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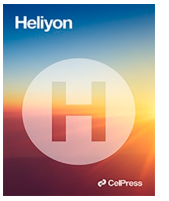

Comparative safety analysis of newly prepackaged backed food products and those approaching the expiry date in Bangladesh

Md Imran Hossain, Md Omor Faruk, Md Akber Subahan Mahbub Tuha, Sanjida Mimi, Khondakar Raisul Islam, Dr Md Sarafat Ali, Md Sahabuddin *

Department of Biotechnology and Genetic Engineering, Bangabandhu Sheikh Mujibur Rahman Science and Technology University, Gopalganj, 8100, Bangladesh

A R T I C L E I N F O

Keywords:
Prepackaged backed food products (bread, Cake, Muffin)
Health risk
Microbiome
Bacterial growth
Manufacturing and expired dates

A B S T R A C T

Purpose: The enrichment of microbial growth in prepackaged, frozen food goods from the day of manufacturing to the day of expiration has been the subject of recurrent concerns. These fortified foods are widely consumed by individuals of all ages in poor nations due to their ability to satisfy even the smallest of appetites. People often disregard the expiration dates printed on food packaging despite the fact that manufacturers are required by law to do so. This research looked into whether or not it was safe to consume packaged foods that were getting close to their expiration date. Finding out if people are exposed to hazardous microorganisms and how much bacteria is created daily on them.

Materials and methods: We collected six prepackaged backed food products samples of three types separately, where three were collected around manufacturing days and three were nearly expired days from different companies. We have assayed and identified the foodborne microbial communities among the samples by morphological study and different types of biochemical tests. After that, we tested how well various popular antibiotics worked against those isolates.

Results: It showed that there are more bacterial communities that grow gradually day by day on prepackaged backed food products and nearly expired products that contain a large number of food-borne disease-causing bacteria that show mostly resistance against commonly used antibiotics.

Conclusion: Although nowadays the demand for prepackaged backed food products is increasing as ready-to-eat processed foods, mostly in developing countries, there's a serious health risk if we take the products that were produced a long time ago.

1. Introduction

Leavened bread items have become increasingly important in family diets over the past century as convenient, ready-to-eat processed foods. With urbanization and more women working in industrial and public sectors, there is a growing demand for processed foods that have a longer shelf life, appealing taste, portability, and high nutritional quality. This demand is observed worldwide [1]. While prepackaged baked food products are one solution to meet these dietary needs, they include items such as bread, cakes, and buns. These products primarily consist of flour, biological or chemical leavening agents, and water. Depending on the final product and

* Corresponding author.
E-mail address: sahabuddin@bsmrstu.edu.bd (M. Sahabuddin).

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intended use, additional components such as eggs, sugar, and shortening may be added [2]. In developing countries, bread and biscuits play a significant role in midday school lunch programs for children [3]. Rolls, sweet dough goods, cookies, crackers, muffins, doughnuts, and cakes are common examples of prepackaged baked food products consumed frequently worldwide during breaks or to satisfy appetites [4].

The per capita consumption of various prepackaged baked food products exceeds 100 kg in Western developed nations, while in Bangladesh it was 0.43 kg in 2008 but increased to 2.09 kg in 2021 [5]. Wheat products typically provide approximately ten to twelve percent of saturated fats, ten to fourteen percent of protein, thirty percent of carbohydrates, twelve percent of calcium, twenty-three to twenty-seven percent of iron, thirty percent of thiamin, sixteen to twenty percent of riboflavin, seventeen to twenty-one percent of niacin, and twenty percent of sodium per 100 gm [6]. Consequently, when it comes to leavened baked food products, the mechanical and sensory aspects of the crumb are vital quality factors [7]. Water, leavening agents (either chemical leavening agents like NaHCO3 or biological leavening agents like yeast), sodium chloride (NaCl), sugar, and shortening are the four fundamental elements for leavened baked food products [8]. Kneading, fermentation, proofing, and baking are several methods employed to transform the ingredients into a fully formed porous structure [9]. Certain key elements can significantly impact the final product, and water stands out as one of the most important ones [10].

Leavened baked goods rely on wheat flour, which is a combination of two types of proteins called gliadins and glutenins [11,12]. These proteins collaborate to create a gluten network that is elastic enough to trap leavening gas during fermentation and baking when mixed and hydrated [13,14]. As the gluten protein denatures during heating, protein-protein crosslinking occurs through the formation of disulfide bonds, resulting in the coagulated gluten and the gelatinization of the starch within the gluten network [15,16]. Baking utilizes moist pressed cakes and dried granules, both containing billions of live Saccharomyces cerevisiae cells [17,18]. Once rehydrated, yeast in wheat flour begins to metabolize and ferment, releasing CO2 in the process [19]. Baked goods are known for their nutritional benefits. Additionally, bread, biscuits, cookies, doughnuts, and crackers have the potential to serve as transport mechanisms for additional proteins, addressing protein deficiency in regions where it is prevalent [20,21].

According to the study titled "Shelf Life and Safety Concerns of Bakery Products," published by the Department of Food Science and Agricultural Chemistry at McGill University's Macdonald Campus, bakery products, like many processed foods, are susceptible to physical, chemical, and microbiological spoilage [22]. Consequently, it can be challenging to prevent contamination. Various types of bakery products have been linked to the presence of Salmonella species, Listeria monocytogenes, and Bacillus cereus, while high-moisture bread items packaged in modified atmospheres raise concerns about Clostridium botulinum [23].

Bakery products are highly popular among people of all ages in developing countries, especially in Bangladesh, due to their nutritional benefits. These ready-to-eat processed foods serve as a convenient solution for satisfying minor hunger at any time. However, if these products become a health risk factor, alternative decisions must be made. In our research study, we aimed to investigate the safety of consuming prepackaged food products nearing their expiration date. Specifically, we examined the bacterial growth in the first three days after production and the three days before expiration, as individuals often consume bakery items close to their expiration date without considering the potential exposure to harmful bacteria.

2. Materials and methods

2.1. Collection of samples

The present research work was conducted at the Biotechnology and Genetic Engineering lab of Bangabandhu Sheikh Mujibur Rahman Science and Technology University (BSMRSTU), Bangladesh. To ensure the reliability of our new findings, the entire experiment was repeated three times, and consistent results were obtained.

For our study, we collected a total of eighteen prepackaged bakery food product samples, specifically bread, cake, and muffin. These samples were obtained separately from a local super-shop in the market. Nine samples were collected that were around their manufacturing days, while the other nine samples were obtained close to their expiration days. These samples represented products from different Bangladeshi companies.

All the collected samples were ready for sale and were packaged in plastic sheeting at the shop. The main ingredients of the bread samples included flour, water, salt, and yeast. The cake samples consisted of flour, eggs, fat (usually butter), sugar, salt, milk, and leavening agents. The muffin samples contained flour, water, sugar, fat, non-fat dry milk powder, baking powder, dry egg white, and salt, along with other ingredients such as rising agents, sweeteners, fats, eggs, salt, and milk or cream.

The collected samples were stored under room temperature conditions, simulating the selling environment, and were analyzed within 24 h of sampling in our laboratory. This ensured that the samples were analyzed promptly and maintained their original properties during the testing process.

2.2. Preparation of sample

Following the standard method, the bakery samples were adequately homogenized in a mortar and pestle using a sterile diluent. This ensured uniformity in the samples. To further facilitate suspension, a vortex machine was employed.

To create a standard solution, each homogenized sample (1.0 g) was taken and mixed with sterile normal saline. Subsequently, the solution was serially diluted at a ratio of 1:10, resulting in dilutions up to 10-3. This was achieved by adding 100 μl of the stock solution to 900 μl of normal saline in test tubes.

The nutrient agar medium was then inoculated with 100 μl of the diluted material using the spread plate method. The inoculated

plates were subsequently incubated at 37 °C for a duration of 24 h. This incubation period allowed for the growth and development of any present microorganisms.

2.3. Isolation of associated bacteria

To analyze the bacterial population, we employed the conventional technique for aerobic bacteria [24,25]. Gram staining was performed on pure colonies obtained from the samples, and microscopic examination was conducted using a UNITRON 14711-PS microscope. This allowed us to observe and analyze the morphological and staining characteristics of the bacteria.

For further identification and verification of the bacterial species, we conducted various biochemical tests, including indole test, MIU (Motility-Indole-Urea) test, TSI (Triple Sugar Iron) test, methyl red test, Voges-Proskauer test, citrate utilization test, and urea utilization test. These biochemical tests provide valuable information about the metabolic capabilities and characteristics of the bacteria, aiding in their identification and classification.

2.4. Total viable count (TVC)

To assess the overall bacterial count, 0.1 mL of each tenfold dilution was transferred using a micropipette and spread evenly on count agar media plates. This process was repeated for each dilution. The diluted samples were quickly distributed across the surface of the plates using a sterile glass spreader.

The plates were then placed in an incubator set at a temperature of 37 °C and incubated for 24 h. During this incubation period, bacterial colonies were allowed to grow and develop on the agar plates.

The results of the total bacterial count were expressed as the number of colony-forming units (CFU) per gram of the tested sample. CFU is a measure of viable bacterial cells capable of forming visible colonies on the agar plates.

2.5. Statistical analysis

The data collected in the study were entered into the statistical software SPSS version 26.00 (SPSS Inc., USA) for analysis. Descriptive statistics, including frequencies, mean, and standard deviation, were computed to summarize the data and provide an overview of the bacterial colonies present in the bread items.

To examine the relationship between the number of bacterial colonies and the number of days, Pearson's linear correlation analysis was performed. This analysis determined the strength and direction of the relationship between these variables. Additionally, regression analysis was conducted to further explore and model this relationship.

Pearson's linear correlation and regression analyses are commonly used statistical methods to assess the association and predictive power between variables. These analyses provide valuable insights into the relationship between the number of bacterial colonies in bread items and the number of days, shedding light on any potential patterns or trends.

2.6. Identification of microorganisms related with the condition

The bacteriological investigation of the samples followed the protocol established by the International Commission on Microbiological Specifications for Foods [26]. Cultural analysis was conducted to examine the samples, and the morphology of bacterial colonies was utilized as part of the bacterial identification process.

In addition, several conventional biochemical tests were performed to aid in the identification of the bacteria. These tests included sugar fermentation, coagulase, catalase, methyl red (MR), and Voges-Proskauer (VP) tests. The Gram staining reaction, which provides information about the cell wall structure, was also carried out.

These standardized protocols and tests are widely recognized and used in microbiological analysis to identify and characterize bacterial species. They help in determining the microbial composition and properties of the samples under investigation, providing important insights into the nature of the bacteria present.

2.7. Antibiotic susceptibility test

To determine the drug sensitivity and resistance patterns of the isolated organisms, various commercially available antibiotic discs with a diameter of 6 mm were used. The selected antibiotics for testing included Ciprofloxacin (5μg/disc), Cefixime (5μg/disc), Azithromycin (30μg/disc), and Gentamicin (10μg/disc) [27]. These antibiotics were chosen based on their availability and frequency of administration for the treatment of bacterial infections in Gopalganj, Bangladesh.

The Kirby-Bauer disc diffusion method, in accordance with the recommendations of the Clinical and Laboratory Standards Institute [28], was employed to test antibiotic resistance. Following overnight incubation at 37 °C, the diameter (in millimeters) of the zones of inhibition around each antimicrobial disc was measured. These measurements were recorded and classified into one of three groups: resistant (R), intermediate (I), and sensitive (S). This classification provides information on the susceptibility of the isolated organisms to the tested antibiotics, indicating whether they are resistant, partially susceptible, or sensitive to the drugs.

3. Result

3.1. Isolated bacteria from samples

The number of bacterial colonies in the bread, cake, and muffin samples increased progressively from the production day to the expiration day. After 24 h of incubation, on the production day, approximately 17, 10, and 23 bacterial colonies were observed in bread, cake, and muffin samples, respectively. However, on the expiration day, a significantly higher number of bacterial colonies was detected. Specifically, there were approximately 247, 63, and 421 bacterial colonies in bread, cake, and muffin samples, respectively, after 24 h of incubation [Fig. 1(a-f)].

These results indicate a substantial increase in bacterial growth over time, suggesting that the presence of bacteria in the pre-packaged bakery items progressively intensifies as the expiration date approaches. The higher bacterial colony counts observed on the expiration day may indicate a potential risk of bacterial contamination and spoilage in these food products as they near the end of their shelf life.

Our findings revealed a consistent pattern of bacterial colony growth in all three bakery products (bread, cake, and muffin) from the manufacturing day to the expiration day. On the manufacturing day, the number of bacterial colonies was relatively low, but it increased exponentially over time. In bread, the colony numbers were 17, 20, and 23 on the first, second, and third day of manufacturing, respectively. Similarly, in cake, the colony numbers were 10, 15, and 18 on the first, second, and third day of manufacturing, respectively. In muffins, the colony numbers were 23, 25, and 32 on the first, second, and third day of manufacturing, respectively [Fig. 2].

To further confirm this increasing trend, we conducted the same study on samples collected one day before the expiration date and two days before the expiration date. Once again, we observed a consistent rise in bacterial colonies over time. In bread, the colony numbers on two days before the expiration date, one day before the expiration date, and the expiration date were 191, 220, and 247, respectively. In cake, the colony numbers on two days before the expiration date, one day before the expiration date, and the expiration date were 30, 52, and 63, respectively. In muffins, the colony numbers on two days before the expiration date, one day before the expiration date, and the expiration date were 390, 411, and 421, respectively [Fig. 3].

These results highlight the progressive increase in bacterial colonization over time, indicating a potential risk of bacterial contamination and spoilage as the bakery products approach their expiration dates. The data suggest the importance of consuming these items well before their expiration dates to minimize the potential health risks associated with bacterial growth.

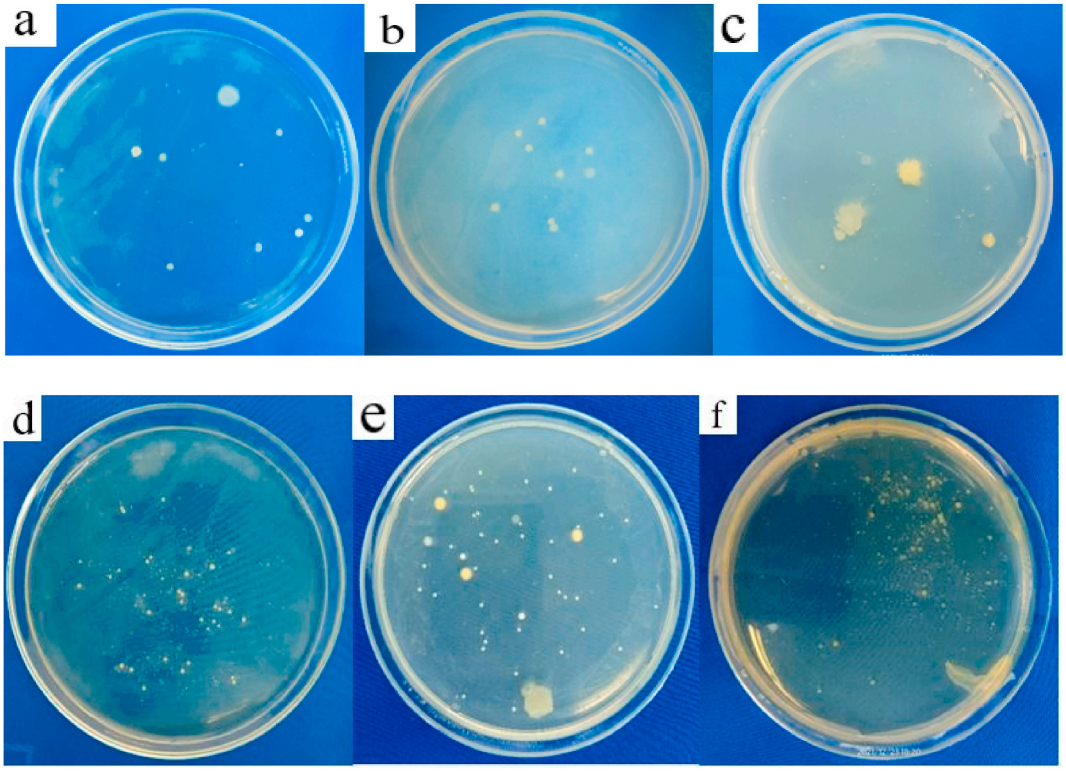

Fig. 1. Isolated bacterial colonies from manufacturing day bakery product samples (a-Bread; b-Cake; c-Muffin); expired day bakery product samples (d-Bread; e-Cake; f-Muffin).

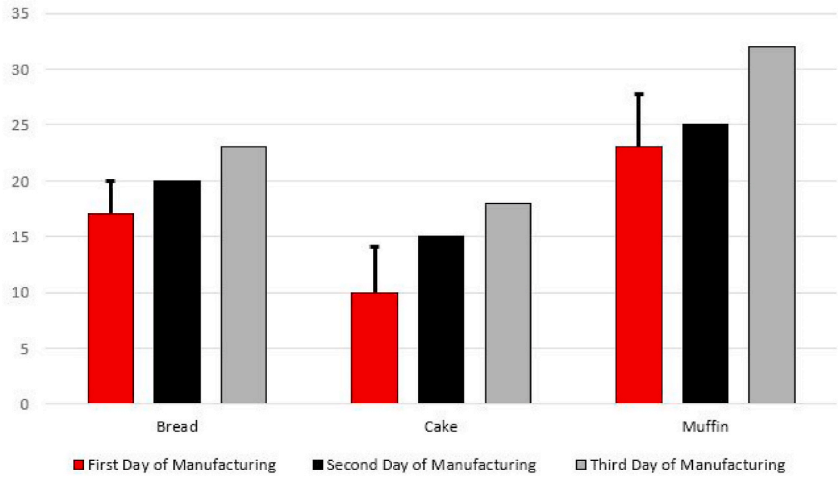

Fig. 2. Bacterial colonies (CFU/g) from first day to third day of manufacturing bakery product samples. (Bread, Cake, Muffin).

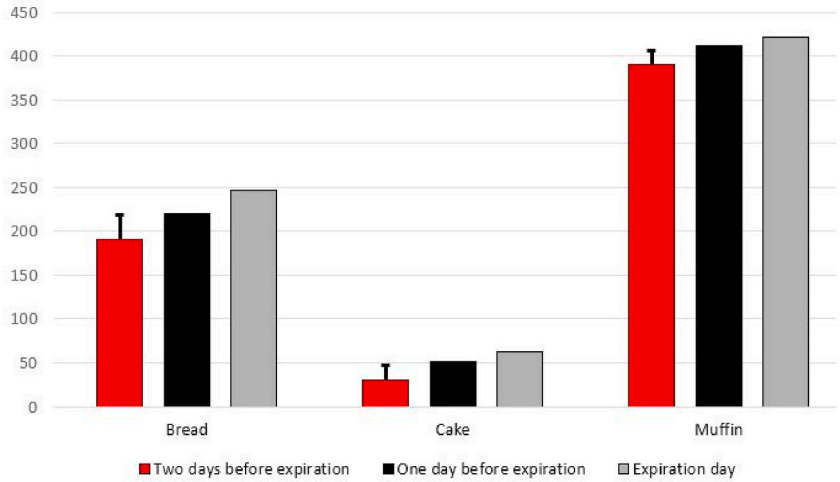

Fig. 3. Bacterial colonies (CFU/g) from two days before to expiration day bakery product samples. (Bread, Cake, Muffin).

3.2. Bacterial enumeration and statistical evaluation

Tables 1 and 2 indicate the total viable count for both manufacture and expiration day samples. From manufacturing until the expiry date, the bacterial concentration in 100 μl bread samples climbed roughly 14.52 times, where the initial values (manufacturing) were 170 and the final values (expiry) were 2.47×10^3. This concentration increased to roughly 6.3 times in the cake, where the initial values (manufacturing) were 100, the final values (expiry) were 630. And in the muffin the bacterial concentration increased to roughly 18.3 times in the muffin, where the initial values (manufacturing) were 230, the final values (expiry) were 4.21×10^3. The rest of this diluted data also confirmed the daily bacterial enrichment of these food goods.

The data shows a strong and significant positive correlation between the number of bacterial colonies on prepackaged bakery food products (bread, cake, and muffins) and the number of days after production ($r = 0.917$, $p < 0.01$) [Fig. 4]. The correlation coefficient (r) indicates a high degree of association between the two variables.

Furthermore, the data analysis reveals that, on average, for each additional day after production, the number of bacterial colonies

Table 1
Total viable count of manufacturing day samples.

Sample	100 μl inoculum	10^{-1} dilution	10^{-2} dilution	10^{-3} dilution
Bread	170	90	5×10^2	2×10^3
Cake	100	70	4×10^2	1×10^3
Muffin	230	130	7×10^2	3×10^3

Table 2
Total viable count of expiration day samples.

Sample	100 μl inoculum	10^{-1} dilution	10^{-2} dilution	10^{-3} dilution
Bread	2.47×10^3	1.190×10^3	6.9×10^3	2.7×10^4
Cake	630	2.90×10^2	1.8×10^3	7×10^3
Muffin	4.21×10^3	2.13×10^3	1.03×10^4	5.3×10^4

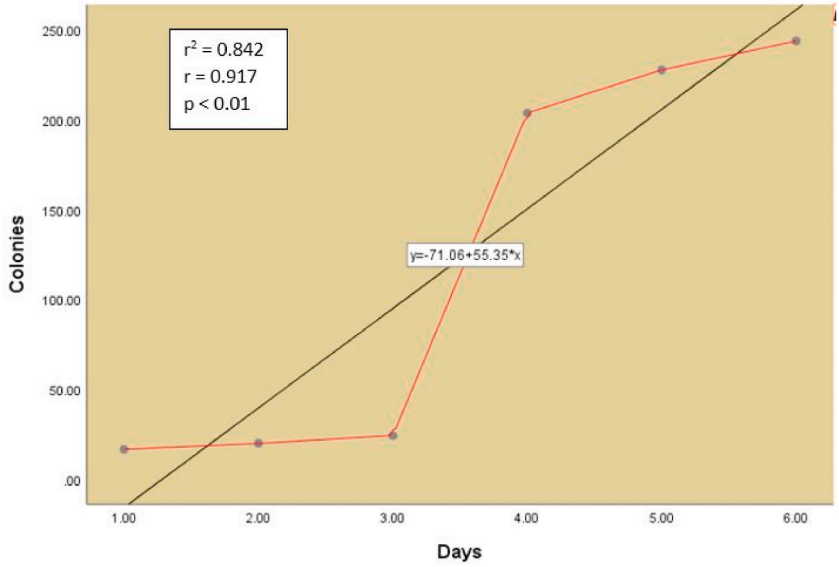

Fig. 4. Pearson's linear correlation and regression of the number of bacterial colonies in bakery products with the number of days from manufacturing to expiration.

increased by approximately 55.35 times, based on the observed value of 71.06 colonies on the first day. This suggests a rapid and exponential growth of bacterial colonies in these food products as the days progress.

The coefficient of determination (r^2 = 0.842) indicates that 84.2% of the total variation in the number of bacterial colonies can be explained by the number of days from manufacturing to expiration. This high value suggests that the number of days is a strong

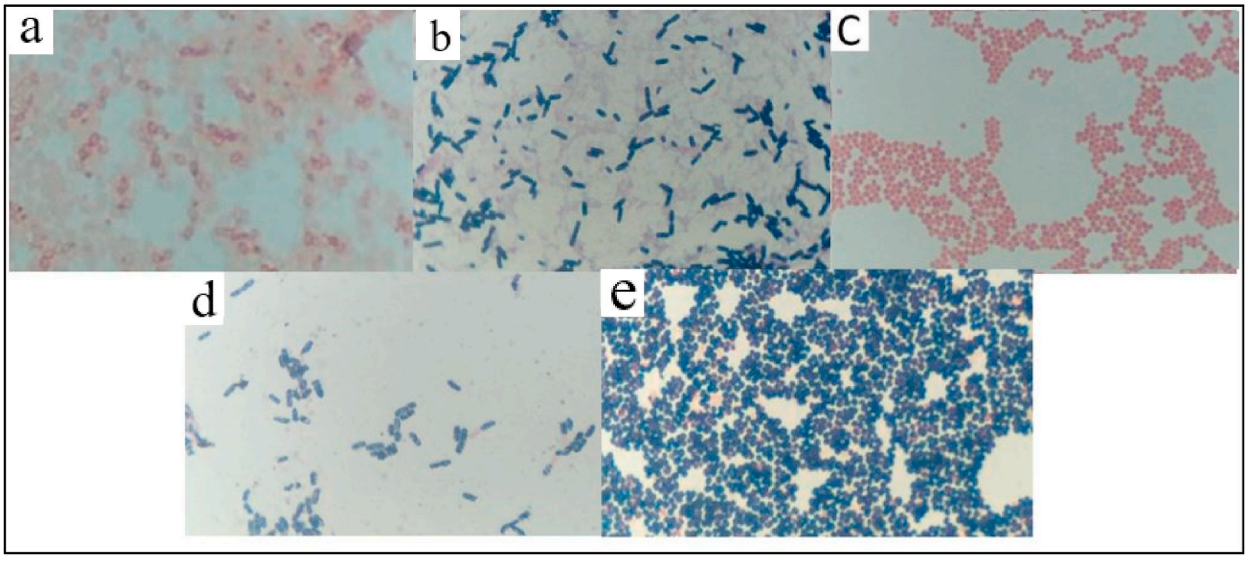

Fig. 5. Light microscopy photograph at 100× magnification of isolated bacteria (a- *Haemophillus*, b-*Bacillus,* c-*Neisseria* and d-*Lactococcus* and e–*Staphylococcus*).

predictor of bacterial colony growth in prepackaged bakery food products.

These findings emphasize the importance of considering the time elapsed since production when assessing the microbial safety and shelf life of bakery products. It underscores the need for proper handling, storage, and timely consumption of these products to minimize the risk of bacterial contamination and ensure food safety.

3.3. Bacterial identification

We used Morphological test, Gram-staining and different biochemical tests for bacteria identification.

3.3.1. Characteristics by morphological study and gram staining

Based on morphological study under microscopic view, along with Gram's staining and different types of biochemical tests, we identified five different types of bacterial species in our collected samples. These are *Haemophillus* spp., *Bacillus* spp., *Neisseria* spp., *Lactococcus* spp., and *Staphylococcus* spp.

Morphological and staining characteristics of bacteria recorded from isolated samples under microscope are presented in [Figures-5 (a-e)] and Table 3.

3.3.2. Characteristics by biochemical tests of isolated samples

We performed seven biochemical tests (indole, MIU, TSI, methyl red, Voges-Proskauer, citrate utilization and urea utilization) for identification of our isolated bacterial species are presented in Table 4.

3.3.3. Antibiotic susceptibility test result

A total of five isolates (*Haemophillus* spp., *Bacillus* spp., *Neisseria* spp., and *Lactococcus* spp., *Staphylococcus* spp) were subjected to antibiotic sensitivity assay. The results of antibiotic sensitivity assay are presented in Table 5. Antibiotic sensitivity test showed that all isolated species were sensitive to Ciprofloxacin. Except *Haemophillus* spp. all were resistance to cefixime. *Bacillus* spp., *Haemophillus* spp., *Neisseria* spp. were resistance to azithromycin while *Lactococcus* spp. were intermediate but *Staphylococcus* spp. were sensitive. All species were sensitive to gentamycin while *Staphylococcus* spp. only intermediate.

4. Discussion

The market for bakery goods as ready-to-eat processed meals is expanding rapidly worldwide. People of all ages enjoy consuming a wide variety of wheat-flour-based baked goods (such as breads, cakes, and biscuits) and pasta products (such as spaghetti, lasagna, noodles, and vermicelli) [29]. These items are primarily made with wheat flour (WF) as the main ingredient. Bakery products are known for their balanced nutritional composition, excellent shelf life, ease of transport, and wide consumer acceptance [30]. However, the increasing demand for bakery products as ready-to-eat processed foods also comes with a significant health risk associated with consuming nearly expired products [31].

In Bangladesh, the Bangladesh Food Safety Authority (BFSA) is responsible for regulating the production, packaging, and marketing of food items. The BFSA carefully monitors the entire process, from production to packaging, before permitting the products to be marketed [32]. Various codex guidelines exist for different aspects of food production, including labeling, packaging, and the use of food additives. For example, the Guidelines on Nutrition Labelling codex is CAC/GL 2–1985; the advisory lists of nutrient compounds for use in foods for special dietary uses intended for infants and young children codex is CAC/GL 10–1979; the principles for the establishment and application of microbiological criteria for foods codex is CAC/GL 21–1997; the principles and guidelines for the conduct of microbiological risk assessment codex is CAC/GL 30–1999; and the guidelines on the application of general principles of food hygiene to the control of Listeria monocytogenes in ready-to-eat foods codex is CAC/GL 61–2007 [33]. It is essential for every manufacturer to adhere to these guidelines in all stages of food production. According to the codex guideline CAC/RCP 48–2001, every company is required to include an expiration date on the packaging. However, some manufacturers provide extended shelf life for their food items, disregarding these codex guidelines, which poses a risk to human health.

Similar to other processed foods, bakery products are susceptible to physical, chemical, and microbiological deterioration [34]. While physical and chemical decomposition may limit the shelf life of low and intermediate moisture bakery items, high moisture products are particularly prone to microbiological spoilage caused by bacteria, yeast, and molds. Certain bacterial species, such as Salmonella, Listeria monocytogenes, Bacillus cereus, and Clostridium botulinum, thrive in high-moisture bakery items packaged in modified atmospheres [35].

Table 3
Microscopic characterization of isolated bacteria after gram staining.

Isolates	Microscopic characterization	Gram's reaction	Identified isolates
a	Non-motile, Rod shaped	Negative	Haemophilus spp.
b	Rod-shaped, endospore-forming aerobic or facultatively anaerobic	Positive	Bacillus spp.
c	Diplococcus that has a flattened shape	Negative	Neisseria spp.
d	Spherical or ovoid shaped remain groups in pairs and short chains	Positive	Lactococcus spp.,
e	Nonmotile, non-spore forming, spherical shape	Positive	Staphylococcus spp.

Table 4
Biochemical properties of isolated bacterial species.

Item	Test	Result
Haemophillus spp	Indole	Positive (+)
	MIU	Negative (−)
	TSI	Positive (+)
	Methyl red	Positive (+)
	Voges-Proskauer	Negative (−)
	Citrate utilization	Negative (−)
	Urea utilization	Positive (+)
Bacillus spp	Indole	Negative (−)
	MIU	Negative (−)
	TSI	Positive (+)
	Methyl red	Positive (+)
	Voges-Proskauer	Negative (−)
	Citrate utilization	Positive (+)
	Urea utilization	Positive (+)
Neisseria spp	Indole	Positive (+)
	MIU	Negative (−)
	TSI	Positive (+)
	Methyl red	Negative (−)
	Voges-Proskauer	No result
	Citrate utilization	Positive (+)
	Urea utilization	Negative (−)
Lactococcus spp.	Indole	Negative (−)
	MIU	Negative (−)
	TSI	Positive (+)
	Methyl red	Positive (+)
	Voges-Proskauer	Positive (+)
	Citrate utilization	Negative (−)
	Urea utilization	Positive (+)
Staphylococcus spp.	Indole	Negative (−)
	M	Positive (+)
	TSI	Positive (+)
	Methyl red	Positive (+)
	Voges-Proskauer	Negative (−)
	Citrate utilization	Negative (−)
	Urea utilization	Positive (+)

Table 5
Antimicrobial profile of identified isolates.

Bacteria Name	Name of the antibiotics	Zone of inhibition (mm)	Interpretation
Haemophillus spp.	Ciprofloxacin (CIP)	20	S
	Cefixime (CFM)	21	S
	Azithromycin (AZN)	0	R
	Gentamycin (GEN)	23	S
Bacillus spp.	Ciprofloxacin (CIP)	23	S
	Cefixime (CFM)	0	R
	Azithromycin (AZN)	0	R
	Gentamycin (GEN)	23	S
Neisseria spp.	Ciprofloxacin (CIP)	21	S
	Cefixime (CFM)	0	R
	Azithromycin (AZN)	9	R
	Gentamycin (GEN)	20	S
Lactococcus spp.	Ciprofloxacin (CIP)	24	S
	Cefixime (CFM)	0	R
	Azithromycin (AZN)	10	I
	Gentamycin (GEN)	23	S
Staphylococcus spp.	Ciprofloxacin (CIP)	22	S
	Cefixime (CFM)	0	R
	Azithromycin (AZN)	22	S
	Gentamycin (GEN)	14	I

In our study, we observed a progressive increase in the growth of disease-causing bacteria on these bakery items over time. Therefore, it is recommended that people consume these baked goods soon after production to minimize the risk. We found a significant difference in bacterial colony counts between the products on the manufacturing date and the expired date. While the manufacturer date products had less than five bacterial colonies, the expired date products had more than fifty colonies. From these

colonies, we isolated five different bacterial genera, namely Haemophillus spp., Bacillus spp., Neisseria spp., Lactococcus spp., and Staphylococcus spp., through morphological studies and various biochemical tests. Gram-positive bacteria were predominant, with three genera being gram-positive and two genera being gram-negative.

These bacteria are associated with various diseases. Haemophilus spp. can cause a wide range of infectious diseases, ranging from mild ear infections to more severe bloodstream infections [36]. Bacillus spp. are naturally present in flour and flour products during bakery production, but they have been increasingly linked to infections in recent years. Bacillus species have been implicated in abscesses, bacteremia/septicemia, wound and burn infections, ear infections, endocarditis, meningitis, ophthalmitis, osteomyelitis, peritonitis, and respiratory and urinary tract infections. Bacillus subtilis, Bacillus licheniformis, Bacillus pumilus, and Bacillus cereus are known to spoil bread, and all species pose risks to human health [37,38].

Neisseria is a gram-negative bacterial genus commonly found in the upper respiratory tract of humans and animals. Within this genus, there are both pathogenic and commensal species [39–41]. Animal models have mainly focused on two species responsible for human diseases: Neisseria meningitidis, an obligate commensal that can cause invasive illnesses, and Neisseria gonorrhoeae, which causes gonorrhea. Both can survive in the upper respiratory tract [42,43].

Consuming fermented baked products and having a compromised immune system due to conditions like pernicious anemia, immunodeficiency, dental or periodontal disease, or bowel disease, along with impaired barriers and immune defense mechanisms, can lead to severe infections caused by less virulent bacteria such as Lactococcus spp [44,45].

The presence of a large number of bacterial colonies on nearly expired prepackaged baked food products poses a significant risk to human health, as these bacteria are responsible for various diseases. People who regularly consume these products are particularly vulnerable to falling ill. Children, especially school-going boys and girls, often choose these items for their small appetites during the tiffin period, placing them at high risk. To assess the antibiotic susceptibility of these bacterial species, we conducted tests and obtained the following results: all isolated species were sensitive to ciprofloxacin, except for Haemophilus spp., which showed resistance. Cefixime resistance was detected in all bacteria except Haemophilus spp. Azithromycin resistance was observed in all Bacillus spp., Haemophilus spp., and Neisseria spp., while Lactococcus spp. showed intermediate resistance and Staphylococcus spp. were susceptible. Gentamycin sensitivity was found in all species except Staphylococcus spp., which showed intermediate sensitivity. These findings indicate that people are likely to encounter antibiotic resistance issues due to the consumption of nearly expired prepackaged baked food products. This poses a significant concern for future generations. Therefore, it is crucial to pay attention to the consumption period of bakery products. The government should address this safety concern regarding prepackaged baked food products promptly. Random inspections of food manufacturing companies should be implemented to minimize any potential malpractice. Manufacturers need to ensure aseptic preparation of these items and adhere to all guidelines provided by the Bangladesh Food Safety Authority (BFSA). Effective communication with consumers about the risks associated with consuming almost expired prepackaged baked food products is essential. This can be achieved through seminars, workshops, and the broadcasting of awareness-raising short films on television channels.

The empirical results reported herein should be considered in light of certain limitations. Firstly, we focused on three popular types of bakery items, overlooking the wide range of other available options. Additionally, although we identified bacterial genera, the use of 16s ribosomal RNA sequencing could have allowed for the identification of bacterial species. Therefore, further research should be conducted with a larger sample size of bakery items and should incorporate 16s rRNA sequencing for a more comprehensive analysis.

5. Conclusion

Bakery products play a crucial role as ready-to-eat processed meals that are consumed immediately. These products, such as bread and biscuits, are widely consumed, particularly in underdeveloped nations where they are included in midday school lunch programs. The bakery industry offers a diverse range of products including bread, rolls, sweet dough products, cookies, crackers, muffins, doughnuts, and cakes, which are enjoyed worldwide.

To ensure our health and safety, it is advisable to consume bakery products around their manufacturing dates, avoiding the consumption of nearly expired products. Bacteria present in these nearly expired products can pose a significant health risk and contribute to the development of various diseases. Therefore, it is essential for manufacturers to prioritize aseptic preparation methods and consider reducing the time between manufacturing and the expiration date indicated on the food packaging.

Furthermore, it is crucial to conduct further research on this issue to address the concern of drug resistance against these microbes and develop effective preventive measures. By investing in research and implementing appropriate strategies, we can mitigate the risks associated with consuming bakery products and safeguard public health.

Ethics approval and consent to participate

This study was performed in accordance with relevant rules, guidelines and regulations. There were no human subjects in this study.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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