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Effect of Storage Temperature on the Microbiological, Physicochemical, and Sensory Properties of Semi-Hard Cheese

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ABSTRACT

This study investigated the impact of different storage temperatures (room temperature: 20°C and refrigeration: 4°C) on semi-hard cheese's microbiological, physicochemical, and sensory properties over 16 days. Cheese samples were made from fresh cow's milk and subjected to two different storage conditions. Various factors, including bacterial count (Standard Plate Count: SPC), pH, acidity, and sensory characteristics (color, texture, odor, taste, and overall acceptability), were determined at different time intervals (Days 1, 4, 7, 10, 13, and 16). Microbial analysis revealed no significant differences in SPC between the two storage conditions. Similarly, physicochemical parameters, including pH and acidity, showed minor fluctuations, with p-values greater than 0.05 in all cases. Sensory analysis indicated that the cheese's texture, color, and taste at room temperature were less acceptable than those stored in refrigeration. A comparison of the tested parameters (SPC, pH, acidity, and sensory attribute rating) and two storage temperatures (environment and fridge) measured under the two storage conditions using the Mann-Whitney U test showed that there were no significant differences between the two ($p > 0.05$). However, Spearman's correlation test demonstrated a significant correlation between storage time, microbial load ($rs = 0.943$), and acidity ($rs = 0.831$), particularly in refrigerated samples. The results suggest that storage temperature affects the spoilage rate in semi-hard cheese. These findings provide valuable insights into preserving semi-hard cheese and highlight the crucial role of refrigeration in slowing the spoilage process.

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INTRODUCTION

Milk is an important nutrient source staff for all animals and humans and provides nearly all nutrients required for the body (Belitz et al., 2009). Today, the above term only points towards cow's milk since it dominates the global production statistics (Lasztity, 2009). The

FAO/WHO and the Codex Alimentarius Commission define milk as a substrate derived from animals for human consumption. It may have undergone processing or partial processing, or it may not have been processed at all. While milk provides numerous health benefits, some individuals may experience lactose intolerance, making it difficult to digest. Proper storage and pasteurization help maintain freshness and prevent bacterial contamination (Widyastuti & Febrisiantosa, 2013).

Most of the milk produced commercially and consumed by humans is cow milk. Cow milk is the base for dairy products such as cheese, yogurt, butter, and cream. Its composition can vary depending on the breed of cow, diet, and farming practices. Cow milk is evaluated based on two critical parameters: milk fat (F) and solids not fat (SNF), which mainly includes protein, lactose, and mineral (Hargrove et al., 1967); however, it contains low levels of iron and folate (Muehlhoff et al., 2013).

Cheese making is one of the oldest known food processes that involves the coagulation of milk to make cheese curd with the assistance of rennet or similar enzymes under the addition of starter microorganisms and lactic acid (Ayeni et al., 2014). This complex process leads to a broad range of types of cheese, which may differ in characteristics such as texture and taste (Moatsou, 2019).

With the increase in demand for cheese across the globe, factors influencing its shelf life are of great concern to producers and consumers. The potential yield of cheese from milk is primarily determined by its composition, especially the fat and protein content. The richness of the flavor and texture, such as softness and smoothness, seem to be directly linked to the cheese's milk fat percentage. Among the various factors that influence yield and can be controlled by the cheese maker, moisture content is likely the most significant (Abd El-Gawad & Ahmed, 2011). Moisture content is an essential factor that primarily influences cheese yield and quality; high moisture content leads to unappetizing tastes and textures, whereas lower moisture content imparts more extended shelf lives to the cheese (Hargrove et al., 1967). Cheese with a higher moisture content is more prone to microbial growth, accelerating spoilage. Water activity (aw) plays a crucial role in determining the shelf life of cheese. Cheeses with higher water activity, such as fresh varieties like ricotta or cottage cheese, spoil faster than harder, low-moisture cheeses like Parmesan (Tamime, 2011). Most microorganisms responsible for food spoilage need a water activity (aw) higher than 0.91 to thrive. However, moulds can grow at an (aw) as low as 0.80. In processed cheese, the water activity typically ranges between 0.91 and 0.96. These water activity levels are sufficient to inhibit the growth of certain strains of *Clostridium botulinum* and prevent the production of their toxin (Buňková & Buňka, 2017). Additionally, the fats in cheese are prone to oxidation, which causes a rancid flavor. This is particularly common in high-fat cheeses. Lipid oxidation occurs due to reduced oxygen availability, which is undesirable for cheese varieties with high-fat content. Understanding the factors contributing to cheese spoilage is crucial for developing strategies to mitigate these risks and improve the quality of the final product. Lipid oxidation is a chemical reaction that occurs when the fats in cheese are exposed to oxygen, leading to rancidity and unpleasant off-flavors. This is especially common in cheeses with high-fat content, particularly if they are stored for extended periods or exposed to light or air (Drake et al., 2010).

Cheese spoilage is a complex problem associated with microbial, chemical, and environmental factors. Bacteria, molds, and yeasts are important microbial communities that affect the cheese quality, and their activities may cause deterioration of cheese quality, including changes in taste, texture, and shrinks. Mold growth, particularly in soft and high-moisture cheeses, is a common sign of spoilage, though some molds, like those in blue cheese, are intentional and safe to consume (Alshareef et al., 2023). Some pathogens, such as *Listeria monocytogenes* and *Salmonella* spp. It threatens consumers' health, while others, such as Pseudomonas spp., affect cheese content and quality through the degradation of the components of the cheese matrix.

Cheeses exposed to air, particularly soft cheeses, are more prone to mold growth. While molds can be cut off from hard cheeses like Cheddar or Parmesan without impacting the rest of the cheese, mold growth on soft cheeses typically means the cheese should be discarded. The presence of mold or surface spoilage can significantly shorten the shelf life of cheese if not properly managed (Fox et al., 2017).

The shelf life of cheese depends on several factors, including the type of cheese, its moisture content, storage conditions, methods, and packaging. Generally, hard cheeses such as Parmesan and Cheddar have a longer shelf life because they contain less moisture, which inhibits microbial growth. In contrast, soft cheeses like Brie and Ricotta have a shorter shelf life due to their higher moisture content, accelerating spoilage (McSweeney, 2007). Temperature control is also crucial for extending shelf life, as most cheeses should be stored between 2°C and 4°C to minimize microbial activity and enzymatic reactions (Muehlhoff et al., 2013). Packaging methods, such as vacuum and modified atmosphere packaging, are key in extending shelf life by limiting oxygen exposure and reducing microbial growth (McSweeney, 2007). The acidity pH of cheese plays a crucial role in its stability and resistance to spoilage. Cheeses with higher pH levels (less acidic) are more susceptible to spoilage by pathogens. In contrast, those with lower pH (more acidic) are generally more resistant, as the acidity inhibits the growth of most spoilage microorganisms (Pentado et al., 2014).

Proper refrigeration is crucial for extending the shelf life of cheese. Most cheeses should be stored between 2°C and 4°C to slow microbial activity and enzymatic reactions. Higher storage temperatures can accelerate spoilage by encouraging bacterial growth, especially in soft cheeses (FDA, 2017).

- This paper aims to identify and analyze the factors influencing cheese shelf life, helping to improve product quality and better meet consumer expectations, thus supporting a sustainable and successful cheese market.
- This research aims to identify factors that influence shelf life in semi-hard cheese, emphasizing the role of microorganisms and storage parameters.

MATERIALS AND METHODS

Research Design

Experimental-laboratory research methodology is adopted to determine the shelf life of semi-hard cheese under different storage temperatures.

Chemicals

NaOH (0.1N) (BDH®, England), NaCl (BDH®, England).

Reagents

MacConkey agar (BD Difco®, USA), Phenol phthalein solution (0.2%) (BDH®, England), Rennet (Jahan e shimi®, Iran), All chemicals and reagents were laboratory grade.

Sample Preparation

Fresh milk was purchased from a local farmer and subsequently boiled in 80°C. The composition of the milk was determined using a milk analyzer (LactoScope™) in the Central Veterinary Diagnostic and Research Laboratory to ensure quality standards (Fat 3.2%, Protein 2.9%, Total Solid 11%, Solid Not Fat 7.8%, Water 89.5% and Ash 0.65%).

To prepare 3 kg of cheese, 10.5 liters of cow's milk were heated at 80°C, and then it was allowed to cool for a while (50°C). Then, yogurt water and renin enzyme were added to the milk (serving as fermenters and starter culture). Once the milk has formed a clot and the heating process has stopped, the mixture is placed into a cheesecloth bag and left for 24 hours to allow the water to separate. During this time, the cheesecloth bag is pressed with weight. Finally, the semi-hard cheese is removed from the cheesecloth bag and is ready for consumption. Later, 14 cheese samples were prepared (200 grams each), and each sample was hygienically packed in a sterile plastic pack. Seven samples were stored in the refrigerator (4°C) and seven at room temperature (20°C).

To conduct the necessary experiments, sampling was performed six times at different intervals (Days 1, 4, 7, 10, 13, and 16), with each collection carried out under strict hygienic conditions to minimize contamination. These sampling dates covered various stages of aging and spoilage in the cheese. PH and acidity were measured for the physicochemical analysis and SPC for microbial evaluation. Additionally, sensory evaluations assessed color, texture, odor, taste, and overall acceptability.

Microbiological Analysis

The bacteriological quality of the cheese samples was determined using the SPC method. About one sample was taken from the interior part of the cheese and diluted with sterile saline solution (1g cheese: 9ml saline solution), then serially diluted. One milliliter of the diluted samples was inoculated onto MacConkey agar (used for mesophilic bacteria, especially Enterobacteriaceae) and incubated for 24 hours at 37°C. Following the incubation period, the plates were observed, and the number of colonies formed was enumerated using a colony counter. Last was determining microbial count per unit weight of the cheese sample based on the number of colonies and dilution factor described by Adams & Moss (2008).

Physico-Chemical Analyses

pH and Acidity determination: To prepare the cheese sample for analysis, 10 grams were transferred into a beaker and thoroughly mixed with hot water at 40°C. Next, this mixture was transferred to a 100 ml beaker, and the volume was adjusted to 100 ml with distilled water. The sample was then homogenized and filtrated using filter paper. From this filtrated sample, 25 ml was taken and transferred to another 100 ml beaker for pH determination. A digital pH meter (HM-25G, DKK.TOA Corporation, Japan) was utilized to measure the pH of the cheese samples. Before measuring the pH, the meter was calibrated with pH buffers of 6.86 and 4.01 to ensure accurate readings.

Titration was performed using 0.1 normal sodium hydroxide solution in the presence of a phenolphthalein indicator. The titration proceeded until the appearance of a pale pink color that persisted for at least 30 seconds. Finally, the result was calculated according to the formula below, which likely involved the volume and concentration of the sodium hydroxide solution used in the titration and was indicative of certain chemical properties or components in the cheese sample (AOAC, 2005).

Acidity in Dornic^{0}=(10 ml×NaOH ml×4)

Acidity= $\frac{Acidity\ in\ Dornic}{100}$

Sensorial Analyses

Cheese samples (they were stored at 4°C and 20°C) were examined by a 10-member panel, including faculty professors and students from the Faculty of Veterinary, Kabul University. Panel members rated cheeses based on color, texture, and flavor (odor and taste) on a of 0 to 5, with zero being the worst and five being the best, according to the IDF (1995) standards. Cheese samples were tested following manufacture and during storage on days 1, 4, 7, 10, 13, and 16.

Statistical analysis

Statistical analysis was performed using SPSS 29 (IBM Inc., Chicago) and Microsoft Excel. Descriptive statistics were used to calculate the central tendencies and measure the dispersions of the test parameters (SPC, pH, acidity, and sensory attribute rating). Mann-Whitney U test was performed to show the mean differences between tested parameters (SPC, pH, acidity, and sensory attribute ratings) and storage temperature (environment temperature and fridge temperature).

In addition, Spearman's correlation coefficient test was performed to find the strength and direction of the relationship between the two variables (duration of storage in two different temperatures [environment and fridge] and cheese parameters [SPC, pH, acidity, and sensory attribute ratings]). The significance level for both tests was set at $p<0.05$.

RESULTS AND DISCUSSION

The findings from the Mann-Whitney U test elucidate that no significant differences were observed in the parameters measured across cheese samples stored under varied temperature conditions (environment and fridge): Standard Plate Count (SPC): p= 0.29; pH: p=0.36; acidity: p=0.51; sensory attribute rating: p=0.89 (Table 1 and Table 2).

Moreover, Spearman's Correlation Coefficient analysis unveiled significant and very strong positive correlations ($p<0.05$) between the Standard Plate Count (SPC) and the duration of cheese storage at fridge temperature (rs =0.943), as well as between acidity and the duration of cheese storage in the fridge (rs =0.831). Conversely, no significant relationships were observed between the duration of storage of cheeses kept in environment or fridge and other parameters (in the environment: SPC: p= nil, rs = 1.000; pH: p= -.500, rs =0.36; acidity: p=0.500, rs =0.667; sensory attribute rating: p=nil, rs =1.000. In the fridge: pH: p= nil, rs = -1.000; sensory attribute rating: p=nil, rs =1.000).

Table 1: SPC, pH, and acidity of cheese samples based on the storage conditions (n=14)

Days	**SPC (cfu/g)**		**pH**		**Acidity (%)**	
	4°C	20°C	4°C	20°C	4°C	20°C
1	9.1×10^{5}	9.1×10^{5}	6.30	6.30	0.36	0.36
4	3.1×10^{7}	8.4×10^{7}	6	4.97	0.4	1.12
7	1.6×10^{8}	1.6×10^{8}	5.9	5.17	0.68	1.04
10	1.7×10^{8}	Spoiled	5.77	Spoiled	0.64	Spoiled
13	$5.5.\times10^{8}$	Spoiled	5.55	Spoiled	0.96	Spoiled
16	1.8×10^{8}	Spoiled	5.23	Spoiled	1.08	Spoiled

*cfu/g=colony form per unit/gram

Table 2: Sensory evaluation of cheese samples stored in two temperature conditions by the panelist (n=14)

Days	**Color**		**Texture**		**Odor**		**Taste**	
	4°C	20°C	4°C	20°C	4°C	20°C	4°C	20°C
1	1	1	1	1	1	1	1	1
4	1.6	2.3	1.8	3	2.1	3.6	1.6	3.3
7	1.8	2.6	2.8	3.4	2.5	4.6	1.8	4.5
10	2.5	Spoiled	3	Spoiled	3	Spoiled	2.5	Spoiled
13	2.8	Spoiled	3.5	Spoiled	3.5	Spoiled	3.5	Spoiled
16	4	Spoiled	4	Spoiled	5	Spoiled	5	Spoiled

*Scores were ranked from 1 (excellent) to 5 (very poor); after 7 days, the room stored semi-hard cheese spoiled.

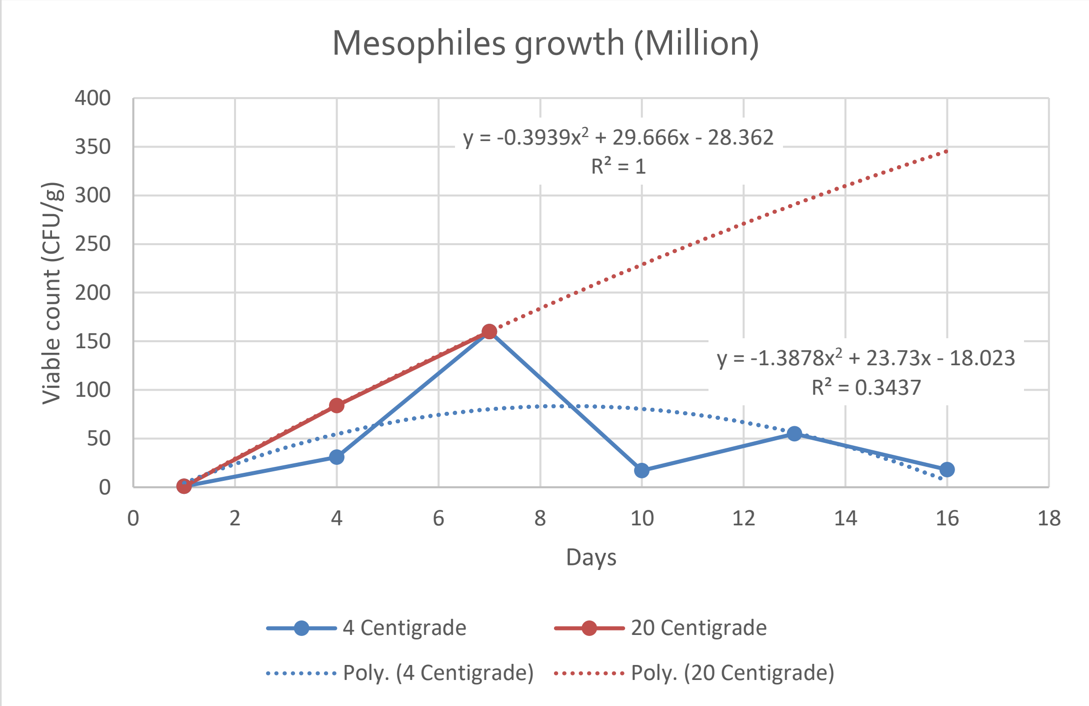

Figure 1: Mesophilic bacterial growth of cheese samples in two different temperature conditions and storage times, "y" describes the linear relationship between x and y, and the "R^2" value indicates how well the regression line fits the data.

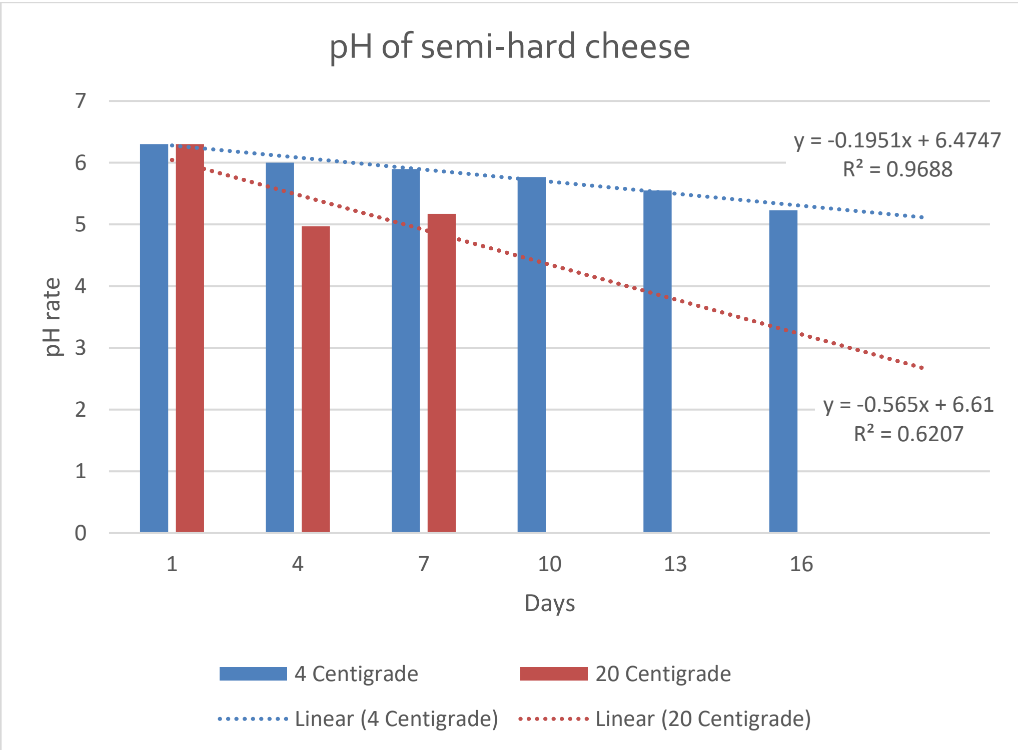

Figure 1: pH rate in room and fridge semi-hard cheese during storage on different days

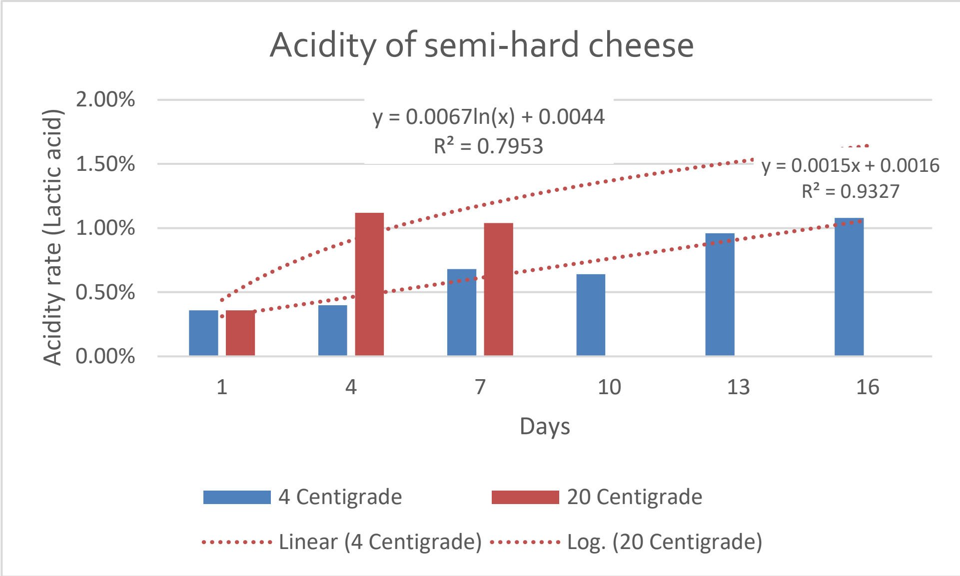

Figure 3: Acidity rate in room and fridge semi-hard cheese during storage on different days

The spoilage observations in this study highlight the significant influence of temperature on the shelf life of semi-hard cheese. Specifically, cheese stored at room temperature spoiled within 7 days, while refrigerated semi-hard cheese remained viable for up to 16 days. These findings align with the work of Memisi et al. (2014), who demonstrated that temperature plays a crucial role in accelerating spoilage of dairy products, particularly semi-hard cheeses.

Further, a positive and strong correlation was observed between the Standard Plate Count (SPC) and storage duration (*rs*=0.943), as well as between acidity and storage time (*rs*=0.831) under refrigerated conditions. This suggests that extended storage enhances microbial growth, leading to increased acidity. This observation supports the findings of Park et al. (2014), who emphasized that both storage temperature and time influence the acidity levels of dairy products, making it essential to monitor these factors closely.

The findings of Kamleh et al. (2012) further corroborate these results, as their study indicated that storage at 5°C adversely affected the quality of Halloumi cheese, causing a high bacterial load and elevated acidity. They highlighted the significant role of microbial growth in altering the acidity and sensory properties of the cheese. Our study found similar effects, with room temperature causing rapid spoilage and increased microbial load.

Additionally, the current research is consistent with Smigic et al. (2018), who observed changes in the color and flavor of processed cheese due to storage temperature. These changes were attributed to an increase in microbial count. While sensory attributes in this study were not significantly affected by the temperature conditions examined, the prolonged shelf life of refrigerated samples suggests that microbiological processes can render the product unsafe for consumption over extended periods, even if the quality remains relatively unchanged.

Supporting evidence from El Owni and Hamed (2009) further reinforces the role of temperature in cheese spoilage. Their study found that cheese stored at room temperature had significantly higher titratable acidity (1.18 ± 0.79%) compared to cheese stored at refrigerated temperatures (0.97 ± 0.45%). Titratable acidity increased with storage time, with a marked difference observed between days 60 and 240. The total bacterial count was significantly higher in cheese stored at room temperature (P = 0.001 on day 60) before it declined, while cheese stored under refrigeration showed a reduced microbial load until day 120, after which it increased before declining again on day 240.

In another study, Zheng et al. (2016) reported a 42% reduction in the firmness of sliced cheeses as storage temperature increased from 4°C to 25°C. This change in texture, along with higher adhesiveness in certain cheeses (e.g., Cheddar and Emmental), demonstrates that temperature significantly affects the physical properties of cheese, making it softer and stickier over time, which can influence sensory characteristics.

Further evidence from Cuffia et al. (2019) also supports the impact of temperature on cheese quality, particularly in pasta filata cheeses. They observed that cheeses stored at temperatures above refrigeration (12°C) showed increased odor intensity, which was attributed to changes in the profile of volatile compounds. This deterioration in quality due to temperature effects is consistent with our findings.

Perveen et al. (2011) also examined the effects of storage temperature on cream cheese, noting that cream cheese stored at 21°C exhibited lower moisture content than those stored at 4°C and 10°C. As the storage period increased, the titratable acidity of cream cheese stored at 21°C rose, accompanied by higher microbial growth. These findings align with the present study's conclusion that microbial activity is closely linked to changes in acidity and pH, particularly at room temperature.

Finally, research on Himalayan cheese by Mushtaq et al. (2015) showed similar trends, with increased pH and acidity in samples stored at room and refrigerated conditions. Their study also highlighted that storage time and temperature significantly influenced the physicochemical properties of the cheese, a finding that supports the current study's results.

CONCLUSION

In summary, the temperature at which cheese is stored is a critical factor influencing its spoilage and quality. A comparison of the tested parameters (SPC, pH, acidity, and sensory attribute rating) and two storage temperatures (environment and fridge) measured under the two storage conditions using the Mann-Whitney U test showed no significant differences between the two temperatures. However, Spearman's correlation test demonstrated a significant correlation between storage time, microbial load, and acidity, particularly in refrigerated samples. The findings of this study support previous research demonstrating that higher storage temperatures promote rapid microbial growth and increased acidity, leading to faster spoilage. While refrigeration can extend shelf life and reduce microbial load, prolonged storage can still lead to safety concerns, even when sensory attributes remain relatively stable. These observations underscore the importance of closely monitoring both storage conditions and time to ensure the safety and quality of dairy products.

Conflict of Interest: The author(s) declared no conflict of interest.

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