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POST-BAKING CONTAMINATION AND MICROBIAL GROWTH DYNAMICS IN BREAD: A STUDY OF INDUSTRIAL AND TRADITIONAL PRODUCTION SYSTEMS IN BAKU, AZERBAIJAN

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The microbiological quality and post-baking contamination dynamics of bread in Baku, Azerbaijan, were evaluated by comparing industrial packaged breads from supermarkets with traditional unpackaged tandir breads. Sixteen freshly baked samples were analyzed on Day 1 and Day 3 under ambient storage conditions for total aerobic bacteria, coliforms, *Escherichia coli*, yeasts, molds, and *S. aureus* using ISO-standard methods. Although baking effectively reduced initial microbial loads, substantial microbial growth was observed during storage. Industrial packaged breads exhibited greater microbiological stability than traditional unpackaged tandir breads, which displayed higher variability and contamination levels. The detection of hygiene indicator organisms in several samples underscores the importance of post-baking handling and storage conditions in ensuring bread safety and shelf life.

Keywords: Bread, microbial dynamics, contamination

INTRODUCTION

Designing healthier bread by considering the gut microbiota has gained increasing attention in recent years [1]. The evolution of breadmaking provides a prime example of how diet-driven host microbial interactions could be altered by the industrialization of food production [2]. Traditional breadmaking involves prolonged spontaneous fermentation using unrefined or whole-grain flours derived from locally available grains and root plants without food additives [2]. Mass production of refined flour, the emergence of commercial yeast starters, and the development of mechanical dough-making processes aided by food additives and bread improvers transformed breadmaking into a rapid and controlled industrial system [2,41].

Bread is one of the most valuable sources of both macro- and micronutrients in the human diet [3]. It also serves as an effective vehicle for functional ingredients such as prebiotics, particularly dietary fibers with selective benefits for the gut microbiota [1]. Recent progress in microbiome research highlights the importance of integrating microbiome-based strategies into functional food development to improve public health [1,4]. When host microbial interactions are considered, healthier bread products may be designed by supplying precursors of beneficial bacterial metabolites or eliminating compounds associated with harmful metabolic pathways [1].

Bread is also a rich source of antioxidant phytochemicals [3]. Phenolic compounds bound to cereal fiber matrices can be released by gut microbial enzymatic activity, enhancing their bioavailability [4]. For

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example, colonic *Bacteroides spp*. can release ferulic acid from arabinoxylan-rich wheat and barley bran without further biotransformation [4].

Flatbread was a staple food of ancient civilizations and evolved over time into modern bread products [2]. It is believed to have spread across Europe during the Sumerian civilization in Mesopotamia [2]. Later, wheat became an essential part of Europeans' diet [3]. Cereal composition, physicochemical flour properties, and fermentation practice all have a significant impact on the bread in Europe [2,5].

Cereals and cereal-based products constitute a major portion of global food resources [3]. Bakery products provide essential nutrients, including carbohydrates, proteins, lipids, vitamins, and minerals [3]. In many countries, up to 50% of daily calories come from bread [3]. Cereal Flours are formed by the grinding of cereal grains into powder and impart structural strength to dough systems [5]. Currently, wheat flour is the dominant raw material in breadmaking worldwide [3]. The quality and nutritional properties of bread are primarily determined by its starch content, proteins, lipids, minerals, and other bioactive compounds [5,3].

During the second half of the twentieth century, domestic bread production declined as industrial bakeries assumed large-scale production [2]. Modern bread varieties are produced from different cereal flours combined with yeast and functional additives to improve quality and shelf stability [2,5]. Both flour and bread are generally considered microbiologically stable because of their low water potential [6]. Although this condition limits microbial growth in bread and flour, pathogenic bacteria can survive for extended periods [6]. Inappropriate storage conditions and poor sanitation during production and storage may facilitate microbial proliferation [6].

Foodborne outbreaks associated with contaminated flour have been reported in Australia, Europe, and the United States. Pathogenic microorganisms such as *Salmonella spp., E. coli*, and *Bacillus cereus* have been detected at low levels in wheat and flour samples [8-11]. In addition to bacterial contamination, mold growth in flour can significantly degrade bread quality [12].

Bread preservation is primarily achieved through baking [5]. The typical shelf life is 5–7 days at room temperature, 1–2 weeks under refrigeration, and up to 3 months when frozen [6]. Microbial spoilage usually results from post-baking contamination originating from air, equipment, cooling, slicing, and packaging operations [6]. The microbial load depends on environmental hygiene, equipment sanitation, ingredient quality, and personal hygiene practices [6].

Bread spoilage microorganisms include genera such as *Bacillus*, *Clostridium*, *Lactobacillus*, *Aspergillus*, *Penicillium*, *Cladosporium*, *Rhizopus*, *Candida*, and *Saccharomyce*s [13-17]. Some of these microorganisms cause ropiness, moldiness, and off-flavors in bread [13].

Rope spoilage is generally associated with endospore-forming bacteria, including *Bacillus subtilis, B. amyloliquefaciens, B. pumilus, B. licheniformis*, and *B. cereus* [14,15,17]. Heat-resistant endospores from these microorganisms can survive after baking and germinate within 1–2 days when the favorable conditions are available [17]. These bacterial species produce extracellular amylases and proteases, which lead to crumb softening, stickiness, discoloration, and unpleasant fruity odors. Specific conditions, such as high dough moisture, slow cooling, and pH values above 5, promote rope development [14]. It has been reported that industrial whole meal flours contain aerobic endospore-forming bacteria up to 3.1 log spores/g, including *Paenibacillus polymyxa. Serratia marcescens* is linked to pigment-related spoilage, also known as bloody bread. Preservative-free bread typically develops visible mold within 5–6 days [13]. Both sliced and packaged bread are vulnerable to fungal contamination, with prevalent spoiling fungi including species of *Penicillium, Aspergillus, Cladosporium, Mucor*, and *Rhizopus* [12,17]. Certain molds can also produce mycotoxins, which can pose a threat to food safety [13]. The crust, with approximately 16% moisture, offers greater resistance to mold growth than the crumb [4]. Spores may be transferred from the air and equipment despite the inhibitory effects of baking yeasts [6].

Microbiological stability of sourdough bread is maintained by natural preservation methods, such as olive leaf extract and selected lactic acid bacteria, which have demonstrated positive effects on moisture, pH, acidity, and texture [17]. Fungal spores are significantly reduced by the combined use of radio-frequency and hot air treatment [18].

Packaging systems also play a critical role in preventing fungal contamination. Chitosan-based coatings and the beeswax–chitosan packaging methods have enhanced sensory and microbiological stability under refrigerated conditions [19]. Although bread is among the most consumed foods, few studies have addressed its microbiological status in specific regions.

Microbiological quality assessment is an essential component of food safety systems, as it provides important information on contamination sources, hygienic conditions, and potential risks associated with

food production and storage. Continuous monitoring of microbial indicators enables the early detection of contamination events and supports evidence-based strategies for quality control and risk management.

Therefore, the aim of this study is to evaluate the microbiological quality and bioburden of bread sold in packaged and unpackaged forms.

MATERIALS AND METHODS

Sample Collection

A total of 16 freshly baked bread samples were collected from retail outlets in Baku, Azerbaijan. To capture potential variability in production practices, samples were obtained from two distinct sources: commercial supermarkets and traditional tandir bakeries, where bread is typically prepared and sold directly on-site. All samples were collected aseptically to prevent exogenous contamination. Each bread loaf was handled using sterile disposable gloves, placed immediately into a sterile polyethylene bag, heat-sealed, and labeled with a unique alphabetical code (e.g., A, B, C, etc.) for traceability. The samples were transported to ChemLab in Baku in insulated containers within 2 h of collection and were stored at 4 ± 2 °C until microbiological analysis, which was performed within 24 h.

Sample Processing

All microbiological procedures were conducted under aseptic conditions in a laminar airflow cabinet, which was disinfected with 70% ethanol before and after use. The experiments were carried out at ChemLab, an accredited laboratory in Baku, Azerbaijan.

From each bread sample, four independent subsamples (10.0 g each) were aseptically weighed to account for sample heterogeneity and minimize analytical bias. Each subsample was homogenized with 90.0 mL of sterile peptone water in a stomacher bag for 1-2 minutes to obtain the initial suspension. Ten-fold serial dilutions were prepared up to 10^{-3} by sterile peptone water. For microbial enumeration, aliquots from appropriate dilutions were transferred into sterile Petri dishes and mixed with molten culture media using the pour plate technique [20]. Microbiological analyses were performed on Day 1 and Day 3 of storage. The selected storage period was based on the shelf-life information provided on most bread labels, where the recommended consumption period was typically indicated as 3 days. This approach allowed the assessment of short-term microbial growth dynamics and changes in microbiological quality during the expected storage period under ambient conditions.

All media were prepared and analyses were performed according to international standards: Plate Count Agar (PCA) for total aerobic mesophilic bacteria (ISO 4833-1:2013), Violet Red Bile Agar (VRB) for coliforms (ISO 4832:2006), Tryptone Bile X-glucuronide Agar (TBX) for E. coli (ISO 16649-2:2001), Yeast Extract Glucose Chloramphenicol Agar (YGC) for yeasts and molds (ISO 21527-1:2008 and ISO 21527-2:2008), and Baird-Parker Agar (BPA) supplemented with egg yolk tellurite emulsion for coagulase-positive staphylococci (ISO 6888-1:2021) [20-31]. Following incubation, all colonies were enumerated, and colony-forming units per gram (CFU/g) were used to express microbial numbers of the sample. The dilution range (10^{-1} to 10^{-3}) was selected based on preliminary trials indicating expected contamination levels in freshly baked bread [32,33]. The dilution range (10^{-1} to 10^{-3}) was selected based on preliminary trials indicating expected contamination levels in freshly baked bread. Throughout the study, microbial counts remained within countable ranges recommended for reliable enumeration, and excessive colony overcrowding was not observed, confirming that dilution up to 10^{-3} was sufficient for accurate microbial counting. All counts were performed in duplicate from four independent subsamples, and results were expressed as mean CFU/g ± standard deviation.

Microbiological Analysis

The microbiological quality of the different bread samples was assessed by determining specific indicator microorganisms and potential pathogen species. Standardized international protocols are followed during the research. It was important to follow the manufacturer's instructions to prepare all culture media, and the media were later autoclaved at 121 °C for 15 minutes, unless otherwise specified. Sterile peptone water was used for the serial dilutions. A summary of the target analytes and the corresponding ISO methods

applied in this study is presented in Table 1, and results were represented as colony-forming units per gram of bread sample, according to the respective international standards listed [22-33].

Table 1. Microbiological analysis methods and corresponding international standards used for bread samples [20-31].

Analyte	Method / International Standard
Total Mesophilic Aerobic Bacteria	ISO 4833-1:2013
Coliform Bacteria	ISO 4832:2006
Escherichia coli	ISO 16649-2:2001
Enterobacteriaceae	ISO 21528-2:2017
Salmonella spp.	ISO 6579-1:2017
Pseudomonas spp.	ISO/TS 11059:2009
Staphylococcus aureus	ISO 6888-1:2021
Listeria monocytogenes	ISO 11290-1:2017
Sulfite-Reducing Clostridia	ISO 15213-1:2023
Yeasts and Molds (aw ≤ 0.95)	ISO 21527-2:2008
Yeasts and Molds (aw > 0.95)	ISO 21527-1:2008

The microbiological quality of bread samples was additionally evaluated according to national microbiological standards for bread products [31,42], which were used as reference criteria for compliance assessment Table 2.

Table 2. Microbiological Standards for Bread Products

Indicator	Limit (per g)	Interpretation
Total aerobic bacteria	≤ 1×10^3 CFU/g	Should not exceed
Coliforms	≤ 1.0 CFU/g	Should not be present
Yeasts and moulds	≤ 25 CFU/g	Limited acceptable level
S. aureus	0 CFU/g	Must be absent
Pathogenic microorganisms	Not permitted	Must be absent

Plate Count Agar (PCA) for Total Mesophilic Aerobic Bacteria

The total mesophilic aerobic bacterial count in bread samples was determined using Plate Count Agar (PCA) according to ISO 4833-1:2013 [20]. Serial dilutions of the bread samples were plated using pour plate technique and then incubated aerobically for 72 hours at 30°C, as specified in the standard, with the upper surfaces facing downwards. Following incubation, all colonies were enumerated, and the total bacterial count was calculated as colony-forming units per gram (CFU/g) of bread sample.

Violet Red Bile Agar (VRBA) for Coliform Bacteria

Coliform bacteria in bread samples were enumerated using Violet Red Bile Agar (VRBA) following ISO 4832:2006 [21]. Appropriate sample dilutions were plated using the pour plate method with an overlay layer to maintain microaerophilic conditions. The plates were incubated inverted at 37±1°C for 24±2 hours. Typical coliform colonies were counted and the results were reported as CFU/g of the bread sample. A counting range of 15–150 colonies was applied for VRBA plates to improve counting accuracy and colony differentiation, as overcrowding and bile precipitation on selective media may complicate reliable enumeration at higher colony densities.

TBX Agar for Escherichia coli

E. coli in bread samples was enumerated on Tryptone Bile X-glucuronide (TBX) agar in accordance with ISO 16649-2:2001 [22]. After preparing the serial dilutions plates were incubated inverted at 44±1°C for 24±2 hours as specified by the standard. Counts were performed on plates and the collected results were expressed as CFU/g of bread sample.

Baird-Parker Agar for Staphylococcus aureus

S. aureus in bread samples was enumerated on Baird-Parker agar according to ISO 6888-1:2021 [26]. For analysis, appropriate sample dilution was plated and incubated aerobically, inverted, at 37±1°C for 24-48 hours. Following incubation suspected colonies were subjected to confirmatory tests, including biochemical testes, including catalase, coagulase assays. After confirmation, the number of typical *S. aureus* colonies was enumerated and reported as CFU/g of bread sample [35].

YGC Agar for Yeasts and Molds

Although bread is generally considered a low water activity product (aw ≤ 0.95), yeasts and molds were also enumerated using Yeast Glucose Chloramphenicol (YGC) Agar following ISO 21527-1:2008 [29]. Sample dilutions were prepared in sterile peptone water were incubated at 25 ± 1 °C for 5 days. The chloramphenicol incorporated into the medium effectively inhibited bacterial growth, allowing for the selective isolation of both yeasts and molds. Following incubation were counted separately and results were calculated and expressed as CFU/g of bread sample.

Confirmatory Tests for Presumptive Isolates

Presumptive colonies of *S. aureus* on Baird-Parker Agar (black, shiny colonies with clear zones) and presumptive *E. coli* on TBX Agar (blue-green colonies) were subjected to confirmatory tests including Gram staining and β-glucuronidase activity following standard microbiological procedures [32,33]. Gram staining and the potassium hydroxide (KOH) tests were performed to evaluate bacterial morphology. Gram-positive bacteria appeared purple to violet, while Gram-negative bacteria appeared pink to red. Cellular morphology and arrangement were recorded for each isolate. *S. aureus* was identified as Gram-positive cocci arranged in grape-like clusters and showed negative KOH reactions, while *E. coli* appeared as Gram-negative rods and produced positive KOH reactions [33]. For *S. aureus* confirmation, the catalase test was performed by transferring a colony to a clean glass slide and adding a drop of 3% hydrogen peroxide, immediate and vigorous bubble formation indicated a positive result. The coagulase test was conducted by inoculating a colony into 0.5 mL of rabbit plasma and incubating at 37°C for 4-6 h, clot formation indicated a positive result for coagulase-positive staphylococci [34].

Statistical Analysis of Microbial Changes

For each bread sample, microbial counts were expressed as CFU/g and transformed as log10(CFU/g + 1) prior to statistical analysis. To normalize the distribution of microbial counts and reduce the impact of extreme values, colony-forming unit (CFU/g) data were transformed using a logarithmic scale. Log transformation stabilizes variance and allows for more reliable comparison between samples with highly variable microbial loads. In this study, a constant value of 1 was added prior to transformation to accommodate zero counts and avoid undefined values [38]. The transformation was performed using the following formula:

$$\log_{10}(\text{CFU/g} + 1)$$

Differences between Day 1 and Day 3 counts for the same bread samples were evaluated using the Wilcoxon signed-rank test. All bread types were included in the paired analysis, and samples with non-detectable counts were recorded as zero. A p-value of less than 0.05 was considered statistically significant.
The Wilcoxon signed-rank test is based on the differences between paired observations. For each pair, the difference is calculated as:

$$d_i = x_{i,\text{Day 3}} - x_{i,\text{Day 1}}$$

The absolute differences are ranked, and the test statistic is calculated as the sum of signed ranks:

$$W = \sum R_i^+$$

Where R_i^+ represents the ranks of positive differences. The resulting test statistic is used to evaluate whether the median difference between paired observations deviates significantly from zero. Data visualization was performed using stacked bar plots to illustrate total microbial load and the relative contribution of different microbial groups (PCA, YGC, VRB, TBX, BPA) represented in the Graph 1.

RESULTS

Microbiological quality and dynamics of bread samples

A total of 16 bread samples representing different production systems and packaging conditions were evaluated for microbiological quality and short-term microbial dynamics. On Day 1, most bread samples showed low microbial counts, confirming the effectiveness of the baking process in reducing microbial load. However, post-processing contamination and ambient storage conditions may contribute to microbial proliferation during storage. Among supermarket breads, sample A showed low bacterial contamination (5.0×10^1–1.3×10^2 CFU/g) and detectable coliform counts (6.5×10^1–1.2×10^2 CFU/g), indicating hygiene deficiencies. Samples D and G demonstrated higher microbial contamination, including elevated total aerobic bacterial counts (3.0×10^2–7.0×10^2 CFU/g and 1.5×10^2–4.0×10^2 CFU/g, respectively), coliforms (1.3×10^1–3.5×10^1 CFU/g and 1.0×10^1–3.0×10^1 CFU/g), yeasts and molds (1.1×10^2–2.5×10^2 CFU/g and 5.0×10^1–1.6×10^2 CFU/g), and detectable *E.coli* (5–12 CFU/g and 2–5 CFU/g), suggesting possible post-baking contamination and inadequate hygienic handling conditions. Among traditional tandir breads, sample F showed moderate contamination characterized by detectable total aerobic bacteria (4–20 CFU/g), yeasts and molds (18–85 CFU/g), and *S. aureus* (3–8 CFU/g). Sample J demonstrated one of the highest contamination levels observed in the study, characterized by elevated bacterial, fungal, and hygiene-indicator microorganism counts. In contrast, samples O, R, and U showed no detectable microbial growth throughout the storage period, reflecting good microbiological quality. The results are presented in Table 3.

Table 3. Microbial counts (CFU/g) of bread samples on Day 1 and Day 3

Sample	PCA D1	PCA D3	YGC D1	YGC D3	VRB D1	VRB D3	TBX D1	TBX D3	BPA D1	BPA D3
A	50	130	4	18	65	120	0	0	1	3
O	0	0	0	0	0	0	0	0	0	0
R	0	0	0	0	0	0	0	0	0	0
U	0	0	0	0	0	0	0	0	0	0
E	200	450	16	70	14	40	0	0	0	0
F	4	20	18	85	0	2	0	0	3	8
I	0	3	0	4	0	1	0	0	0	0
M	0	5	12	45	0	1	0	0	8	18
B	35	100	8	25	0	2	0	0	0	0
C	10	60	4	16	0	1	0	0	0	0
D	300	700	110	250	13	35	5	12	2	5
Z	1	8	0	2	4	15	1	3	1	2
G	150	400	50	160	10	30	2	5	2	5
H	50	150	35	95	20	55	0	0	6	12
J	250	600	25	90	0	2	30	65	3	8
K	60	180	10	40	3	12	12	25	2	5

During storage (Day 3), microbial counts increased in previously contaminated samples, particularly for total aerobic bacteria, fungi (YGC), and hygiene-indicator microorganisms. Increased fungal growth was especially observed in samples D, G, H, J, F, and E.

When evaluated according to national microbiological standards for bread products (Table 2), all analyzed samples complied with the acceptable limit for total mesophilic aerobic bacteria ($\leq 1 \times 10^3$ CFU/g). However, non-compliance was observed for coliforms, *E. coli*, yeasts and molds, and *S. aureus* in several samples. A compliance assessment against national microbiological standards is presented in Table 4.

Table 4. Compliance assessment against national microbiological standards.

Indicator	Standard Limit	Samples Exceeding Limit	Non-Compliant Samples	Compliance Rate

Total aerobic bacteria	≤ 1 × 10³ CFU/g	None	—	100%
Coliforms	≤ 1 CFU/g	A, B, D, E, F, G, H, J, K, Z	10	38%
E. coli	0 CFU/g	D, G, J, K, Z	5	69%
Yeasts and molds	≤ 25 CFU/g	D, E, F, G, H, J, K, M	8	50%
S. aureus	0 CFU/g	A, D, F, G, H, J, K, M, Z	9	44%

Overall, factory-produced and packaged breads demonstrated better microbiological quality, whereas traditional unpackaged tandir breads showed greater variability, higher contamination levels, and more pronounced microbial growth during storage. Although most samples complied with acceptable limits for total aerobic bacterial counts, several breads failed to meet strict hygienic microbiological criteria due to the presence of indicator and potentially pathogenic microorganisms.

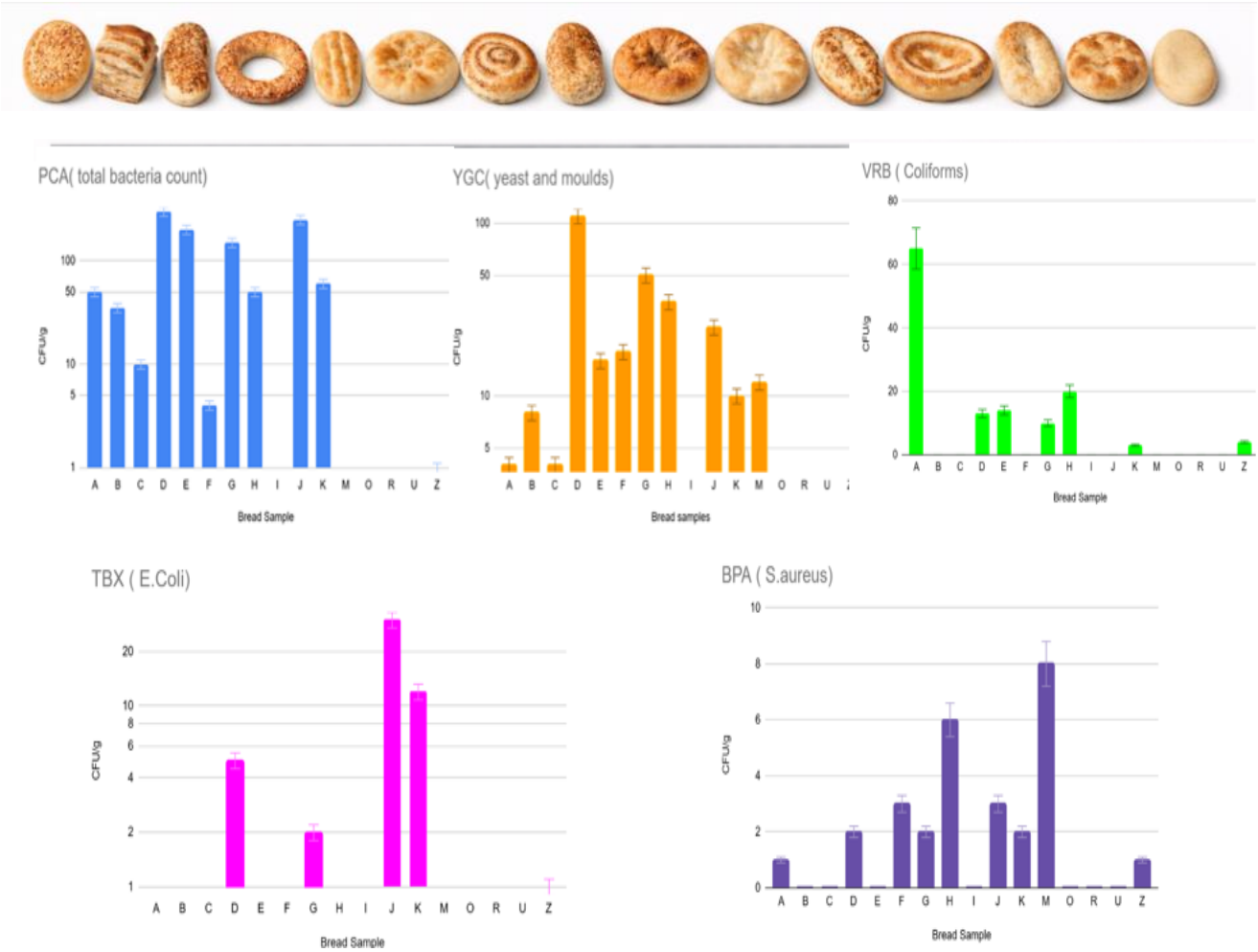

Figure 1. Distribution of microbial counts across the five culture media used for bread sample analysis. Higher microbial loads were mainly observed in samples D, E, G, H, J, K, whereas samples O, R, and U showed no detectable microbial growth. Greater microbial variability was observed among unpackaged/traditional bread samples compared with packaged supermarket breads.

The highest total bacterial counts (PCA) were observed in samples D and J, followed by sample E. Elevated coliform counts (VRB) were detected in samples A, B, D, E, F, G, H, J, K, and Z, indicating hygiene deficiencies and possible post-processing contamination. *E. coli* (TBX) was detected in samples D, G, J, K, and Z, with the highest counts observed in sample J. Elevated fungal counts (YGC) were particularly

observed in samples D, G, H, J, F, and E, suggesting increased spoilage risk during storage. *S. aureus* (BPA) was detected in samples A, D, F, G, H, J, K, M, and Z, indicating possible contamination associated with human handling. Overall, traditional unpackaged tandir breads demonstrated higher microbial contamination compared with packaged supermarket breads.

Changes in microbial counts (log10 CFU/g + 1) in bread samples between Day 1 and Day 3. (A) Total aerobic bacteria (PCA), (B) yeasts and molds (YGC), (C) coliforms (VRB), (D) *E. coli (TBX), and (E) S. aureus (BPA).* A significant increase in microbial counts was observed during storage.

Results of Statistical Analysis

Comparison of microbial counts between Day 1 and Day 3 using the Wilcoxon signed-rank test showed significant increases across all analyzed microbial groups. Total aerobic bacterial counts (PCA) increased significantly during storage (W = 0.0, p = 0.0015), as did yeast and molds counts on YGC agar (W = 0.0, p = 0.0015). Coliform counts on VRB agar also showed a significant increase (W = 0.0, p = 0.0014). For specific hygiene-related microorganisms, significant increases were observed for E. coli on TBX agar (W = 0.0, p = 0.0422) and for *S. aureus* on Baird-Parker agar (W = 0.0, p = 0.0071). Overall, these results indicate progressive microbial proliferation during storage. The temporal changes in microbial counts between Day 1 and Day 3 across different microbial groups are illustrated in Figure 2. The detailed statistical results of the Wilcoxon signed-rank test, including p-values and significance levels for each microbial parameter, are presented in Table 5.

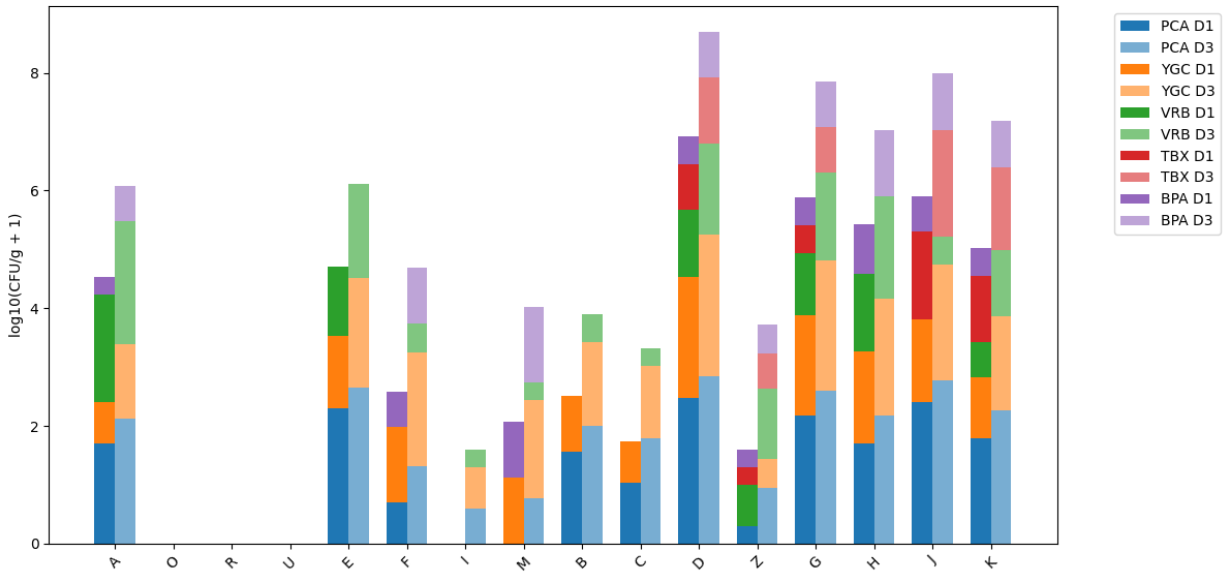

Figure 2. Changes in microbial counts [log10(CFU/g + 1)] between Day 1 and Day 3 for total aerobic bacteria (PCA), yeasts and molds (YGC), coliforms (VRB), *E. coli* (TBX), and *S. aureus* (BPA). Increased microbial loads were observed during storage.

Table 5. Results of the Wilcoxon signed-rank test comparing microbial counts (log_{10} CFU/g + 1) between Day 1 and Day 3.

Microbial Parameter	Total Paired Samples (n)	Non-Zero Pairs Used in Test	Test Statistic (W)	p-value	Significance	Interpretation
PCA (Total aerobic bacteria)	16	13	0.0	0.0015	Significant	Significant increase
YGC (Yeasts & molds)	16	13	0.0	0.0015	Significant	Significant increase
VRB (Coliforms)	16	13	0.0	0.0014	Significant	Significant increase
TBX (*E. coli*)	16	5	0.0	0.0422	Significant	Significant

						increase
BPA (*S. aureus*)	16	9	0.0	0.0071	Significant	Significant increase

DISCUSSION

The present study indicates that the microbiological quality of bread samples sold in Baku, Azerbaijan, was generally acceptable immediately after baking but weakened during storage, with significant increases in total aerobic bacteria, yeasts and moulds, coliforms, *E. coli*, and *S. aureus* by Day 3. This overall pattern is in agreement with previous studies showing that bread is usually microbiologically safer at the end of baking, whereas contamination and microbial multiplication become more evident during cooling, handling, packaging, retail display, and storage. Regarding this, the results of this investigation support the broader view that bread spoilage is not determined only by baking efficiency, but also by post-baking environmental and hygiene conditions [36].

In our study, most samples showed low or undetectable counts on Day 1, indicating that the baking process was initially effective at reducing vegetative microbial populations. However, the subsequent increase in counts by Day 3 suggests that thermal treatment alone was insufficient to ensure microbiological stability throughout storage. This interpretation is consistent with the review by Vermelho et al. (2024), which notes that after baking, mainly spore-formers and environmentally introduced contaminants remain relevant, and that air, equipment, packaging materials, insects, and food handlers are major entry points for microorganisms in bread production chains. That is why the results of this study fit well with the established concept that bread contamination is predominantly post-baking rather than solely raw-material driven [38].

The most significant finding of this work is the detection of coliforms and *E. coli* in selected samples, together with their significant increase during storage. This points to hygiene deficiencies in at least part of the production, handling, or distribution chain. Ali et al. (2023) in Alexandria, Egypt, came to a similar conclusion in their work, where unpacked bread and bread handled without gloves or displayed outside shops had markedly higher coliform counts, higher yeast and mould counts, and more frequent *S. aureus* contamination than better-protected products. In that study, unpacked breads had much higher coliform burdens than packed breads, and the researchers directly linked these differences to inadequate coverage, handling, and display practices. In this investigation, observations are similar; the contaminated bread samples in Baku likewise appear to reflect insufficient hygienic barriers after baking [37].

The significant increase in *S. aureus* observed in our data is also noteworthy. Because *S. aureus* is commonly associated with human skin, hands, and the upper respiratory tract, its presence in bread is often interpreted as evidence of contamination during manual handling. Ali et al. (2023) similarly reported that *S. aureus* was more frequent in flat, unpacked bread, in bread displayed outside shops, and in bread handled without gloves. In our study, the increase in *S. aureus* during storage suggests that even low initial contamination levels may become more significant over time if bread is stored under ambient conditions after exposure to human contact or to inadequately sanitised surfaces. This strengthens the argument that hygiene practices during retail and post-production handling are just as important as hygiene during mixing and baking [37].

Another major outcome of the present study was the pronounced rise in yeasts and moulds during storage, especially in samples already showing contamination on Day 1. This is particularly important because fungal proliferation is one of the main determinants of bread shelf life. Garcia et al. (2019), in their study of spoilage fungi in a Brazilian bread factory, showed that spoilage fungi were present not only in raw materials but also in air samples from cooling, slicing, and packaging areas, and concluded that recontamination after baking can drive early spoilage. Their work identified Penicillium and related fungi as major spoilage agents, directly linking the production environment to the fungal deterioration of bread. Our findings are consistent with this mechanism: the marked Day 3 increase in fungal counts strongly suggests that ambient storage and post-baking exposure created favourable conditions for fungal development in the contaminated samples [39].

The comparison between factory-produced/packaged breads and traditional unpackaged tandir breads in our study also follows a pattern seen in the literature. We observed that packaged industrial breads generally showed better microbiological quality and lower contamination than traditional unpackaged samples, which displayed greater variability and stronger microbial growth over time. A related trend was described by Caro et al. (2023), who found that breads from small stores had higher microbial contamination than breads from supermarkets. Although the retail context in that study differs from our direct comparison

of production systems, the direction of the evidence is very similar: products handled and distributed in more controlled commercial environments tend to have lower microbial loads than those exposed to less standardised conditions. This supports the interpretation that environmental control, standardised processing, and protective packaging are decisive factors in limiting bread contamination [40].

Overall, the present study contributes region-specific evidence from Baku, Azerbaijan, to a growing international body of literature showing that bread microbiological quality is shaped by interactions among baking, environmental contamination, packaging, and storage time. In comparison with previous authors, our work confirms three recurring conclusions: first, baking substantially lowers the initial microbial load but does not eliminate later contamination risks; second, unpackaged or less-controlled bread systems are more vulnerable to hygiene-related contamination; and third, fungi and other post-baking contaminants become increasingly important during storage. In this sense, our results are not isolated findings, but rather fit a consistent pattern already reported in Egypt, Brazil, and broader bakery-system reviews. At the same time, our study adds local comparative data between industrial and tandir breads and demonstrates, through paired Day 1/Day 3 analysis, that microbial proliferation during short-term ambient storage is statistically significant across several microbial groups [39].

In addition to its implications for food safety, the present study highlights the value of continuous microbiological monitoring for identifying biological risks associated with food production and storage systems. The systematic collection and evaluation of microbiological data provide important indicators of hygienic conditions, environmental exposure, and contamination pathways. Such monitoring approaches support evidence-based risk assessment and contribute to the development of preventive management strategies. The importance of systematic biological monitoring and risk-based assessment has also been emphasized by Mammadova et al. (2024), who highlighted the role of biodiagnostic and biomonitoring frameworks in supporting environmental management and sustainable development strategies in Azerbaijan [43].

CONCLUSION

This study evaluated the microbiological quality and temporal microbial dynamics of bread produced in industrial and traditional production systems in Baku, Azerbaijan. The findings demonstrated that freshly baked bread generally exhibited low microbial contamination, confirming the effectiveness of thermal processing in reducing microbial load. However, microbial proliferation increased considerably during storage, highlighting the critical role of post-baking handling, environmental exposure, and hygiene conditions in determining final product safety and quality.

The most pronounced microbial increases during storage were observed among total aerobic bacteria, yeasts, and molds, indicating that ambient storage conditions strongly promote microbial growth and contribute to bread spoilage. In particular, fungal proliferation emerged as a major factor limiting shelf life. The detection of indicator microorganisms, including coliforms, *E. coli*, and *S. aureus* in several samples further emphasizes the importance of hygienic processing, handling, and storage practices to minimize post-baking contamination risks.

Comparative observations suggested that industrially produced and packaged breads generally demonstrated better microbiological stability, whereas traditional unpackaged tandir breads exhibited greater variability and increased susceptibility to microbial contamination. These findings highlight the importance of controlled production environments, effective packaging systems, and strengthened sanitation measures, particularly in traditional bakery settings, to preserve bread quality and improve consumer safety.

Overall, this study demonstrates that bread, despite being considered relatively microbiologically stable immediately after baking, remains highly susceptible to microbial changes during storage. Storage duration represents an important determinant of spoilage progression and microbiological quality deterioration. Future studies should integrate advanced molecular approaches, including next-generation sequencing (NGS) and metagenomic analysis, to characterize both culturable and non-culturable microbial communities and provide deeper insight into microbial succession, spoilage mechanisms, and microbiome-based indicators of bread quality and safety. Larger-scale investigations across different production systems and storage conditions are also recommended to support evidence-based improvements in bakery product safety and shelf-life management.

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