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IDENTIFICATION OF HEAT-RESISTANT MICROORGANISMS IN STERILIZED MILK AND THEIR EFFECT ON SHELF LIFE

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Abstract

The study investigated the survival and growth of heat-tolerant microorganisms, including *Bacillus cereus, Geobacillus stearothermophilus,* and *Clostridium sporogenes* in ultra-heat treatment (UHT) processed milk stored under different temperature conditions. The quantitative polymerase chain reaction (qPCR) was employed to measure the proliferation and detection of microbes. The effect of storage temperatures on the viability, durability, and safety of UHT milk was also investigated. The results showed that the extent of biofilm growth when stored at higher temperatures was variable with the *Bacillus cereus* growing in abundance (79.3% rise), followed by *Geobacillus stearothermophilus* (77.6%), and *Clostridium sporogenes* (62.7%). In addition, the lowest counts for these microorganisms were at 10°C which increased with longer storage times. This highlights the need for stringent temperature-permitting UHT milk storage to minimize the number of bacteria in the interest of product safety. This study contributes to existing knowledge on the complications and microbial dynamics of UHT milk as a means towards developing improved food storage systems and safety protocols.

Keywords: UHT milk, Heat-tolerant microorganisms, Microbial growth, Shelf life.

تحديد الكائنات الحية الدقيقة المقاومة للحرارة في الحليب المعقم وتأثيرها على مدة الصلاحية

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الخلاصة

تبحث هذه الدراسة الحالية في بقاء ونمو الكائنات الحية الدقيقة المقاومة للحرارة، بما في ذلك *Bacillus cereus* و *Geobacillus stearothermophilus* و *Clostridium sporogenes*، في الحليب المعالج بدرجات حرارة عالية جدًا (Ultra Heat Treatment) (يتم ادراج درجة الحرارة لطفا) المخزن في ظل ظروف درجات حرارة مختلفة (تذكر الدرجات الحرارية لطفا). تم استخدام تفاعل البوليميراز المتسلسل الكمي (qPCR) لقياس تكاثر واكتشاف الميكروبات. كما تم التحقق في تأثير درجات حرارة التخزين على قابلية بقاء ومتانة وسلامة حليب UHT. تثبت نتائجنا أن مدى نمو الأغشية الحيوية عند تخزينها في درجات حرارة أعلى كان متغيرًا حيث وجد أن – نمت بكثرة (ارتفاع بنسبة 79.3%)، تليها *Geobacillus stearothermophilus* (77.6٪) و*Clostridium sporogenes* (62.7٪). كما وجد أن أقل عدد لهذه الكائنات الحية الدقيقة كان عند 10 درجات مئوية، ويزداد هذا العدد مع زيادة وقت التخزين. علاوة على ذلك، تم تسليط الضوء على متطلبات تخزين الحليب المعقم بدرجة حرارة صارمة تسمح بتقليل عدد البكتيريا من أجل سلامة المنتج. تساعد هذه الدراسة في تطوير الديناميكيات الميكروبية في قاعدة المعرفة الخاصة بمضاعفات الحليب المعقم وتخلق أنماط تخزين أفضل وبروتوكولات سلامة الغذاء.

كلمات مفتاحية: حليب UHT، الكائنات الحية الدقيقة المقاومة للحرارة، النمو الميكروبي، مدة الصلاحية.

Introduction

Knowing the microbial population dynamics in ultra heat treatment milk is critical for developing more effective sterilization strategies and for improving the product's quality and safety. The outcomes of this study add to the scientific community's efforts in promoting the safety of high-temperature processed foods against microbe resistance. Moreover, this study contributes to improvements in food safety regulations, which in turn boosts consumer confidence in dairy foods available in the market. This research on the inextricable link between microbial viability and product life-span offers important points on improving UHT methods in ensuring dairy milk manufacturing processes will be at optimum safety and quality levels (4).

A common method employed in UHT milk treatment is to sterilize it by heating at over 135°C for a few seconds. The pasteurization of dairy products by heating them at temperatures above the boiling point of water helps ensure they are free of harmful microorganisms and extends their shelf-life without the need for refrigeration (5 and 14).

Knowledge of the effect on the growth of thermos-tolerant microorganisms on the shelf life of UHT milk in various storage conditions is critical for the dairy industry in tackling product stability and safety issues. It is well established that higher store temperatures substantially increase the proliferation of organisms consisting of *Bacillus cereus, Geobacillus stearothermophilus, and Clostridium sporogenes*.

Among those, *Bacillus cereus* proliferated the most, mainly at 30°C, followed by *Geobacillus stearothermophilus* and *Clostridium sporogenes*. This temperature-structured growth underscores the necessity for stringent temperature controls in the UHT milk storage to inhibit microbial growth and promote food safety (6).

However, heat-resistant microbes can resist UHT pasteurisation at high temperatures. The organisms present considerable issues to the dairy sector, including product contamination and shelf-life reduction (12). Recent UHT milk deterioration highlights the need to better understand these products' post-processing microbial characteristics (15). Some of the numerous studies done were able to detect bacteria and spores that could withstand UHT. Nevertheless, overall knowledge of microbe diversity, resistance mechanisms, and their effect on milk quality remains scant. This is not just an economic issue as it also raises concerns over the idea of self-stable food. Hence, the question remains whether UHT processing, which includes sterilization, is as effective as it is often claimed to be (10).

Research by (16) focussed on the effectiveness of various preservatives in controlling microbial growth in UHT milk. While they suggested some success with the usage of natural preservatives, this study explores the continued importance of temperature control, suggesting that a mix of both methods is probably the appropriate solution for enhancing product protection (16).

This study is a systematic process that enabled the characterization and identification of heat-resistant microorganisms associated with the extended shelf life of UHT milk products. By applying microbiology and molecular methodologies, this project helps distinguish how resistant strains have their recognizable taxonomy and how they in reality force living organisms to adapt to their survival strategies. Also, this study determined the potential biological microorganisms based on how they affect the sensory and chemical features of UHT milk during its shelf life (2).

Ultra-heat treatment processing revolutionized the dairy business by presenting a method that allowed milk to retain much longer on the shelves. It significantly improves the method of heating milk to over 135°C for a few seconds thereby eliminating microbial life that causes disease or spoilage (2).

While UHT treatments have come to be one of the dominant symbols of product safety and durability in milk, the presence of thermophile microorganisms remain an issue of concern. Such organisms have developed a resistance mechanism to these temperatures, creating the risk of sterilization in milk treated by the UHT process (7). Research into the living conditions of bacteria exposed to ultra-high temperatures brings up a range of microorganisms, mainly those that form spores, which can survive such elevated temperatures (11). Species consisting of *Bacillus cereus* and *Geobacillus stearothermophilus* were regularly recognized in spoiled UHT milk samples. The resilience of these microorganisms is frequently attributed to their spore-forming abilities, which enable them to endure environments that could be lethal to regular bacterial cells (3).

The presence of heat-resistant microorganisms in UHT milk does not only impact product quality and safety it also poses full-size monetary risks to producers due to increased spoilage charges and customer rejection (8). Furthermore, the resilience of these organisms to the UHT procedure raises questions on the effectiveness of modern-day heat treatments and the need for appropriate strategies or supplementary safety measures

Materials and Methods

Experimental Design**:** The experiment was conducted using three different strains of bacteria known for their heat resistance, namely Clostridium sporogenes, Bacillus cereus, and Geobacillus stearothermophilus. All strains were inoculated in sterile UTM-equivalent milk samples at a concentration of ~10^3 spores/mL. The inoculated samples were preserved at three different temperatures of 10°C, 20°C, and 30°C in various storage situations. These bacteria were added to the milk before heat treatment to prove that they could survive the treatment

Sampling**:** The experiment involved a pool of 90 samples (3 microbe strains × 3 temperatures × 10 time points). Inoculums were sampled on day 0 (immediately after inoculation) and 1, 2, 3, 5, 7, 10, and 14 days after inoculation. At any given time, samples were taken under aseptic conditions to assess the concentrations of viable bacteria.

Microbial Analysis**:** The presence of each microorganism was confirmed through both conventional culturing and quantitative PCR (qPCR), followed by a quantitative assessment of their growth rates. Cultures were developed on selective media specific to the sugar added and the colonies used to estimate viable cell concentrations. Quantitative PCR is a more sensitive method for monitoring microbial composition, targeting specific DNA sequences unique to each strain of bacteria. This method was used to because the milk may contain other types than those added.

Statistical Analysis**:** Data analysis on the obtained growth data was done using statistical software. Growth curves were plotted to visualize the microorganism populations that had increased over time at every storage temperature. For this purpose, analysis of variance (ANOVA) was conducted to detect the statistically significant variations in microbial growth between different temperatures and specific time intervals. To extend the analysis, additional regression tests were carried out to determine the effect of temperature, storage time, and quantity of microbial load on the microbiological growth of the food using SPSS software.

Results and Discussion

Table 1 shows that culture growth at 10°C was low over 14 days, only doubling from the original position. At 20°C there was moderate growth that multiplied nine times over the 14 days, while growth at 30°C was rapid increasing fifteen-fold over 14 days. The number 1.0 x 10^3 on day 0 was obtained using the plate-counting method.

Table 1: Microbial Growth in UHT Milk.

Sample ID	Microorganism	Storage Temperature (°C)	Day	Viable Count (CFU/mL)
1	*Bacillus cereus*	10	0	1.0 x 10^3
1	*Bacillus cereus*	10	7	1.5 x 10^3
1	*Bacillus cereus*	10	14	2.0 x 10^3
2	*Bacillus cereus*	20	0	1.0 x 10^3
2	*Bacillus cereus*	20	7	3.0 x 10^3
2	*Bacillus cereus*	20	14	6.0 x 10^3
3	*Bacillus cereus*	30	0	1.0 x 10^3
3	*Bacillus cereus*	30	7	5.0 x 10^3
3	*Bacillus cereus*	30	14	10.0 x 10^3

This result means that the 10°C temperature does not allow microbial species to multiply fast, suggesting that low storage temperatures can be used to increase the shelf life of UHT milk by limiting the growth of bacteria. The bacteria grew faster at 30°C, implying that temperatures above 10°C, even moderate ones, have a major impact on UHT milk freshness. High temperatures greatly accelerate bacterial proliferation, dramatically shortening shelf life and posing a significant risk to product safety and quality. Alterations in membrane composition also play an essential part in heat resistance. Microorganisms can adjust the degree of saturation of fatty acids in their membranes or the duration in their lipid chains to reduce membrane fluidity thus enhancing their balance at higher temperatures (1).

These results align with the findings of comparable research on microbial dynamics in UHT and pasteurized milk products. For instance, (9) suggested that thermophile microorganisms undergo a surge at higher temperatures, corroborating our observations on *Geobacillus stearothermophilus*. However, this study extends those findings by providing an instantaneous comparison of growth rates amongst distinct microorganisms under similar experimental conditions, highlighting the especially aggressive proliferation of *Bacillus cereus* at elevated temperatures (9).

As shown in Table 2, the *Geobacillus stearothermophilus* experienced stronger growth at higher temperatures, which is clear evidence of its thermophilic character. This microbe contributes more to contamination only at 30°C, posing a storage risk where temperatures are higher. (9) focused on the survivability of *Geobacillus stearothermophilus* in dairy products subjected to excessive-temperature processing. Similar to our findings, they noted that the microorganism continued to survive in extreme temperatures, underscoring the challenges in completely eliminating it at some stage in UHT processing (9). However, this study extends these results by quantitatively evaluating increased contamination rates across various temperature ranges and presenting a more comprehensive view of how temperature variations affect microbial dynamics.

Table 2: Growth of Geobacillus Stearothermophilus in UHT Milk.

Sample ID	Microorganism	Storage Temperature (°C)	Day	Viable Count (CFU/mL)
4	*Geobacillus stearothermophilus*	10	0	1.0×10^3
4	*Geobacillus stearothermophilus*	10	7	1.1×10^3
4	*Geobacillus stearothermophilus*	10	14	1.2×10^3
5	*Geobacillus stearothermophilus*	20	0	1.0×10^3
5	*Geobacillus stearothermophilus*	20	7	2.0×10^3
5	*Geobacillus stearothermophilus*	20	14	4.0×10^3
6	*Geobacillus stearothermophilus*	30	0	1.0×10^3
6	*Geobacillus stearothermophilus*	30	7	3.5×10^3
6	*Geobacillus stearothermophilus*	30	14	7.0×10^3

Table 3 shows that although *Clostridium Sporogenes* growth declined the most at the 10°C benchmark, major increases were noted in the bacterial population and viable count at higher temperatures. This cyclicality indicates the importance of maintaining low storage temperatures to inhibit the growth of these spores.

Table 3: Growth of Clostridium Sporogenes in UHT Milk.

Sample ID	Microorganism	Storage Temperature (°C)	Day	Viable Count (CFU/mL)
7	*Clostridium sporogenes*	10	0	1.0×10^3
7	*Clostridium sporogenes*	10	7	1.1×10^3
7	*Clostridium sporogenes*	10	14	1.2×10^3
8	*Clostridium sporogenes*	20	0	1.0×10^3
8	*Clostridium sporogenes*	20	7	1.5×10^3
8	*Clostridium sporogenes*	20	14	2.0×10^3
9	*Clostridium sporogenes*	30	0	1.0×10^3
9	*Clostridium sporogenes*	30	7	2.5×10^3
9	*Clostridium sporogenes*	30	14	5.0×10^3

(13) found that *Clostridium sporogenes* exhibited markedly lower increases in their UHT milk experiments compared to this study, and suggested that variations in dietary content and initial spore masses may be the reason. This discrepancy underscores the

importance of standardized experimental processes, or alternatively, highlights how special UHT formulations can impact microbial behaviour (13). Contrastingly, studies by (13). (9) determined lower growth rates of *Clostridium sporogenes* in UHT milk, which they attributed to different spore activation methods and milk formulations. This discrepancy suggests that the composition of UHT milk and precise processing conditions can also significantly impact the survival and growth of microorganisms, indicating an area for further similar research.

Figures 1 and 2 show the heat resistance levels of the three varieties of microbes in UHT milk stored for 14 days at 30°C.

Bacillus cereus shows a sharp increase in the curve, i.e., 4 (log CFU) by day 14, suggesting high proliferation during this period and indicating a high likelihood of spoilage and safety issues if temperatures are not maintained.

Geobacillus stearothermophilus registered remarkable growth, but its count nearly doubled from 3.54 (log CFU) on day 7 to 3.84 (log CFU) on day 14. This shows that the bacteria are thermophile and thrive at above-normal temperatures. The use of the 7 and 14-day incubation periods demonstrates the actions of the heat-loving microbials and their rapid multiplication at high temperatures.

Clostridium sporogenes displays an initially slower growth pattern compared to the other two microorganisms but increased markedly over time in colony-forming units to 3.69 (log CFU) at the end of the experimental period. This indicates the proliferation possibility of even less dominant spore-formers given that its initial count had doubled by day 14. Nevertheless, despite having a lower growth rate than the other two its steady increment can affect product safety as well.

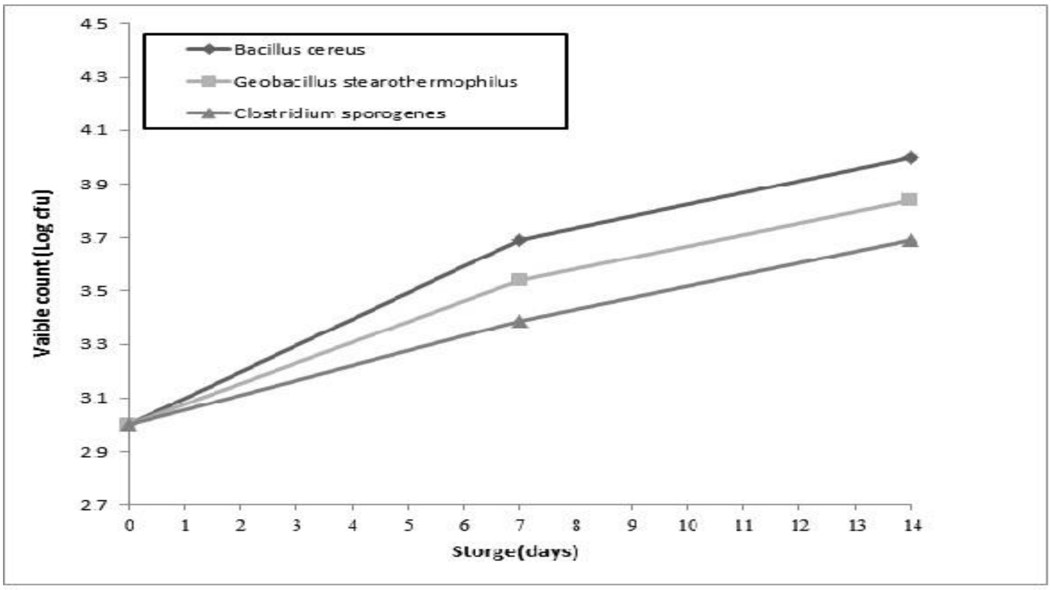

Fig. 1: Growth of heat-resistant microorganisms in UHT milk over time at 30°C.

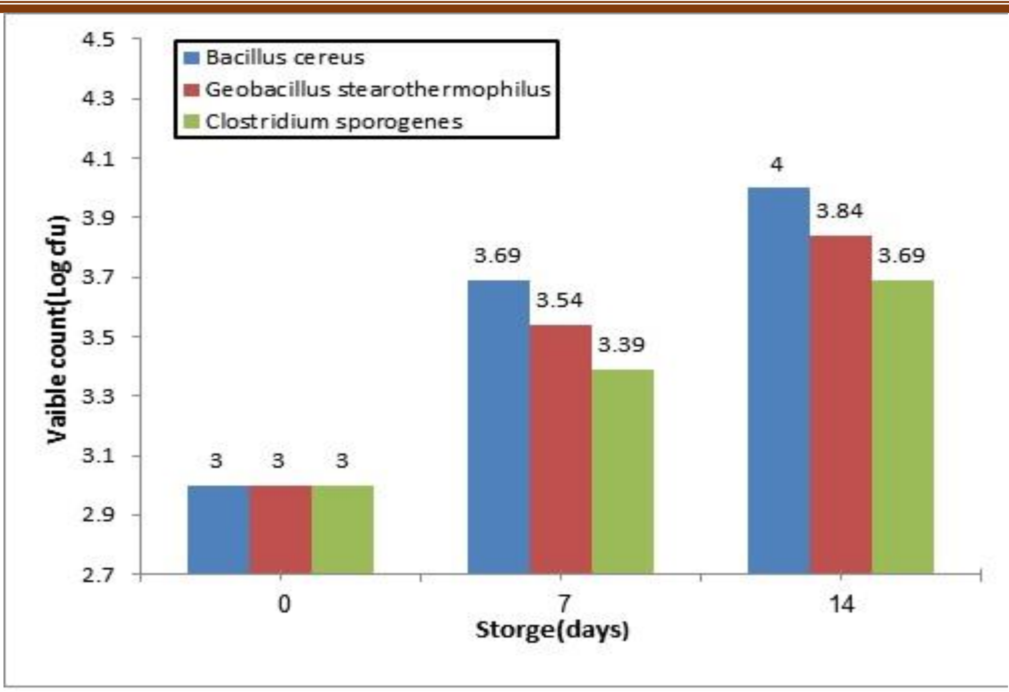

Fig. 2: Comparison of microbial growth over time at 30°C.

(17) explored the impact of storage conditions on the shelf life of UHT milk, with special focus on microbial spoilage. They found that lower storage temperatures drastically inhibited microbial growth, which is consistent with our observations (17). However, our research extends that finding by demonstrating how incremental increases in temperature can differentially accelerate the growth of particular microorganisms.

The heat map in Figure 3 depicts the progression of the three bacteria over the course of the experiment, starting from 30°C. Each cell in the heat map stands for the viable count (log CFU) of a certain microorganism per particular date. Such a visualization offers a color-coded overview of the growth dynamics of microorganisms.

In terms of color gradient, the shade becomes more intense together with the growth of bacteria; the greater the growth over time the higher the color value attained. For growth patterns, the *Cereus bacillus* attains the highest growth, reaching up to 4 (log CFU) on day 14, followed by *Geobacillus stearothermophilus* and *Clostridium sporogenesis*.

Such visualization helps in comparing the growth rates of the microorganisms for the different processes. It also allows tracking of how different temperature values affect microbial metabolism in UHT milk.

These findings have important implications for public health and the dairy industry. The vigorous growth of *Bacillus cereus* poses notable health risks due to its ability to produce toxins, emphasizing the need for the industry to reassess storage and distribution practices. Moreover, the persistence of *Geobacillus stearothermophilus* and *Clostridium sporogenes* even at lower temperatures highlights the challenge of ensuring long-term stability and safety of UHT milk, which is often stored and transported without refrigeration.

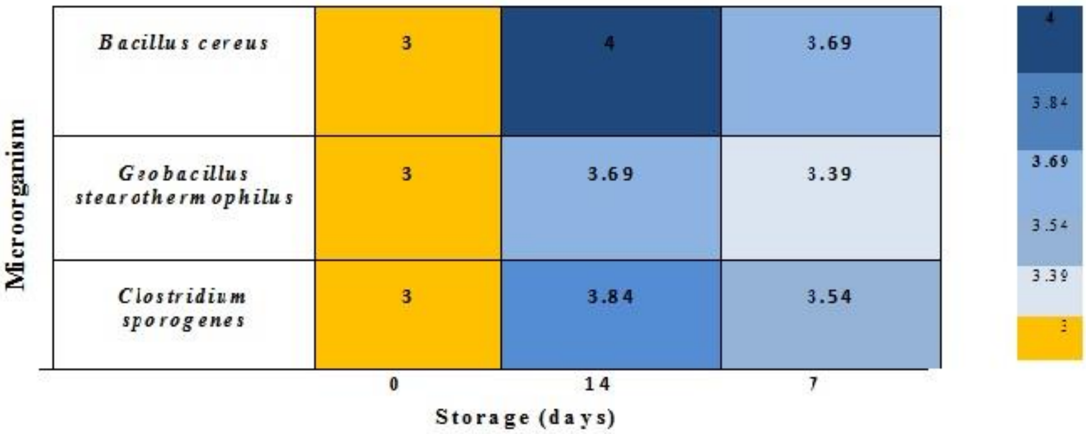

Fig. 3: Heat map of microbial growth over time at 30°C.

Given the complexity of microbial behaviour in processed milk, future research needs to focus on multi-faceted techniques. This must encompass investigating alternative processing strategies that would lessen the viability of warmth-resistant spores, along with higher-stress processing or the inclusion of natural antimicrobial retailers. Additionally, genetic research on those microorganisms could provide insights into precise resistance mechanisms, providing goals for new antimicrobial techniques.

Conclusions

This study offers valuable insights on the ongoing efforts to improve the safety and quality of UHT milk. By systematically analysing the growth patterns of key heat-resistant microorganisms under different temperature conditions, it provided empirical evidence for supporting stricter control of storage temperatures as a critical factor in extending shelf life and ensuring the safety of UHT milk. As the dairy industry continues to evolve, incorporating advanced processing technologies and more effective preservation strategies are essential for addressing the challenges posed by microbial contamination and ensuring consumer trust in dairy products. Moreover, studies are needed to investigate the impact of milk formula changes, including versions in protein and fat content material, on the increased dynamics of those microorganisms. Understanding these relationships will help tailor processing techniques to particular milk formulations, thereby improving the overall safety of UHT milk.

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No Supplementary Materials.

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Author 1 and 2; writing—review and editing. Both authors have read and agreed to the published version of the manuscript.

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The authors declare no conflict of interest.

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References

1. Álvarez-Ordóñez, A., Fernández, A., López, M., Arenas, R., and Bernardo, A. (2008). Modifications in membrane fatty acid composition of Salmonella typhimurium in response to growth conditions and their effect on heat resistance. International Journal of food microbiology, 123(3): 212-219. https://doi.org/10.1016/j.ijfoodmicro.2008.01.015.
2. Burton, H. (2012). Ultra-high-temperature processing of milk and milk products: Springer Science and Business Media.
3. Checinska, A., Paszczynski, A., and Burbank, M. (2015). Bacillus and other spore-forming genera: variations in responses and mechanisms for survival. Annual review of food science and technology, 6(1): 351-369. https://doi.org/10.1146/annurev-food-030713-092332.
4. Dash, K. K., Fayaz, U., Dar, A. H., Shams, R., Manzoor, S., Sundarsingh, A., ... and Khan, S. A. (2022). A comprehensive review on heat treatments and related impact on the quality and microbial safety of milk and milk-based products. Food Chemistry Advances, 1: 100041. https://doi.org/10.1016/j.focha.2022.100041.
5. Karlsson, M. A., Langton, M., Innings, F., Malmgren, B., Höjer, A., Wikström, M., and Lundh, Å. (2019). Changes in stability and shelf-life of ultra-high temperature treated milk during long term storage at different temperatures. Heliyon, *5*(9). https://doi.org/10.1016/j.heliyon.2019.e02431.
6. Kumar, J., Hunge, S. S., Jaiswal, S., and Kumar, A. (2024). A Textbook of Dairy Chemistry: Academic Guru Publishing House.
7. Lewis, M. J., and Deeth, H. C. (2008). Heat treatment of milk. Milk processing and quality management, 168-204. DOI:10.1002/9781444301649.

8. Melini, F., Melini, V., Luziatelli, F., and Ruzzi, M. (2017). Raw and heat-treated milk: From public health risks to nutritional quality. Beverages, 3(4): 54. https://doi.org/10.3390/beverages3040054.
9. Milojevic, T., Cramm, M. A., Hubert, C. R., and Westall, F. (2022). “Freezing” thermophiles: From one temperature extreme to another. Microorganisms, 10(12): 2417. https://doi.org/10.3390/microorganisms10122417.
10. Ntuli, V., Sibanda, T., Elegbeleye, J. A., Mugadza, D. T., Seifu, E., and Buys, E. M. (2023). Dairy production: Microbial safety of raw milk and processed milk products Present Knowledge in Food Safety (pp. 439-454): Elsevier. https://doi.org/10.1016/B978-0-12-819470-6.00076-7.
11. Owusu-Darko, R. N. (2019). Molecular characterization of Bacillus sporothermodurans in Ultra High Treatment (UHT) milk: University of Pretoria (South Africa).
12. Owusu-Kwarteng, J., Akabanda, F., Agyei, D., and Jespersen, L. (2020). Microbial safety of milk production and fermented dairy products in Africa. Microorganisms, *8*(5): 752. https://doi.org/10.3390/microorganisms8050752.
13. Patel, A. S., Bariya, A. R., Ghodasara, S. N., Chavda, J. A., and Patil, S. S. (2020). Total carotene content and quality characteristics of pumpkin flavoured buffalo milk. Heliyon, 6(7): e04509. https://doi.org/10.1016/j.heliyon.2020.e04509.
14. Rauh, V., and Xiao, Y. (2022). The shelf life of heat-treated dairy products. International Dairy Journal, 125: 105235. https://doi.org/10.1016/j.idairyj.2021.105235.
15. Roberts, T., Cordier, J.-L., Gram, L., Tompkin, R., Pitt, J., Gorris, L., and Swanson, K. (2005). Milk and dairy products Micro-Organisms in Foods 6: Microbial Ecology of Food Commodities (pp. 643-715): Springer. https://doi.org/10.1007/0-387-28801-5_16.
16. Smith, M. M. (2019). Control of Biofilm and Planktonic Cells of Cronobacter Sakazakii, Listeria Monocytogenes, Shiga Toxin-Producing Escherichia Coli, and Salmonella Serovars Using Pressure-and Quaternary Ammonium Compound-Based Interventions (Master's thesis, Tennessee State University).
17. Zhao, S., Chen, J., Fei, P., Feng, H., Wang, Y., Ali, M. A., ... and Yang, W. (2020). Prevalence, molecular characterization, and antibiotic susceptibility of Bacillus cereus isolated from dairy products in China. Journal of Dairy Science, 103(5): 3994-4001. https://doi.org/10.3168/jds.2019-17541.