Proceedings Series on Health & Medical Sciences, Volume 5
Proceedings of the 5th International Nursing and Health Sciences
ISBN: **xxx**-xxx-xxxx-xx-x, ISSN: 2808-1021

CORRELATION BETWEEN STORAGE DURATION AND TEMPERATURE OF UHT MILK AND THE GROWTH OF Streptococcus sp.

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ARTICLE INFO

Article history:

DOI:
10.30595/pshms.v9i1.2220

Submitted:
February 23, 2026

Accepted:
April 03, 2026

Published:
April 11, 2026

Keywords:

Duration, Storage, Streptococcus, Temperature, UHT Milk,

ABSTRACT

Ultra-High Temperature (UHT) milk is processed by brief exposure to extremely high temperatures (approximately 135150C) to eliminate pathogenic microorganisms and extend shelf life. Streptococcus lactis, a member of the normal microflora in milk, contributes to fermentation processes; however, once the package is opened, the milk becomes susceptible to environmental contamination. Key factors influencing the growth of Streptococcus sp. in UHT milk include storage temperature, duration of storage, and nutrient availability. This study employed a time series design and was conducted in July 2025. Sixteen UHT milk samples were stored at 4C and 25C, with bacterial isolation performed on days 0, 1, 7, and 14. Data were analyzed using a repeated measures test. The results demonstrated a statistically significant correlation between storage duration and temperature and the growth of Streptococcus sp., with a p-value of 0.000 (p 0.05). These findings indicate that prolonged storage and higher temperatures are associated with increased bacterial proliferation.

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1. INTRODUCTION

Milk is a nutrient-rich biological fluid containing water, fat, protein, lactose, vitamins, and minerals, making it an important food source with significant health benefits (Nababan et al., 2014). Ultra-High Temperature (UHT) milk undergoes rapid heating at approximately 135–150°C to destroy pathogenic microorganisms and prolong shelf life while maintaining nutritional quality (Rizkika et al., 2023). Although UHT processing ensures sterility in sealed conditions, contamination risk increases significantly after the package is opened due to environmental exposure such as air and surfaces, which may reduce product quality and shelf life (Putra & Jumiono, 2017).

Previous studies reported that bacteria from the genus Streptococcus, particularly Streptococcus lactis, may contaminate milk after opening, contributing to fermentation processes that alter taste, texture, and acidity (Demarigny et al., 2015). Factors influencing Streptococcus sp. growth include storage temperature, duration, pH, and nutrient availability, particularly lactose as a carbohydrate source (Lubis et al., 2024). Improper storage practices, such as leaving milk at room temperature or using unsealed containers, further accelerate bacterial proliferation (Arini, 2017). Food safety standards establish acceptable microbial limits of approximately 1×10^6 CFU/mL to ensure safe consumption and quality control (Putri & Kurnia, 2018).

ISBN: xxx-xxx-xxxx-xx-x

Despite the long shelf life of unopened UHT milk, limited information exists regarding bacterial growth dynamics after opening under different storage conditions. Therefore, this study aimed to analyze the relationship between storage duration and temperature and the growth of Streptococcus sp. in UHT milk, providing scientific evidence relevant to consumer safety and product handling practices. The hypothesis proposed that both storage duration and temperature significantly influence bacterial growth.

2. RESEARCH METHOD (Font 10, Times New Roman, Spacing 1.15)

This study employed a quasi-experimental design with a time series approach, where bacterial growth observations were conducted repeatedly on days 0, 1, 7, and 14. The research was performed in July 2025 at the Microbiology Laboratory of the Medical Laboratory Technology Program, Faculty of Health Sciences, Universitas Muhammadiyah Purwokerto. Sixteen UHT milk samples obtained from supermarkets in Purwokerto were selected using purposive sampling based on inclusion criteria (pure UHT milk, intact packaging, available across multiple stores) and exclusion criteria (flavored milk, expired products, or damaged packaging). Samples were divided into two storage groups: 4°C and 25°C.

Bacterial isolation followed the pour plate method (Suwito et al., 2018). One milliliter of milk was serially diluted to 10^{-4} using sterile distilled water, inoculated onto Blood Agar Plate (BAP), and incubated at 37°C for 24 hours. Pure cultures were obtained by streaking isolates onto Tryptic Soy Agar (TSA) and incubating under the same conditions (Sahputra et al., 2016). Identification included Gram staining and microscopic examination (Admi et al., 2024), catalase testing using 3% H_2O_2 (Fadilah et al., 2022), lactose fermentation testing with lactose broth medium (Susanti et al., 2018), and Voges–Proskauer testing with α-naphthol and KOH reagents (Sari et al., 2019).

Primary data consisted of Streptococcus sp. colony counts expressed as CFU/mL. Normality was assessed using the Shapiro–Wilk test, and normally distributed data were analyzed using repeated measures ANOVA to determine the effect of storage duration and temperature on bacterial growth.

3. RESULT AND DISCUSSIONS (Font 10, Times New Roman, Spacing 1.15)

3.1 Univariat Analysis

Univariate analysis was conducted to describe the morphological and biochemical characteristics of bacterial isolates obtained from UHT milk samples stored at different temperatures and storage durations. The identification process included Gram staining examination and biochemical testing to confirm the presence of *Streptococcus* sp.

The Gram staining results demonstrated that most isolates showed purple coloration with cocci morphology arranged in chains, which is consistent with the characteristics of Gram-positive *Streptococcus* bacteria. A few samples showed red coloration with rod morphology, indicating the presence of other bacterial types that were not included in the *Streptococcus* group. These findings suggest that microbial contamination may occur after package opening due to environmental exposure, handling, or storage conditions.

Table 4.2 Gram Staining Results

Sample Code	Temperature (°C)	Color	Shape
P01	4	Purple	Cocci Chain
P02	4	Purple	Cocci Chain
P03	4	Red	Rod
P04	4	Purple	Cocci Chain

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P05	4	Red	Rod
P06	4	Purple	Cocci Chain
P07	4	Purple	Cocci
P08	4	Purple	Cocci Chain
P09	25	Red	Rod
P10	25	Purple	Cocci Chain
P11	25	Purple	Cocci
P12	25	Purple	Cocci Chain
P13	25	Red	Rod
P14	25	Purple	Cocci Chain
P15	25	Red	Rod
P16	25	Purple	Cocci Chain

Table 4.2 shows that 11 samples were identified as Gram-positive cocci, indicating the presence of *Streptococcus* sp. isolates.

Biochemical testing further supported the identification results. The catalase test showed negative reactions in most isolates, which is a distinguishing characteristic of *Streptococcus* species compared with catalase-positive bacteria such as *Staphylococcus*. Lactose fermentation testing indicated the ability of isolates to utilize lactose as a carbohydrate source, producing acid and gas formation in some samples. The Voges–Proskauer (VP) test results also supported the metabolic characteristics associated with lactic acid bacteria.

These findings confirm that *Streptococcus* sp. bacteria were present in UHT milk after package opening, indicating that contamination may occur during storage even when refrigeration is applied.

3.2 Bivariat Analysis

Bivariate analysis was conducted to determine the relationship between storage duration and temperature and the growth of *Streptococcus* sp. bacteria. Observations were carried out on days 0, 1, 7, and 14 for samples stored at 4°C and 25°C. The results showed a progressive increase in bacterial counts over time at both temperatures, with a more pronounced increase at 25°C.

Table 4.1 Growth of *Streptococcus* sp. in UHT Milk Stored at 4°C and 25°C

Temperature (°C)	Day 0 (Mean±SD)	Day 1 (Mean±SD)	Day 7 (Mean±SD)	Day 14 (Mean±SD)	P value
4	8.00 ± 0.756	8.75 ± 1.282	12.00 ± 1.309	19.63 ± 1.408	0.000
25	7.75 ± 0.707	21.38 ± 1.685	42.75 ± 2.605	138.50 ± 2.449	

ISBN: xxx-xxx-xxxx-xx-x

Based on Table 4.1, bacterial growth at 4°C increased gradually from day 0 to day 14, indicating that refrigeration slowed but did not completely inhibit bacterial proliferation. In contrast, samples stored at 25°C showed a rapid increase in bacterial counts, particularly after day 7, demonstrating the strong influence of temperature on microbial growth dynamics.

Statistical analysis using the repeated measures ANOVA test showed a significant effect of storage duration and temperature on bacterial growth (p=0.000), indicating that both variables are significantly associated with the proliferation of *Streptococcus* sp. in opened UHT milk.

Discussion

The results of this study demonstrate that storage duration has a significant effect on bacterial growth. The longer the storage period, the more time bacteria have to adapt, multiply, and metabolize available nutrients, particularly lactose present in milk. Microbial growth typically follows a logarithmic pattern when environmental conditions are favorable, explaining the sharp increase observed after several days of storage.

Temperature was also identified as a critical factor influencing bacterial growth. Higher temperatures accelerate enzymatic reactions, cellular metabolism, and replication rates, leading to faster bacterial proliferation. Conversely, refrigeration at 4°C reduced microbial activity by slowing metabolic processes but did not completely eliminate bacterial growth. This finding indicates that contamination may still occur during refrigerated storage if the product is exposed to environmental microorganisms after opening.

The presence of *Streptococcus* sp. in UHT milk after opening is consistent with previous studies reporting that lactic acid bacteria can contaminate dairy products through environmental exposure, handling, or storage containers. These bacteria are capable of fermenting lactose into lactic acid, contributing to spoilage characteristics such as sour taste, odor changes, and texture alterations.

The significant relationship between storage duration and temperature observed in this study confirms that improper storage practices increase the risk of microbial contamination and spoilage in UHT milk. The combination of longer storage duration and higher temperature resulted in the highest bacterial counts, indicating synergistic effects between these factors.

From a public health perspective, these findings highlight the importance of proper storage practices after opening UHT milk packages. Although UHT processing ensures sterility before opening, consumer handling and storage conditions play a major role in determining product safety afterward. Refrigeration and timely consumption are therefore essential to minimize microbial growth and maintain product quality.

2. CONCLUSION AND RECOMMENDATION (Font 10, Times New Roman)

Conclusion

Based on the results of this study, it can be concluded that storage duration and temperature significantly influence the growth of *Streptococcus* sp. bacteria in UHT milk after package opening. Bacterial counts increased progressively over time at both storage temperatures, indicating that longer storage duration provides more opportunity for bacterial adaptation, metabolism, and proliferation. However, the increase was substantially higher at 25°C compared with 4°C, demonstrating that higher temperatures accelerate bacterial growth.

Refrigerated storage at 4°C was able to slow bacterial proliferation but did not completely inhibit microbial growth, suggesting that contamination can still occur during storage even under cold conditions. The highest bacterial growth was observed in samples stored at 25°C for 14 days, indicating that the combination of prolonged storage duration and higher temperature creates optimal conditions for bacterial multiplication. Statistical analysis confirmed a significant relationship between storage duration and temperature and the growth of *Streptococcus* sp. (p=0.000).

These findings indicate that proper storage conditions after opening UHT milk packaging are essential to maintain microbiological quality and safety. Although UHT processing ensures sterility before opening, post-opening handling and storage practices play a critical role in determining product safety.

ISBN: xxx-xxx-xxxx-xx-x

Recommendations

Based on the research findings, several recommendations can be proposed:

1. **For the community or consumers**
   Consumers are advised to store UHT milk at refrigeration temperature (approximately 4°C) immediately after opening and to consume it within a limited time period to minimize bacterial growth and maintain product quality.
2. **For health and laboratory professionals**
   Monitoring and education regarding proper food storage practices should be improved to reduce the risk of microbial contamination in dairy products after opening.
3. **For future researchers**
   Further studies are recommended to include larger sample sizes, additional types of microorganisms, and different storage conditions to obtain more comprehensive data regarding microbial growth dynamics in dairy products.

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