g). Unpreserved bread showed the highest coliform, Staphylococcal, and fungal counts, suggesting post-baking contamination and rapid spoilage. Visible mold growth appeared by day 9 in UB and PC, whereas PB and RC remained visually mold-free throughout storage. A diverse range of bacterial and fungal genera associated with spoilage and food safety concerns were isolated across samples. Physicochemical analysis revealed declining pH and increasing moisture content in bread samples, conditions that favored microbial growth. In contrast, RC maintained relatively stable pH and moisture levels, contributing to its superior microbial stability. Sensory evaluation showed a gradual decline in acceptability across all samples; however, RC retained the highest overall sensory scores by day 12. The study demonstrates that process-based interventions, such as hot-milk treatment, can significantly enhance the microbial stability and sensory quality of bakery products. While calcium propionate remains effective, combining preservatives with improved processing, hygiene, and moisture control offers a more sustainable approach to extending bakery product shelf life.

**Keywords:** Bakery products, preservatives, shelf life, formulation factor, ambient storage, physicochemical properties, sensory quality

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## Introduction

Bakery products refer to a wide variety of food items such as bread, cakes, pastries, cookies, crackers, and more. Despite their diversity, these products are generally connected by the common characteristic of being made from recipes that primarily use wheat flour (Cauvain and Young, 2006). They are a staple food globally (Hunt and Robbins, 2009) and are highly susceptible to microbial spoilage due to their nutrient-rich composition and moisture content. Spoiled food may be defined as food that has been damaged or injured to make it undesirable for human consumption (Saranraj and Geetha, 2011).

Bakery products are an important part of a balanced diet, and a wide variety of such products can be found on supermarket shelves. However, like many processed foods, bakery products are subject to physical, chemical and microbiological spoilage. Changes in the physical and chemical properties of bakery products can lead to a decline in freshness, along with negative effects on their texture and flavor. In contrast, microbial spoilage is typically marked by visible alterations caused by the growth of bacteria, yeasts, and molds (Melini and Melini, 2018). While physical and chemical spoilage limits the shelf life of low and intermediate-moisture bakery products, microbiological spoilage is a concern in high-moisture products.

Spoilage can be caused by a range of microorganisms, including bacteria, yeasts, and molds. Fungi such as *Aspergillus*, *Penicillium*, and *Rhizopus* are often implicated in

mold spoilage, while bacteria like *Bacillus* spp. can cause ropiness in bread (Saranraj & Sivasakthi, 2021; Kumar et al., 2018). Many industrially produced baked goods emerge from the baking process with an essentially sterile surface, but post-bake handling can quickly lead to fungal, microbial surface contamination as a result of exposure to airborne contaminants as well as equipment contact (Nakhchian et al.,2014). The most common factor of bakery products is water activity, microbiological spoilage, in particular mould growth is of major economic importance to bakery products, causing significant economic losses and also presents potential health risks to consumers.

Preservatives such as calcium propionate and sorbic acid are widely used to inhibit microbial growth in bakery products and extend their shelf life (Sofos and Busta, 1991). In general, bread is preserved using propionic acid and its salts, as well as acetic acid and its salts, while cakes are typically treated with sorbic acid and its salts. However, a key issue with sorbic acid and its derivatives is their ability to suppress yeast activity, which can negatively impact dough development, fermentation, and baking if added directly to the dough (Cauvain and Young, 2008). Therefore, it is essential to consider the type of bakery product and its production process when selecting preservatives. However, with the growing consumer demand for natural and clean-label products, there is a pressing need to explore alternative methods to control spoilage effectively without compromising product performance or sensory quality.

In addition to preservatives, formulation and processing methods may also influence microbial growth and spoilage in bakery products. Variations in ingredient handling, such as the use of heated dairy components (e.g., hot milk), can alter the initial microbial load in the batter and subsequently affect spoilage dynamics. Heat treatment of milk is known to reduce spoilage microorganisms including bacteria, yeasts, and molds, thereby improving microbial safety and extending product shelf life (Keogh & O'Callaghan, 2022). In this study, the use of hot milk in one cake formulation was considered as a formulation variable alongside use of preservatives such as calcium propionate and vinegar in evaluating spoilage patterns.Bread and bakery products have been part of the human diet since ancient times (10,000 BC) and are one of the most consumed staple foods worldwide (Rosell, et al; 2015). More than 9 billion kg of bread products are produced annually (Cho and Peterson, 2010), with an average consumption of 41 to 303kg per year per capita (Bajerska, et al., 2015).

The spoilage of bakery products by microorganisms such as moulds, bacteria, and yeasts is a major concern for the food industry. Overall losses of bakery products due to mould spoilage vary between 1-5 % depending on seasons, type of products and methods of processing (Jarvis, 2001.). Additionally, preservative-free products are more susceptible to microbial growth, leading to reduced shelf life and quality, as the stability of bakery products against spoilage is mainly due to preservatives. Preservatives help to reduce or prevent wastage of food through spoilage caused by microorganisms, enabling a longer shelf life of bakery products. In food preservation, the application of a single preservative agent is often insufficient or requires concentrations above recommended levels. The emerging concept of hurdle technology combines multiple preservation strategies such as mild chemical preservatives, pH reduction, and process modifications to achieve enhanced microbial control and extended shelf life, while maintaining desirable product quality (Gupta. et al, 2019)

Despite the effectiveness of chemical preservatives, their usage has raised health concerns among consumers and when used in higher concentrations may have undesirable effects on the taste of bakery products (Ramos 2003). This has led to a rising interest in various methods of bio-preservation; defined as the use of microorganisms and their metabolites to prevent spoilage and to extend the shelf life of foods. While preservatives are effective in reducing spoilage, their use raises concerns. High concentrations may adversely affect taste (Ramos, 2003), and health-conscious consumers increasingly demand products with minimal synthetic additives. This has prompted research into alternative preservation strategies, including bio-preservation and process-based interventions.

Despite the availability of several preservation methods, the spoilage of bakery products remains a persistent challenge, particularly in regions with warm and humid climates where microbial growth is accelerated. The formulation and choice of ingredients strongly influence the rate and nature of spoilage, as factors such as moisture content, pH, and nutrient composition create favorable conditions for microbial proliferation. Understanding how preservatives and formulation modifications interact to influence microbial activity is therefore essential for improving product quality and extending shelf life.

This study aims to address these concerns by isolating and identifying the spoilage organisms associated with different bakery formulations and by comparing the effects of chemical preservatives and process-based interventions. Specifically, it focuses on bread prepared with calcium propionate and a standard recipe, and cake prepared with calcium propionate and vinegar, as well as cake formulated with hot milk. The findings from this comparative study are expected to provide valuable insights into the microbial ecology of bakery products and guide the development of safer, more effective preservation and reformulation strategies in the baking industry.The overall aim of this study is to isolate and identify spoilage organisms in bakery products and to evaluate factors influencing their growth during storage.

## Materials and Methods

### Sample Collection and Preparation

Two types of bakery products were prepared: bread and cake. Bread samples included one batch containing preservatives (calcium propionate) and another without preservatives. Cake samples were prepared using a vanilla cake recipe, with one batch incorporating hot milk without preservatives and another using homemade buttermilk (milk and vinegar) with calcium propionate. Ingredients such as flour, sugar, eggs, and fat were combined according to standard procedures, mixed, baked, cooled, and then packaged in sterile Ziploc bags. All samples were clearly labeled and stored at ambient temperature (25–

28 °C) for microbial, physicochemical, and sensory analyses over a 12-day period.

**Microbiological Analysis of Samples**

Twenty-five grams (25 g) of each bread and cake sample was weighed and mixed in 225 ml of sterile peptone solution under aseptic conditions. Microbiological analysis was carried out on day 0, 3, 6, 9, and 12 of storage. Serial dilutions of the bread and cake samples were prepared by transferring 1 mL of the stock solution into a test tube containing 9 mL of sterile saline solution, The process was repeated for subsequent dilutions to achieve appropriate concentrations for plating.

**Total Heterotrophic Bacteria Count (THBC)**

The total heterotrophic bacteria count (THBC) of the bread and cake samples was determined by inoculating aliquots onto Plate Count Agar (PCA) using the spread plate method. The test sample solution was serially diluted five times ($10^{-1}$ to $10^{-5}$), and a 0.1 mL aliquot from each dilution ($10^{-2}$ to $10^{-5}$) was inoculated onto PCA plates. Plates were incubated at 35°C for 24 hours as described by Moroof et al. (2022). Colonies were manually counted, and results were recorded as CFU/g.

**Total Coliform Count**

A 0.1 mL aliquot from the appropriate dilutions ($10^{-2}$ to $10^{-5}$) was inoculated onto sterile Petri dishes containing MacConkey Agar. The inoculum was spread evenly, allowed to dry, and incubated at 37°C for 24 hours, as described by Moroof et al. (2022). Colonies were manually counted, and results were recorded as CFU/g.

**Total Staphylococcal Count (TSC)**

A 0.1 mL aliquot from the appropriate dilutions ($10^{-2}$ to $10^{-5}$) was inoculated onto Mannitol Salt Agar (MSA) plates using the spread plate method. Plates were incubated at 37°C for 24 hours as described by Moroof et al. (2022). Colonies were manually counted, and results were recorded as CFU/g.

**Total Fungal Count (TFC)**

A 0.1 mL aliquot from the appropriate dilutions of the samples was inoculated onto freshly prepared Potato Dextrose Agar (PDA) plates using the spread plate method. Plates were labeled and incubated at room temperature (28 ± 2°C) for 5 days. Fungal colonies, including yeasts and molds, were manually counted and recorded as CFU/g.

**Isolation and Identification of Isolates**

Single colonies from culture plates were identified and streaked as primary inoculants on freshly prepared Nutrient Agar (NA). Plates were incubated at 37°C for 24 hours for bacteria to obtain pure cultures after repeated sub-culturing. Pure cultures were maintained on NA slants stored at 4°C until identification. For fungi, PDA was used for isolation, and cultures were incubated for 5 days.

**Microscopic and Morphological Identification of bacterial and Fungal Isolates**

Fungal isolates were first characterized based on their colonial appearance on Potato Dextrose Agar (PDA). Observations were made after 5 days of incubation at room temperature (28 ± 2°C). The colony characteristics recorded and provided preliminary information to the identity of the fungi. Microscopic characterization was carried out using the lactophenol cotton blue staining technique. A small portion of the fungal mycelium was placed on a clean glass slide, stained with lactophenol cotton blue, covered with a coverslip, and observed under the microscope at ×40 objective. Distinguishing microscopic features such as hyphal structure (septate or non-septate) and conidial arrangement were noted and recorded. Bacterial isolates from the bread and cake samples were first examined for their colonial morphology on selective and differential media, including Plate Count Agar (PCA), MacConkey Agar, and Mannitol Salt Agar (MSA). Colony characteristics such as color, size, shape, margin, surface, opacity, and elevation were recorded after incubation. The results were used to identify and differentiate bacteria based on their biochemical reactions (Prescott et al., 2005).

**Physiochemical Analysis**

**pH Determination**

The pH of the bread and cake was measured at specific intervals to monitor changes in acidity or alkalinity that may occur due to microbial activity or ingredient interaction during storage. A calibrated digital pH meter was used for the measurements.

**Moisture Content**

The moisture content of bread and cake was determined at regular intervals using the method described by the Association of Official Analytical Chemists (AOAC, 2006). The oven-drying method was used to determine moisture content. 5g of each sample was placed in a pre-weighed ceramic dish and dried in a hot air oven at 105°C for 3 hours. The final weight was recorded, and moisture content was calculated as:

$$Moisture\ Content\ (\%) = [(Initial\ Weight - Final\ Weight)\ /\ Initial\ Weight] \times 100$$

**Sensory Evaluation**

A total of 10 untrained assessors served as panelists to assess the bread and cake samples. Sensory evaluation was carried out on all the samples using a 9-point hedonic scale, ranging from 1 (dislike extremely) to 9 (like extremely). The following attributes: appearance, aroma, texture, taste, and overall acceptability of the bread and cake samples with or without preservative were evaluated at intervals during the storage period to determine acceptability and quality.

## Results

**Total Heterotrophic Bacteria Count of Bread Samples**

The total heterotrophic bacteria counts of the bread samples are illustrated in Figure 1, showing the growth of heterotrophic bacteria in preserved and unpreserved bread during storage at room temperature. In the unpreserved bread, counts ranged from no detectable growth on day 0 to $6.6 \times 10^3$ CFU/g on day 3 and $1.5 \times 10^7$ CFU/g on day 12. For the preserved bread, counts also showed no growth on day 0 but were higher on day 3 at $2.05 \times 10^4$ CFU/g and reached $1.4 \times 10^8$ CFU/g by day 12. Based on these results, bacterial counts increased with storage time in both samples, with preserved bread recording higher values than unpreserved bread at each time point. The counts for days 6 and 9 are presented in Figure 1 below.

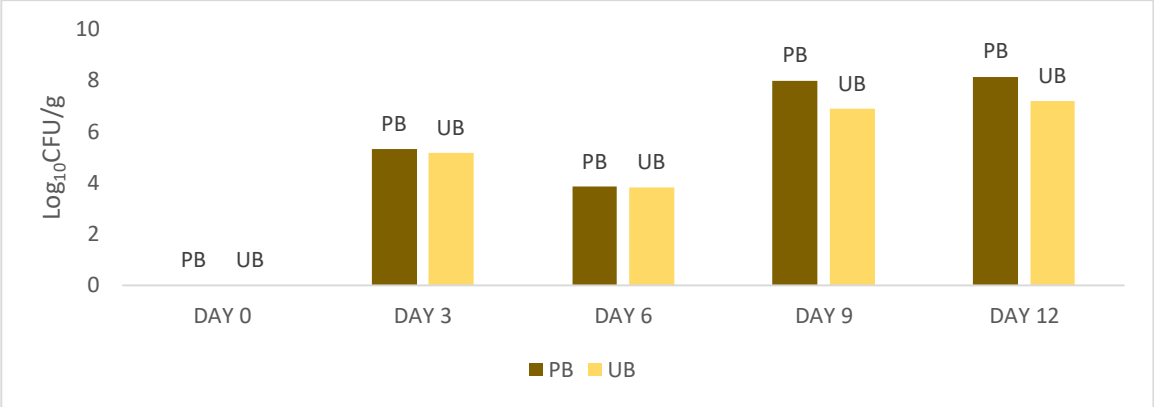


**Figure 1:** Total heterotrophic bacteria count of the bread samples**.**
**Key: PB** = Preserved Bread (bread with calcium propionate; **UB**=Unpreserved Bread (bread without preservatives)

**Total Staphylococcal Counts of Bread Samples**
The staphylococcal counts of the bread samples are llustrated in Figure 2. In the unpreserved bread, no growth was detected on days 0 and 3, but counts reached 2.0 × $10^2$ CFU/g on day 6 and then declined to 1.0 × $10^2$ CFU/g on day 12. In the preserved bread, counts were also undetectable on days 0 and 3, but increased to 6.0 × $10^2$ CFU/g on day 6 and further to 1.8 × $10^3$ CFU/g on day 12. Overall, preserved bread recorded higher staphylococcal counts than unpreserved bread at each time point.

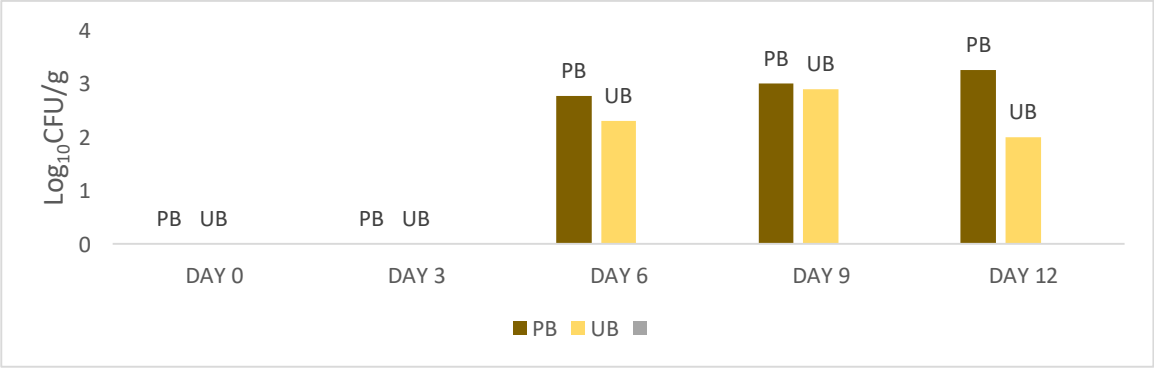


**Figure 2:** Total staphylococcal counts of bread samples
**Key: PB** = Preserved Bread (bread with calcium propionate; **UB**=Unpreserved Bread (bread without preservatives)

**Total Coliform Counts Bread Samples**
The coliform counts of the bread samples are illustrated in Figure 3. In both preserved and unpreserved bread, no growth was detected on day 0. For the unpreserved bread, counts increased to 2.3 × $10^3$ CFU/g on day 3, decreased slightly to 1.6 × $10^3$ CFU/g on day 6, and were undetectable again by day 12. In the preserved bread, counts were 2.5 × $10^3$ CFU/g on day 3 and remained stable at 2.5 × $10^3$ CFU/g on day 6, before also reducing to no detectable growth by day 12. Overall, both samples showed similar patterns, with detectable coliforms during early storage that were absent by day 12.

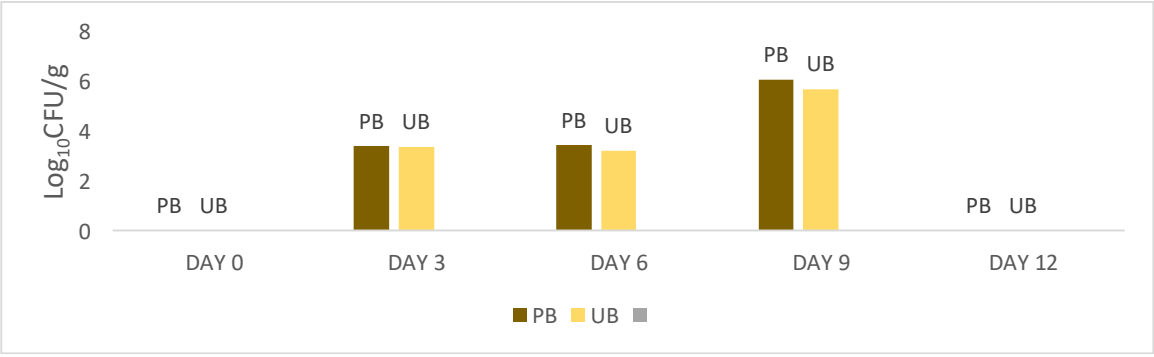


**Figure 3:** Total coliform count of bread samples
**Key: PB** = Preserved Bread (bread with calcium propionate; **UB**=Unpreserved Bread (bread without preservatives)

**Total Fungal Counts of Bread Samples**

The fungal counts of the bread samples are illustrated in Figure 4. No fungal growth was detected on day 0 in either preserved or unpreserved bread. In the unpreserved bread, counts increased to $1.03 \times 10^6$ CFU/g on day 3 and reached $7.7 \times 10^6$ CFU/g by day 12. For the preserved bread, counts were lower at both time points, with $1.03 \times 10^5$ CFU/g on day 3 and $3.9 \times 10^6$ CFU/g on day 12. Overall, fungal counts increased with storage time in both samples, though the unpreserved bread consistently showed higher values than the preserved bread.

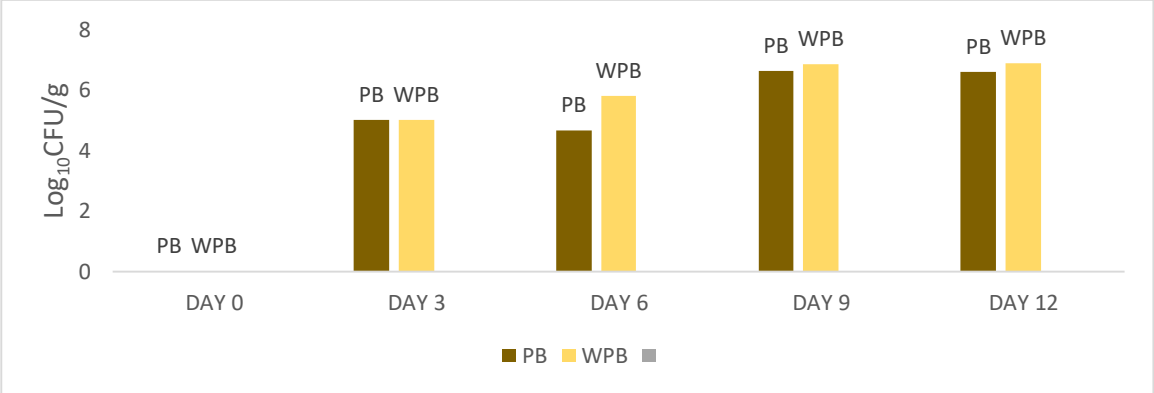


**Figure 4:** Total Fungal Count of Bread Samples
**Key: PB** = Preserved Bread (bread with calcium propionate); **UB**=Unpreserved Bread (bread without preservatives)

**Total Heterotrophic Bacteria Count of Cake Samples**

The total heterotrophic bacteria counts of the cake samples are presented in Figure 5. No bacterial growth was observed on day 0 for either sample. In the reformulated cake, counts rose to $9.0 \times 10^2$ CFU/g on day 3 and $1.0 \times 10^4$ CFU/g by day 12. For the preserved cake, higher values were observed, with $5.0 \times 10^3$ CFU/g on day 3 and $1.2 \times 10^6$ CFU/g on day 12. Overall, bacterial counts increased with storage time in both samples, with preserved cake recording consistently higher values than the reformulated cake.

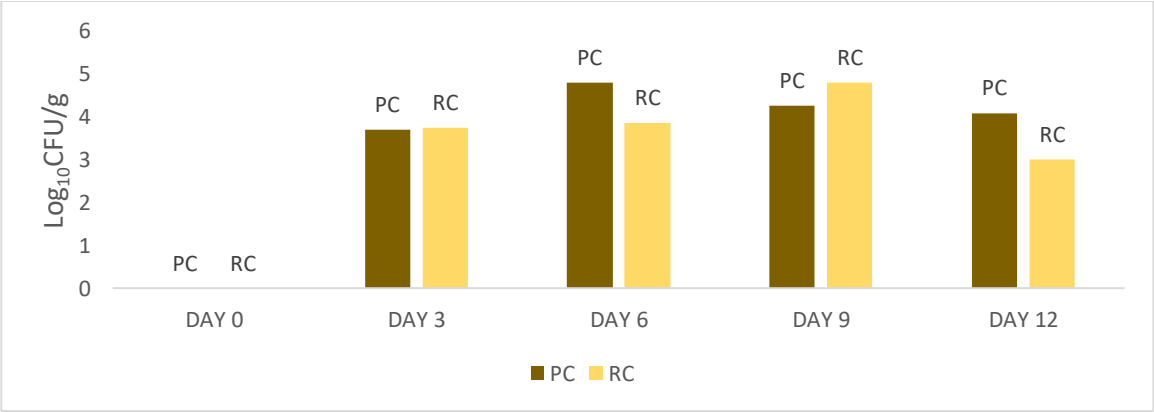


**Figure 5:** Total Heterotrophic Bacteria Count of Cake Samples
**Key: PC** = Preserved Cake (cake with vinegar and calcium propionate); **RC** = Reformulated Cake (cake with hot milk, no preservatives)

**Total Staphylococcal Counts of Cake Samples**

No growth was observed on any of the sampling days (0, 3, 6, 9, and 12) for both preserved and reformulated cake samples, indicating the absence of staphylococcal contamination in the samples throughout the storage period.

**Total Coliform Count of Cake Samples**

The coliform counts of the cake samples are illustrated in Figure 7. No growth was detected on day 0 for either sample. In the reformulated cake, counts rose slightly to $1.0 \times 10^2$ CFU/g on day 3 but showed no detectable growth on days 6, 9, and 12. For the preserved cake, no growth was observed for subsequent days. The results indicate that coliforms were only transiently detected at the early stage of storage.

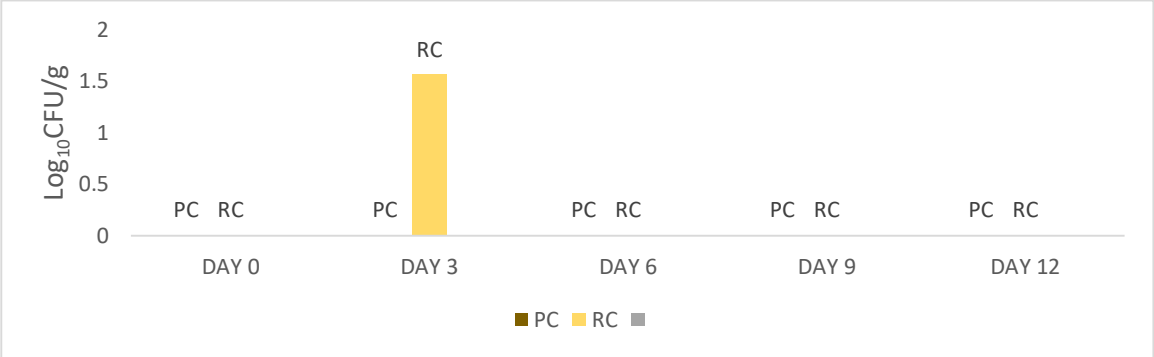


**Figure 6:** Total Coliform Count of Cake Samples
**Key: PC** = Preserved Cake (cake with vinegar and calcium propionate); **RC** = Reformulated Cake (cake with hot milk, no preservatives)

**Total Fungal Count of Cake Samples**

The fungal counts of the cake samples are presented in Figure 8 showing the growth of fungi in preserved and reformulated cake during storage at room temperature. In the reformulated cake, counts ranged from no detectable growth on day 0 to 2.0 × $10^2$ CFU/g on day 3 and 3.0 × $10^3$ CFU/g on day 12. For the preserved cake, counts also showed no growth on day 0 but were higher on day 3 at 5.2 × $10^3$ CFU/g and reached 3.0 × $10^5$ CFU/g by day 12, consistent with the appearance of visible mould growth from day 8.

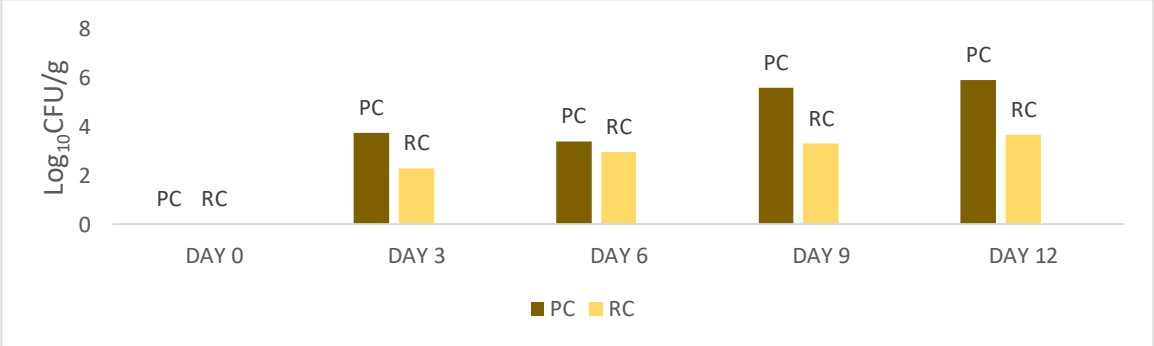


**Figure 7:** Total Fungal Count of Cake Samples
**Key: PC =** Preserved Cake (cake with vinegar and calcium propionate); **RC** = Reformulated Cake (cake with hot milk, no preservatives).

**Table 1:** Frequency of Occurrence Bacteria in the Bread Samples

| Isolates | PB<br>**Frequency of Occurrence** | **Percentage of occurrence %** | UB<br>**Frequency of Occurrence** | **Percentage of occurrence %** |
|---|---|---|---|---|
| *Bacillus sp* | 4 | 80 | 4 | 80 |
| *Staphylococcus aureus* | 2 | 40 | 2 | 40 |
| *Lactobacillus sp* | 1 | 20 | 1 | 20 |
| *Micrococcus sp* | 1 | 20 | - | - |
| *Enterobacter sp* | - | | 2 | 40 |
| *Pseudomonas sp* | 3 | 60 | 2 | 40 |
| *Sporolactobacillus sp* | 2 | | - | |

**Key: PB** = Preserved Bread (bread with calcium propionate); **UB**=Unpreserved Bread (bread without preservative

**Table 2:** Frequency of Occurrence Bacteria in the Cake Samples

| Isolates | PC<br>**Frequency of Occurrence** | **Percentage of occurrence %** | RC<br>**Frequency of Occurrence** | **Percentage of occurrence %** |
|---|---|---|---|---|
| *Bacillus sp* | **4** | **80** | **4** | **80** |
| *Staphylococcus aureus* | **1** | **20** | **-** | **-** |
| *Lactobacillus sp* | **1** | **20** | **1** | **20** |
| *Leuconostoc sp* | **2** | **40** | **1** | **20** |
| *Aeromonas sp* | **2** | **40** | **1** | **20** |
| *Clostridium sp* | **1** | **20** | **-** | **-** |
| *Pseudomonas sp* | **3** | **60** | **2** | **40** |
| *Sporolactobacillus sp* | **1** | **20** | **-** | **-** |

**Key: PC** = Preserved Cake (cake with vinegar and calcium propionate); **RC** = Reformulated Cake (cake with hot milk, no preservatives)

**Table 3:** Frequency of Occurrence Fungi in the Bread Samples

| Isolates | PB **Frequency of Occurrence** | **Percentage Occurrence (%)** | UB **Frequency of Occurrence** | **Percentage occurrence (%)** |
|---|---|---|---|---|
| *Candida sp* | 2 | 40 | 2 | 40 |
| *Fusarium sp* | 2 | 40 | - | - |
| *Penicillum sp* | 2 | 40 | 4 | 80 |
| *Aspergillus niger* | 2 | 40 | 2 | 40 |
| *Aspergillus flavus* | 2 | 40 | 4 | 80 |
| *Mucor sp* | 2 | 40 | 1 | 20 |
| *Saccharomyces cerevisiae* | 3 | 60 | 3 | 60 |
| *Rhizopus sp* | 2 | 40 | 4 | 80 |

**Key: PB** = Preserved Bread (bread with calcium propionate); **UB**=Unpreserved Bread (bread without preservatives)

**Table 5:** Frequency of Occurrence Fungi in the Cake Samples

| Isolates | PC **Frequency of Occurrence** | **Percentage Occurrence (%)** | RC **Frequency of Occurrence** | **Percentage occurrence (%)** |
|---|---|---|---|---|
| *Candida sp* | 2 | 40 | 1 | 20 |
| *Fusarium sp* | 3 | 60 | 2 | 40 |
| *Penicillum sp* | 3 | 60 | 3 | 60 |
| *Aspergillus niger* | 3 | 60 | 2 | 40 |
| *Aspergillus flavus* | 2 | 40 | 3 | 60 |
| *Mucor sp* | 3 | 60 | 4 | 80 |

**Key: PC** = Preserved Cake (cake with vinegar and calcium propionate); **RC** = Reformulated Cake (cake with hot milk, no preservatives)

**Physicochemical parameters**

The physicochemical properties (moisture content (Table 6) and pH (Table 7)) and sensory attributes (Tables 8-9) of the bread and cake samples were evaluated over a 12-day storage period to assess quality changes and consumer acceptability.

**Table 6:** Moisture Content (MC) for Bread and Cake samples

| Sample | Day 0 | Day 6 | Day 12 |
|---|---|---|---|
| PB | 37% | 39.6% | 40.4% |
| UB | 39.8% | 41.2% | 43.1% |
| PC | % | % | % |
| RC | 32.4% | % | 33.8% |

**Key: PB** = Preserved Bread (bread with calcium propionate)
**UB**=Unpreserved Bread (bread without preservatives); **PC** = Preserved Cake (cake with vinegar and calcium propionate); **RC** = Reformulated Cake (cake with hot milk, no preservatives)

**Table 7:** pH of the Bread and Cake samples

| Sample | Day 0 | Day 6 | Day 12 |
|---|---|---|---|
| PB | 5.37 | 5.47 | 4.33 |
| UB | 5.39 | 5.13 | 4.12 |
| PC | 5.2 | 5.5 | 6.1 |
| RC | 6.71 | 6.64 | 6.42 |

**Key: PB** = Preserved Bread (bread with calcium propionate); **UB**=Unpreserved Bread (bread without preservatives); **PC** = Preserved Cake (cake with vinegar and calcium propionate); **RC** = Reformulated Cake (cake with hot milk, no preservatives)

Table 8: **Sensory Evaluation of Bread Samples**

| | Day 0 | | | | | Day 6 | | | | | Day 12 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample | **Appearance** | **Aroma** | **Texture** | **Taste** | **Overall acceptability** | **Appearance** | **Aroma** | **Texture** | **Taste** | **Overall acceptability** | **Appearance** | **Aroma** | **Texture** | **Taste** | **Overall acceptability** |
| PB | 9.0 | 8.7 | 8.5 | 9.0 | 9.0 | 8.0 | 7.1 | 7.9 | 7.0 | 7.5 | 5.2 | 5.0 | 4.8 | 4.3 | 4.8 |
| UB | 9.0 | 8.9 | 8.3 | 9.0 | 9.0 | 6.9 | 6.2 | 6.0 | 5.9 | 6.0 | 2.4 | 2.2 | 2.5 | 1.5 | 2.6 |

**9-point hedonistic scale: 9= Like extremely, 8= Like very much, 7= Like moderately, 6= Like slightly, 5= Neither like nor dislike, 4= Dislike slightly, 3= Dislike moderately, 2= Dislike very much, 1= Dislike extremely.**
**Key: PB** = Preserved Bread (bread with calcium propionate); **UB**=Unpreserved Bread (bread without preservatives

**Table 9: Sensory Evaluation of Cake Samples**

| | Day 0 | | | | | Day 6 | | | | | Day 12 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample | **Appearance** | **Aroma** | **Texture** | **Taste** | **Overall acceptability** | **Appearance** | **Aroma** | **Texture** | **Taste** | **Overall acceptability** | **Appearance** | **Aroma** | **Texture** | **Taste** | **Overall acceptability** |
| PC | 9.0 | 8.5 | 9.0 | 9.0 | 9.0 | 7.0 | 7.8 | 6.5 | 6.0 | 6.5 | 4.8 | 5.5 | 4.9 | 3.7 | 4.8 |
| RC | 9.0 | 8.8 | 9.2 | 9.0 | 9.0 | 7.2 | 7.9 | 7.5 | 7.7 | 7.7 | 6.8 | 5.5 | 5.9 | 4.5 | 5.6 |

**9-point hedonistic scale: 9= Like extremely, 8= Like very much, 7= Like moderately, 6= Like slightly, 5= Neither like nor dislike, 4= Dislike slightly, 3= Dislike moderately, 2= Dislike very much, 1= Dislike extremely.**

**Key: PC** = Preserved Cake (cake with vinegar and calcium propionate); **RC** = Reformulated Cake (cake with hot milk, no preservatives)

## Discussion

### Microbial Quality of Bakery Products

This study investigated the microbial spoilage of bakery products, focusing on the comparative effects of preservatives and formulation factors. Four sample types were evaluated: cake with calcium propionate and vinegar (PC), cake prepared with hot milk without preservatives (RC), bread with calcium propionate (PB), and bread with a standard recipe (UB). Differences in bacterial and fungal growth, as well as changes in pH, moisture content, and sensory characteristics, were monitored over a 12-day storage period to assess the efficacy of preservatives and formulation factors (ingredient composition) on microbial proliferation and shelf life.

The Total Heterotrophic Bacteria Counts of bread and cake samples are presented in Fig 1 and 5 respectively. The THBC analysis revealed marked differences between the preserved cake (PC) containing calcium propionate and vinegar and the reformulated cake prepared with hot milk (RC). No bacterial growth was observed in either sample on day 0. By day 3, PC had a THBC of $5.0 \times 10^3$ CFU/g, which steadily increased to $1.2 \times 10^6$ CFU/g by day 12. In contrast, RC maintained very low bacterial counts throughout the storage period, ranging from $9.0 \times 10^2$ CFU/g on day 3 to $1.0 \times 10^4$ CFU/g by day 12. When compared with ICMSF standards for bakery products, bacterial counts exceeding $10^5$ CFU/g are generally considered indicative of potential spoilage and reduced product quality. By day 12, the THBC in PC ($1.2 \times 10^6$ CFU/g) surpassed this threshold, suggesting that the preservative combination was insufficient to fully control bacterial proliferation under the storage conditions used. In contrast, RC remained well below the ICMSF limit throughout the study likely due to the heat induced inactivation of vegetative cells and modifications to the cake matrix that reduced water activity and nutrient availability. This aligns with previous findings that flash or rapid pasteurization can inactivate the majority of spoilage organisms and extend shelf life without chemical preservatives (Tamime 2009).

The high bacterial counts in PC can be explained in part by the pH values measured over the storage period, which increased from 5.2 on day 0 to 5.5 on day 6 and 6.1 on day 12. The antimicrobial activity of weak organic acids like vinegar is strongest when the pH is near the acid's pKa (Theron & Lues, 2007). As the pH increased during storage, the proportion of undissociated acid molecules decreased, reducing their inhibitory effect on bacteria (Theron & Lues, 2007; Coban, 2020). Additionally, several bacterial species, including *Bacillus subtilis* and *Bacillus cereus*, are known to tolerate acidic and preservative stress via mechanisms such as spore formation, enzyme-mediated detoxification, and biofilm production.

For bread samples, similar trends were observed. The preserved bread (PB) recorded a THBC of $2.05 \times 10^4$ CFU/g on day 3, rising sharply to $1.4 \times 10^8$ CFU/g by day 12, while the unpreserved bread (UB) had slightly lower counts of $6.0 \times 10^3$ CFU/g and $1.5 \times 10^7$ CFU/g at the same intervals. Almost all the bread samples evaluated in this study did not meet the recommended standards. The TBC of the preserved bread (PB) and unpreserved bread (UB) ranged from 0–8.15 $\log_{10}$ CFU/g and 0–5.89 $\log_{10}$ CFU/g, respectively, indicating progressive microbial proliferation during storage. These values are higher than those reported by Dinnaya and Ahaotu (2023), who found bacterial counts ranging between 2.85–4.46 $\log_{10}$ CFU/g for preserved bread and 2.95–3.90 $\log_{10}$ CFU/g for the control. Their study on the microbiological quality, shelf life, and sensorial properties of bread preserved with sorbic acid and calcium propionate also observed that samples containing these preservatives recorded higher bacterial counts than the control in some cases. This agrees with the present findings, where calcium propionate delayed but did not fully prevent microbial proliferation as PB showed the highest bacterial load by the end of storage, suggesting that preservative efficacy diminishes over time and may vary depending on formulation and storage conditions. The predominance of *Bacillus* species, observed in both bread types, aligns with reports that identified Bacillus as the major bacterial genus associated with rope spoilage in bakery products (Lindsay et al., 2004). The high proportion of *Bacillus* isolates supports their well-documented ability to survive baking temperatures and later germinate under favorable post-baking conditions (Pepe et al., 2003).

The presence of calcium propionate in PB may have applied selective pressure favoring acid-tolerant or preservative-resistant bacterial strains. *Bacillus* species are particularly resilient: their endospore formation allows survival of heat and desiccation, while biofilm formation enhances resistance to acidity, osmotic stress, and antimicrobial agents (Berlanga & Guerrero, 2016). Biofilm-associated cells are known to tolerate a wider range of pH and stressors compared to planktonic cells due to their protective extracellular matrix and altered physiological states. The final TBC values in both PB ($1.4 \times 10^8$ CFU/g) and UB ($1.5 \times 10^7$ CFU/g) far exceeded the

ICMSF acceptable limit of $10^5$ CFU/g for ready-to-eat bakery products, indicating significant bacterial spoilage by day 12.

The Total Staphylococcal Count (TSC) results for bread and cake samples shown in Fig. 2 and Fig. 6, highlight the impact of handling practices on microbial contamination. Both cake samples (PC and RC) showed no detectable *Staphylococcus* counts from Day 0 to Day 12, suggesting that the formulation and processing conditions effectively inhibited this bacterium. In contrast, bread samples exhibited low but detectable *S. aureus* growth over time. Preserved bread (PB) showed no growth on Days 0 and 3, with counts increasing to $6.0 \times 10^2$ CFU/g on Day 6 and $1.8 \times 10^3$ CFU/g on Day 12. Unpreserved bread (UB) followed a similar trend, with counts of $2.0 \times 10^2$ CFU/g on Day 6 and $1.0 \times 10^2$ CFU/g on Day 12. According to ICMSF standards, ready-to-eat bakery products are considered acceptable if *S. aureus* counts are below $10^3$ CFU/g, marginally acceptable between $10^3$–$10^4$ CFU/g, and potentially hazardous above $10^4$ CFU/g (ICMSF, 2011). Based on this standard, the TSC observed in PB and UB remains within acceptable limits, although PB approaches the marginal range by Day 12, indicating a slight risk of spoilage or toxin production if storage is prolonged or handling practices are poor. These findings indicate that handling, in addition to preservatives, plays a significant role in controlling contamination in bakery products.

The presence of *Staphylococcus aureus* in bread is consistent with previous studies in Nigeria. Daniyan and Nwokwu (2011) observed *Staphylococcus aureus* counts of $2.2 \times 10^4$ CFU/g in bread samples after baking, attributing the contamination to handlers, since *Staphylococcus aureus* is a normal flora of human skin. Similarly, Olusegun et al. (2015) reported low glove usage among bakery workers in Nigeria, with only 45% wearing gloves regularly and 45% not perceiving any need to use gloves, indicating a significant risk of post-baking contamination. The findings suggest that improper handling practices can introduce *S.aureus* into bakery products even after heat treatments.

Supporting evidence from other regions shows similar trends. In Alexandria, Egypt, *S. aureus* was detected in packaged bread samples, with 11% testing positive, underscoring that post-production contamination during handling and packaging is a critical factor in Staphylococcal prevalence (Ali et al., 2023). Overall, these results highlight that while preservatives may help limit microbial growth, hygienic handling practices are equally essential. The combination of chemical or process-based preservation with proper handling and adherence to food safety protocols is critical to minimizing S. aureus contamination and ensuring bakery product safety.

The Total Coliform Counts (TCC) for bread and cake samples are presented in Figures 3 and 7, respectively. Both preserved (PB) and unpreserved (UB) bread samples showed no coliform growth on day 0, indicating effective initial hygienic conditions post-baking. However, by day 3, coliforms were detected in both samples $2.5 \times 10^3$ CFU/g in PB and $2.3 \times 10^3$ CFU/g in UB suggesting post-baking contamination likely introduced during handling or packaging. The unpreserved bread showed an increase to $4.8 \times 10^5$ CFU/g by day 9 before declining to no detectable growth by day 12, while the preserved bread peaked at $1.14 \times 10^6$ CFU/g on day 9, also showing no growth by day 12. This pattern suggests that coliform proliferation occurred under favorable moisture and temperature conditions during mid-storage but declined as the bread became drier and less supportive of microbial activity.

Coliforms are Gram-negative, rod-shaped, non-spore-forming bacteria that can ferment lactose with gas production within 48 hours at 35–37 ℃. They include *Escherichia coli*, *Enterobacter*, *Klebsiella*, and *Citrobacter* species and are widely used as indicator organisms of hygienic quality and potential fecal or environmental contamination in food systems (Jay et al., 2005; Tortora et al., 2019). In bakery microbiology, coliforms generally do not survive baking temperatures; therefore, their presence after baking is a strong indicator of post-baking contamination through handling, packaging, or environmental exposure.

According to the ICMSF standards, bakery products should ideally contain less than $10^2$ CFU/g of coliforms, with values above $10^4$ CFU/g considered unsatisfactory. The high counts observed on day 9 in both PB and UB exceeded this threshold, reflecting possible lapses in hygiene during storage and handling. These findings align with Ali, Hashish, and Fekry (2023), who reported significantly higher coliform loads in unpacked bread compared with packaged varieties in Alexandria, Egypt, attributing contamination to manual handling and exposure to ambient air. Similarly, Gill et al. (2020) found coliform counts up to 450 MPN/g in bread samples from Lahore, Pakistan, indicating inadequate sanitary control. In Bangladesh, Talukder et al. (2019) also documented progressive coliform multiplication in stored bakery products, exceeding WHO limits and reinforcing the impact of post-processing contamination on spoilage dynamics.

In contrast, both cake samples preserved cake (PC) and reformulated cake (RC) showed no coliform growth on days 0, 6, 9, and 12, with only minimal counts ($4.0 \times 10^2$ CFU/g) on day 3. This suggests cakes were less prone to coliform contamination, possibly due to their lower water activity, higher sugar content, and shorter handling exposure compared to bread. The transient appearance of coliforms on day 3 may represent brief surface contamination that did not persist under the less favorable conditions for bacterial survival. The elevated coliform counts observed particularly in bread samples reflect the influence of environmental hygiene and handling practices on microbial quality.

The Total Fungal Count (TFC) results for bread and cake samples as shown in Fig 4 and Fig 8, indicate significant differences depending on formulation and preservative use. Both cake samples, preserved cake with calcium propionate and vinegar (PC) and reformulated cake with hot milk (RC), showed no fungal growth on day 0. By day 3, PC recorded a TFC of $5.2 \times 10^3$ CFU/g, which increased sharply to $7.7 \times 10^6$ CFU/g by day 12. RC maintained lower counts of $2.0 \times 10^2$ CFU/g on day 3 and $4.7 \times 10^3$ CFU/g on day 12. Visible fungal growth was observed in PC on day 9, whereas RC remained free of visible molds throughout the 12-day storage period. Both bread samples, preserved bread with calcium propionate

(PB) and unpreserved bread (UB), showed no fungal growth on day 0. By day 3, UB exhibited a TFC of $1.03 \times 10^6$ CFU/g, increasing to $7.7 \times 10^6$ CFU/g by day 12. PB recorded lower counts of $9.9 \times 10^4$ CFU/g on day 3 and $4.3 \times 10^6$ CFU/g on day 12, showing no visible fungal growth, while UB displayed visible molds by day 9. This pattern indicates that calcium propionate delayed fungal growth but did not fully prevent it over prolonged storage, whereas unpreserved bread showed rapid proliferation.

According to ICMSF (2011), total fungal counts of $0–10^3$ CFU/g in cake are considered safe for human consumption, while the Institute of Food Science and Technology (IFST) recommends maximum permissible mold counts of $10^4$ CFU/g in pastries and cakes (Chaudhari et al., 2017). Samsudin et al. (2019) also suggest that yeast and mold count below 2 $\log_{10}$ CFU/g are satisfactory, and borderline acceptable counts fall in the 4–6 $\log_{10}$ CFU/g range. By these standards, RC remained within acceptable limits throughout the storage period, demonstrating the effectiveness of hot milk treatment as a process-based intervention. In contrast, PC exceeded acceptable TFC levels by day 12, suggesting that chemical preservatives delayed but did not fully prevent fungal proliferation over time.

These findings align with previous reports showing that calcium propionate can inhibit mold growth for short periods but may be insufficient against tolerant fungal species such as *Penicillium roqueforti* and *Aspergillus niger*, which can metabolically adapt to preservatives through decarboxylation or other mechanisms (Debonne et al., 2019; Saranraj et al., 2012). Similarly, spoilage yeasts such as *Saccharomyces cerevisiae* can develop resistance to weak acids over time, reducing the long-term efficacy of preservatives. The delayed fungal growth in PB and RC compared to UB and PC demonstrates that both chemical and process-based interventions influence spoilage dynamics, with hot milk reformulation showing higher efficacy in cakes.
These observations are consistent with studies reporting fungal loads in baked products from various regions. Williams et al. (2020) found total fungal counts in cakes sold in Port Harcourt ranging from $2.0 \times 10^6$ to $3.0 \times 10^6$ CFU/g, while Das et al. (2020) reported lower counts ($1.5 \times 10^2 – 2.4 \times 10^3$ CFU/g) in freshly baked cakes, reflecting the impact of storage time and conditions. Nawawi et al. (2016) also observed mold growth in banana cake stored at room temperature on the sixth day, supporting the trend seen in PC and UB in this study. The results highlight that freshly baked products typically have low fungal counts, but without effective preservatives or formulation strategies, fungal proliferation increases significantly during storage.

The TFC data suggest that process-based interventions such as hot milk treatment can be more effective than chemical preservatives in limiting fungal proliferation in cakes. For bread, calcium propionate delayed fungal growth but was insufficient to fully prevent spoilage during prolonged storage. These results emphasize the importance of combining preservative use with optimized processing and handling practices to maintain microbial stability and extend the shelf life of bakery products.

The Bacterial Genera identified in this study revealed notable trends influenced by both preservatives and formulation methods. Across all samples, *Bacillus* species were the most prevalent, occurring in 80% of both cake and bread samples. This aligns with findings by Lindsay et al. (2004), where *Bacillus* was reported to constitute 98% of isolates from bakery products. This high prevalence reflects the ability of *Bacillus* spores to survive baking temperatures and later germinate during storage; a phenomenon frequently associated with rope spoilage.

In the bread samples, preserved bread (PB) and unpreserved bread (UB) both showed *Bacillus spp.* at 80% frequency, *Staphylococcus aureus* at 40%, and *Lactobacillus spp.* at 20%. PB additionally contained *Micrococcus spp.* at 20%, while UB contained *Enterobacter spp.* at 40%. *Pseudomonas spp.* was present in 60% of PB and 40% of UB samples, and *Sporolactobacillus spp.* was identified only in PB. These results indicate that while chemical preservatives such as calcium propionate can suppress certain microbial populations, they are less effective against *Bacillus* and *Pseudomonas* species, which tolerate preservative stress through spore formation and biofilm production.

For cake samples, preserved cake (PC) and reformulated cake (RC) both had *Bacillus spp.* at 80%, with *Staphylococcus aureus* detected only in PC at 20% and absent in RC. *Lactobacillus spp.* was found in both at 20%, *Leuconostoc spp.* occurred in PC at 40% and in RC at 20%, *Aeromonas spp.* was identified in 40% of PC and 20% of RC, *Clostridium spp.* appeared in PC at 20%, and Pseudomonas spp. in 60% of PC and 40% of RC. *Sporolactobacillus spp.* was observed only in PC. These patterns suggest that the hot milk formulation (RC) maintained lower diversity of spoilage organisms and fewer opportunistic pathogens compared to PC, indicating that heat treatment effectively reduced microbial proliferation while still allowing the dominance of spore-forming Bacillus.

Notably, no Escherichia coli was detected in any of the homemade samples, contrasting with bakery studies in Aliero, Kebbi State (Muhammad & Galadima, 2023) and Dhaka, Bangladesh (Hossain et al., 2020), where *E. coli* and other *Enterobacteriaceae* were common due to environmental contamination and poor hygienic practices. This difference likely reflects the controlled preparation environment of homemade samples and the absence of post-baking contamination from bakery handling, water, or packaging surfaces.

*Staphylococcus aureus* was detected in both bread and cake samples, but their frequency varied depending on formulation and preservative use. In bread, *Staphylococcus aureus* was present in 40% of both PB and UB, whereas in cake it was detected only in PC at 20% and absent in RC. *Staphylococcus aureus* is commonly found on human skin and mucous membranes, and its presence in baked products is often linked to post-baking contamination from handlers (Daniyan & Nwokwu, 2011; Ali et al., 2023). The lower frequency in RC cake suggests that minimal handling and the use of hot milk pasteurization helped limit contamination. *Enterobacter*, a Gram-negative indicator of environmental or fecal

contamination, was detected only in UB bread at 40%, while absent in all other samples. This is consistent with the understanding that *Enterobacter* species are not heat-resistant and are typically introduced post-baking through handling, water, or packaging surfaces (Jay et al., 2005). The absence of *Enterobacter spp.* in cakes and PB further suggests that either chemical preservatives or careful handling reduced their occurrence.

The presence of S*taphylococcus aureus* and *Enterobacter spp.* in certain bread samples highlights lapses in hygiene during handling and storage, even when preservatives are used. These findings align with previous studies in Dhaka, Bangladesh, and Aliero, Kebbi State, where bakery breads were contaminated with *Staphylococcus*, *Pseudomonas*, and *Enterobacter* species due to poor hygienic practices (Das et al., 2020; Ibrahim Galadima et al., 2023). In the context of this study, the absence of *Enterobacter spp.* and the lower occurrence of *Staphylococcus aureus* in cakes indicate that process-based interventions such as hot milk treatment and careful handling can effectively reduce post-baking contamination.

The Fungal Genera identified included *Candida spp, Fusarium spp., Penicillium spp., Aspergillus niger, Aspergillus flavus, Mucor spp., Saccharomyces cerevisiae,* and Rhizopus spp. In the cake samples, F*usarium, Penicillium, Aspergillus niger*, and *Mucor* species showed the highest frequency of occurrence (60%) in the preserved cake (PC), while *Candida* and *A. flavus* occurred at 40%. In contrast, the reformulated cake (RC) showed *Mucor spp.* (80%) as most frequent, followed by *A. flavus* (60%), *Penicillium spp.*(60%), and *A. niger* (40%), with *Candida* and *Fusarium* occurring at lower frequencies. Among the bread samples, preserved bread (PB) showed *Saccharomyces cerevisiae* (60%) as dominant, followed by *Candida spp, Fusarium spp, Penicillium spp, A. niger, A. flavus, Mucor spp,* and *Rhizopus spp* (each 40%). In unpreserved bread (UB), *Penicillium* and *A. flavus* showed the highest occurrence (80%), while *Mucor* had the lowest (20%), with *Rhizopus* and *Saccharomyces cerevisiae* both appearing at 80% and 60%, respectively.

These fungal species are known to be associated with bakery spoilage. *Fusarium, Aspergillus flavus, A. niger, Cladosporium,* and *Penicillium* have been frequently isolated from baked foods and their processing environments (Morassi et al., 2018; Nakhchian et al., 2014; Ibejekwe & Nyam, 2018). Similarly, Sudawa et al. (2022) identified *Aspergillus*, *Mucor*, and *Rhizopus spp*. in retail cakes sold in Kano, while Williams et al. (2020) reported *A. niger* in cakes sold within Port Harcourt metropolis. These findings are consistent with the present study, where *Aspergillus* and *Penicillium* species were among the most frequent isolates.

According to Ahaotu et al., (2024), *Aspergillus niger* can tolerate high concentrations of sugar and salt, enabling its survival in cakes and other baked goods. Its dominance in PC samples aligns with its ability to withstand osmotic stress and resist mild heat during baking. The lower fungal diversity and absence of visible growth in RC throughout the 12-day storage period can be attributed to the hot milk treatment, which provides a mild pasteurization effect, reducing initial spore load. The absence of preservatives in RC likely limited nutrient changes that favor fungal colonization, while the heat treatment improved product stability by inactivating spores and denaturing fungal enzymes.

In contrast, visible fungal growth was first observed in PC samples on day 9 of storage, consistent with reports by Ahaotu et al., (2024) that fungal growth in fruit cakes typically begins between days 9 and 10 at ambient temperature. The predominance of *Aspergillus spp*. and *Fusarium spp.* agrees with Ahaotu et al., (2024), who reported these as the most frequent contaminants in stored cakes, while *Penicillium* and *Mucor* remain ubiquitous spoilage molds. *Fusarium spp* are of public health concern due to their production of moniliformin toxin, while *Penicillium spp*. can secrete citrinin and cyclopiazonic acid, which cause nephrotoxic and hepatotoxic effects (Ire et al., 2020).

**Physiochemical Quality of Bakery Products**

The pH and Moisture Content of the bakery samples over the 12-day storage period reflected differences in formulation and preservative use. Bread samples, preserved bread (PB) and unpreserved bread (UB), started at mildly acidic levels (5.37 and 5.39, respectively). PB showed a slight increase to 5.47 on day 6 followed by a sharp decline to 4.33 on day 12, while UB gradually decreased to 5.13 on day 6 and 4.12 on day 12. These changes are consistent with microbial fermentation and acid accumulation from spoilage bacteria and yeasts, particularly in UB, and align with observed high total bacterial and fungal counts. According to ICMSF guidelines, pH changes can create conditions that support pathogen growth, with a pH of 4.6 often cited as a control boundary for spore-forming organisms. The decline in bread pH, coupled with increasing moisture (PB: 37.0 → 40.4 %; UB: 39.8 → 43.1 %), reflects the role of water activity and acidification in promoting microbial proliferation, consistent with previous findings that moisture is a critical determinant of shelf life in bakery products ( Coppock et al., 2009).

Cake samples exhibited different pH dynamics. Preserved cake (PC) increased from 5.21 to 6.10, reducing the antimicrobial efficacy of weak acids like vinegar and allowing mold growth during late storage. Reformulated cake (RC) remained relatively stable (6.71 → 6.42), reflecting minimal microbial activity and demonstrating the effectiveness of hot-milk pasteurization in limiting microbial proliferation (Coban, 2020; Tamime, 2009; Varnam & Sutherland, 2001). The final pH of cakes is influenced by ingredients such as leavening agents, eggs, and acids, which affect chemical reactions, texture, volume, and stability during baking. This aligns with the understanding that pH in batters and doughs can be modulated by ingredient selection: acidity can be increased by adding acids such as vinegar or cream of tartar, whereas alkalinity can be raised using baking soda (Ward et al., 2022). In the case of RC, the hot-milk treatment likely reduced microbial activity and maintained pH stability. The rise in PC pH can be attributed to the buffering capacity of preservatives and formulation components, which diminished over time, permitting fungal growth despite initial inhibition. Moisture content followed similar trends: PC increased from 36.2 % to 40.0 %, while RC remained lower and stable (32.4 → 33.8 %).

RC's lower and stable moisture likely reduced water activity, contributing to enhanced microbial stability and limited spoilage.

These results align with typical pH ranges reported for bakery products. Bread pH generally ranges from 5.0 to 6.0, with white bread around 5.5 and whole wheat slightly more acidic (~6.1), while cake pH varies by type, from acidic fruitcakes (4.4–5.0) to yellow layer cakes (6.7–7.5) and sponge or chocolate cakes (7.3–8.0) (Ward et al., 2022). The stability observed in RC reflects the influence of processing interventions, ingredient selection, and controlled handling on microbial proliferation.

Microbial stability in bakery products depends on formulation, preservative use, and processing techniques. Process-based interventions such as hot-milk treatment effectively limit microbial growth, maintain pH and moisture stability, and extend shelf life, whereas chemical preservatives alone, including calcium propionate, may delay spoilage but cannot fully compensate for high moisture, pH fluctuations, or suboptimal handling.

**Sensory Evaluation of Bakery Products**

The Sensory Evaluation of bakery samples over the 12-day storage period was assessed using a 9-point hedonic scale for appearance, aroma, texture, taste, and overall acceptability as seen Table 7 and 8 . Bread samples, preserved bread (PB) and unpreserved bread (UB), showed gradual declines in sensory scores over time. On day 0, both PB and UB were rated highly across all parameters, with overall acceptability at 9.0. By day 6, PB maintained moderate ratings (overall acceptability 7.5), while UB declined more sharply (overall acceptability 6.0). By day 12, PB received lower ratings (overall acceptability 4.8), whereas UB was least preferred (overall acceptability 2.6).

Cake samples, preserved cake (PC) and reformulated cake (RC), followed similar trends. On day 0, both samples were highly rated. By day 6, RC maintained better sensory scores than PC, particularly in texture and taste (overall acceptability 7.7 vs. 6.5). On day 12, RC still received higher scores (overall acceptability 5.6) compared to PC (4.8), indicating greater stability in sensory qualities over storage.

These observations are consistent with previous studies. Dinnaya and Ahaotu (2023) reported that bread preserved with calcium propionate was most preferred by panelists, whereas sorbic acid-preserved bread was least favored. The taste, aroma, and mouthfeel of calcium propionate bread were liked moderately, with overall acceptability rated very high. Similarly, Shahnawaz et al. (2012) reported that bread preserved with 0.8 g calcium propionate maintained superior sensory qualities compared to sorbic acid, which was rated poorly across all parameters. The results suggest that calcium propionate effectively preserves sensory attributes while delaying spoilage. Reformulated cake (RC), treated with hot milk and without chemical preservatives, demonstrated greater sensory stability compared to preserved cake (PC), likely due to reduced microbial growth and minimal biochemical changes affecting texture and taste. This study evaluated microbial stability, physicochemical changes, and the effects of preservatives and processing interventions in bakery products, focusing on four sample types: preserved cake (PC) with calcium propionate and vinegar, reformulated cake (RC) prepared with hot milk and no preservatives, preserved bread (PB) with calcium propionate, and unpreserved bread (UB) with a standard recipe. Microbial analysis showed that bread samples generally had higher microbial loads than cakes. Total bacterial, coliform, Staphylococcal, and fungal counts were highest in unpreserved bread, reflecting post-baking contamination and higher moisture content, with bread pH decreasing and moisture increasing over time, supporting microbial proliferation. . The hot milk treatment in RC likely acted as mild pasteurization, reducing microbial load, inactivating spores and enzymes, and limiting water activity, thereby slowing microbial proliferation and extending shelf life.

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