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Evaluating the Shelf Life of Chilled Raw and Pasteurized Goat Milk: An Analysis of Physicochemical and Microbial Content

N. S. Ibrahim*, N. A. Zahari, and N. I. Shamsul Nizam

School of Animal Science, Aquatic Science, and Environment, Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Malaysia

Abstract. Goat milk offers a variety of health benefits. The objective of this study is to evaluate the influence of refrigerated storage on the quality of unprocessed and pasteurized goat milk within a defined timeframe. In addition, also analyzes the chemical and microbiological composition of both raw and pasteurized goat milk during refrigerated storage to assess its longevity. Goat milk samples were obtained from healthy Saanen female goats, aged 3-4 years, with a body condition score (BCS) ranging from 2 to 3. The samples were taken from UniSZA Pasir Akar farm in Besut, Terengganu, Malaysia. The milk samples were processed through pasteurization. Exactly 10 goats were meticulously chosen, with each goat yielding 1 liter of milk. The goats were segregated into two cohorts: one cohort for raw milk (n=5) and the other for pasteurized milk (n=5). Subsequently,the milk samples from both groups were subjected to chilling storage experimentation at the foodtechnology laboratory of Universiti Sultan Zainal Abidin (UniSZA) in Besut. The milk samples(duplicates) were monitored for 42 days to assess their physical characteristics. The pasteurizedgoat milk samples were evaluated for milk composition (fat, solid non-fat, protein, and lactose)and microbial contents (TPC and EMB) from Week 0 to Week 6. The effect of shelf life, composition values, and bacterial growth were measured weekly, and the data were presented descriptively (SEM±STD) with paired T-Test as the statistical analysis, considering $p<0.15$ as asignificant difference. The findings revealed a downward trajectory in the physical attributes and chemical makeup of both unprocessed and pasteurized goat milk throughout the storage duration, resulting in decreases in fat, solid non-fat, protein, and lactose. The microbiological investigation revealed increased microbial levels in both raw and pasteurized milk after two weeks. Nevertheless, there was a subsequent decrease in the number of microorganisms from week 3 to week 6, indicating a phase of natural bacterial decline caused by limited nutrients and competition among bacteria. The study emphasizes the significance of maintaining sustainability in dairy production and addressing food safety concerns. It is crucial for customers to be informed about the duration of safe consumption of pasteurized milk and the potential threat of bacterial growth during prolonged cold storage.

Keywords: goat milk, microbial, pasteurized, Saanen, shelf life

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*Corresponding author at: School of Animal Science, Aquatic Science, and Environment, Faculty of Bioresources and Food Industry, Universiti Sultan Zinal Abidin, Besut Campus, 22200 Besut, Terengganu Darul Iman, Malaysia

E-mail address: syaheeraibrahim@unisza.edu.my

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1. Introduction

The worldwide demand for goat milk is consistently increasing. This substance is characterized by its high nutritional content and is associated with a diverse range of positive effects on human health. The digestibility of goat milk is a determining factor in individuals' preference for it over cow milk. Lactose, an inherent milk sugar, is found in all cow's milk and can offer difficulties for full digestion in specific individuals as they grow older [1]. Hence, goat milk is highly digestible and suitable for consumption by those with lactose intolerance [2].

Fresh milk either raw or pasteurized is known for its limited shelf life, rendering them susceptible tospoilage if consumed beyond its expiration date. Consuming expired raw or pasteurized milk can lead to health issues and illness [3]. Nevertheless, there is a common notion held by certain persons that raw milk possesses superior nutritional content and health advantages when compared to pasteurized milk [4]. Pasteurization is a process that uses heat to treat food goods at a specific temperature and for a specific amount of time to eliminate or deactivate potentially hazardous germs. Pasteurization serves the dual function of guaranteeing the safety of fresh milk for human consumption and extending its longevity on store shelves. The process involves exposing unprocessed, unpasteurized milk to high temperatures for a short duration. Hence, the consumption of pasteurized milk is deemed to be secure, and when maintained in optimal conditions, it possesses a lengthier lifespan in comparison to unpasteurized milk. Pasteurized milk is subjected to precise thermal treatment using regulated machinery at a set temperature and is constantly maintained for a specified duration. Conversely, raw milk is acquired directly from ruminant animals, particularly cows and goats [5].

The shelf-life of milk is reduced due to microbial growth, leading to the development of undesirablecharacteristics and alterations in sensory attributes. Factors such as limited production capacity, climatic conditions, and logistical challenges sometimes impede the widespread industrialization of goat milk. As a result, farmers are obligated to keep raw milk at cold temperatures for more than a day until it is processed [6]. However, this method raises significant quality problems in the industry, as it negatively impacts the overall quality of milk. Moreover, the existing information on the durability of unprocessed and pasteurized goat milk is severely limited, especially in Malaysia. Therefore, thepresent study seeks to provide valuable insights into the shelf life of both raw and pasteurized goat milk,exploring their impact on milk composition, and microbial content. This study has two main objectives:firstly, to assess the physical and chemical composition of raw and pasteurized goat milk during chillerstorage, and secondly, to observe the microbial content of raw and pasteurized goat milk throughout theduration of the experiment.

2. Materials and Methods

2.1. Animal Ethics Application

The sampling method and experimental protocols used in this study were approved by the UniSZA Animal and Plant Research Ethics Committee (UAPREC) under reference number UAPREC/008/002.

2.2. Animal Management and Milk Collection Samples

The collection of goat milk samples was conducted at the UniSZA Pasir Akar Farm situated in Jerteh, Terengganu. This procedure involved the manual extraction of milk. A deliberate selection was made to include a sample size of ten female Saanen goats (n=10) for this study. The goats were in excellent condition and fell within the age bracket of 3 to 4 years. Furthermore, their body condition ratings (BCS) varied between 2 and 3. The selection criteria also mandated that the goats have symmetrical udder traits, as specified in a prior investigation [7]. The Saanen goats were housed in a confined housing arrangement and their diet consisted of concentrates in the morning, accounting for 10% of their dry matter intake (DMI), and fresh Bracharia humidicola in the afternoon.

To ensure the freshness of the samples, the goat milk was collected in the morning at 9 am. Great care was taken during the collection process to maintain aseptic conditions, and all raw milk samples were obtained exclusively from healthy female goats to prevent any potential contamination that couldadversely affect the analytical results. To preserve the integrity of the samples, the collected goat milk was then stored in sterilized plastic bottles, following the protocol established by Suguna et al., (2012) [8].

2.3. Process of Milk Pasteurization

The pasteurization process was carried out using vat pasteurization at a temperature of 63°C for a period of 30 minutes [9]. The technique was carried out at the UniSZA Pasir Akar Farm, where the milk was gathered. Every pasteurized sample of goat milk was correctly labeled. However, due to the significant distance to the Food Technology Laboratory, the pasteurized milk was stored in the icebox to maintain its quality.

2.4. Chilling Milk Samples for a Shelf-life Experiment

The experiment involved transferring the milk samples into twelve small plastic containers, each with avolume of 50 mL, with duplicate samples assigned for each week. The containers were carefully labeled,ranging from "Week 0" to "Week 6," and subsequently stored in a temperature-controlled chiller set at 4°C. Additionally, the milk samples were duplicated into two pasteurized and raw groups labeled. Overa period of six weeks, these samples were subjected to chemical composition and microbiological analyses to evaluate their stability and quality during storage.

2.5. Evaluation of Chemical Composition

The investigation into the composition of goat milk was conducted at the Microbiology Laboratory of Universiti Sultan Zainal Abidin (UniSZA). The Milkotester Ultrasonic Milk Analyzer (Bulgaria), was employed to ascertain the milk's various components. Specifically, duplicate samples of goat milk collected during "Week 0" were subjected to comprehensive analyses to determine their protein, fat, lactose, and SNF. The operational procedure for utilizing the Milkotester was executed in accordance with the established protocol as outlined in a prior study [7].

2.6. Statistical Analysis

The mean value together with the standard deviation (STD) of the chemical composition and microbiological analysis of milk samples were reported. The evaluation of these samples was performed using Paired Sample T-test analysis, with a significance level of $p<0.15$. The study sought to assess the statistical significance of variations in milk composition between raw and pasteurized milk varieties over a six-week duration. The study employed Minitab statistical software to analyze the disparities in means regarding the colony count and composition of raw and pasteurized goat milk samples. The data was analyzed using an 85% confidence interval and a 15% significance threshold. Plate enumeration and milk composition analyses were conducted on both raw and pasteurized goat milk samples to ensure accuracy and consistency. The colony count findings were quantified as colony-forming units per milliliter (CFU/mL).

3. Results and Discussion

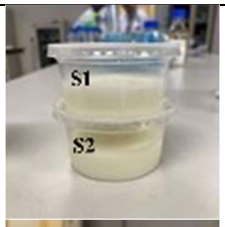
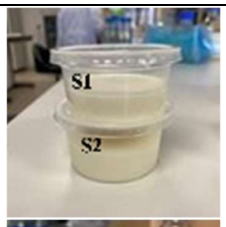
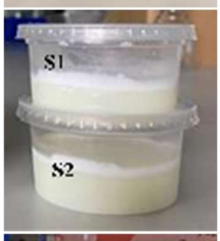
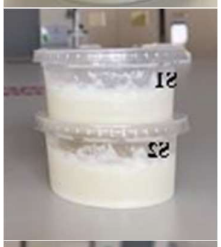
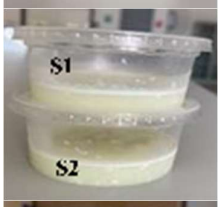
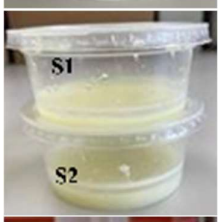
3.1. Physical Characteristics of Raw and Pasteurized Goat Milk Samples

The goat milk samples, comprising both raw and pasteurized, were subjected to cold storage at 4°C fora duration of 6 weeks, and weekly analyses were conducted. The physical attributes of these milk samples exhibited distinct changes as the storage period progressed, and these variations are summarizedin Table 1.

The fundamental drivers of milk deterioration are the growth and multiplication of various microorganisms, including bacteria, yeast, and molds. The microorganisms in question exhibit active degradation of the components of milk and generate by-products that contribute to its deterioration [10] – [11]. The results of the investigation revealed that the goat milk samples exhibited indications of spoiling starting from the second week, as illustrated in Table 1. The deterioration seen in this study was defined by the occurrence of clumps in both raw and pasteurized milk samples, along with the noticeable separation or stratification of their constituents. In the milk samples, the fat content exhibited a tendency to separate and accumulate at the uppermost layer, resulting in the formation of a distinct cream layer. Concurrently, the

remaining liquid component had a dilution effect, manifesting as an increase in its watery consistency.

Table 1. Physical Characteristic Changes of Raw and Pasteurized Goat Milk Samples

Week	Raw	Pasteurized
Week 0		
Week 1		
Week 2		
Week 3		
Week 4		
Week 5		
Week 6		

Note: S1: sample 1; S2: sample 2

The current results align with prior research, indicating that unpasteurized goat milk can remain suitable for consumption for a duration of 12 to 16 days when stored at a temperature of 4°C. Furthermore, studies have shown that milk can maintain its microbiological safety for a maximum of 12 hours when stored at room temperature, as long as the level of microbial contamination stays below the recommended limit [12] – [13]. Over a span of six weeks, the quality of both raw and pasteurized goat milk samples gradually declined. By the end of the sixth week, a noticeable change in the color of the raw milk samples was seen, as they appeared slightly greenish, whilst the pasteurized goat milk samples had a yellowish tinge. Consequently, the deterioration of the milk significantly affected the chemical composition and microbiological studies conducted in this study. According to reference [14], the study on pasteurized milk stored in HDPE bottles found that it can last for 43 days when kept at a temperature of 2°C.

3.2. Chemical Composition of Raw and Pasteurized Goat Milk During Chilling Storage for 6 Week

An investigation was carried out to analyze the effect of the chemical composition of goat milk samples when stored at a temperature of 4oC for a period of 6 weeks. The components analyzed in this investigation consist of fat, protein, lactose, and solid non-fat (SNF), as outlined in Table 2.

Table 2. Physiochemical Analysis Result of Milk Samples

Week	Sample	Physio-Chemical Quality Parameters			
		Fat (%)*	SNF (%)*	Protein (%)*	Lactose (%)*
0	Raw	2.95	7.61	2.70	4.60
	Pasteurized	2.75	7.40	2.50	4.00
1	Raw	2.85	7.20	2.60	4.35
	Pasteurized	2.60	7.00	2.60	3.90
2	Raw	3.50	7.04	2.50	4.35
	Pasteurized	3.40	6.70	2.25	3.50
3	Raw	3.40	6.76	2.30	4.20
	Pasteurized	3.20	6.26	2.10	3.30
4	Raw	3.35	6.55	2.10	3.85
	Pasteurized	3.30	6.15	1.80	3.20
5	Raw	3.15	6.54	1.70	3.65
	Pasteurized	3.10	6.00	1.35	3.00
6	Raw	3.00	6.27	1.60	3.50
	Pasteurized	2.90	5.80	1.30	2.80

Note: An analysis was conducted on the milk composition of both raw and pasteurized goat milk samples on a weekly basis, with a 7-day interval between each analysis. (*) The symbols within a column show significant differences for up to 6 weeks of storage at 4oC, with a p-value of less than 0.1

The results indicate a clear decline in the fat, protein, solid non-fat (SNF), and lactose contents of both raw and pasteurized goat milk over the 6-week storage period at a temperature of 4°C, as shown in Table 2. The observed downward trajectory suggests a correlation between the temporal duration of milk's storage and a decrease in its chemical composition. The results of this study are consistent with a prior investigation which observed a drop in the levels of fat, protein, and lactose

in Sapera goat milk during a storage period of 40 days at a temperature of -18°C. Additionally, it was noted that the solid non-fat component exhibited a decline after 30 days of storage [15].

The present study presents compelling evidence of a significant influence on the quantities of fat, SNF (solids-not-fat), protein, and lactose in both unprocessed and pasteurized goat milk ($p<0.15$). The implementation of a chilling storage method for Saanen goat milk leads to a decrease in chemical and microbiological processes associated with milk spoilage. Refrigerating food products can lead to the decay of lactose due to the growth of lactic acid bacteria, which use lactose as a source of energy [16]. This conclusion aligns with the viewpoints of Amit et al. (2017), who emphasize that chilling methods can result in reductions in chemical, microbiological, and biochemical activities linked to the degradation of food [17]. Milk's high nutrient content creates an ideal environment for the growth and metabolic processes of various microorganisms, such as bacteria, yeast, and molds. Therefore, suboptimal storage conditions may enhance the proliferation and metabolic activities of these microbes, leading to the deterioration of milk quality over time.

As per the findings reported previously, protein and lactose represent constituents of the solid non-fat (SNF) fraction, which in turn reflects the percentage of SNF present in milk [11]. This study revealeda decline in SNF content over a 40-day storage period at 4°C, followed by reductions in protein and lactose levels, demonstrating significant differences ($p<0.15$). SNF is regulated by the interplay of lactose and milk protein components, with the quality of the feed having a notable impact on SNF levels,which are in turn associated with milk protein levels [18]. The decrease in lactose levels observed in thisstudy may be attributed to the activity of psychotropic bacteria [19].

3.3. Analysis of Microbial Content of Raw and Pasteurized Goat Milk

In this microbiological analysis, the utilization of Plate Count Agar (PCA) and Eosine Methylene Blue (EMB) agar was employed to discern the presence of microbial growth in both raw and pasteurized goat milk. To facilitate the quantification of E. coli and other bacterial species, a series of dilutions were made in a sterile saline solution. These dilutions included 10-1, 10-2, 10-3, 10-4, 10-5, and 10-6 dilutions. The 10-3 serial dilution method was chosen for the microbiological study based on its exceptional performance, and all data points were accurately documented.

3.3.1 Analysis TPC

The Total Plate Count (TPC) is a crucial metric used to monitor adherence to sanitary protocols during all stages of milk production, transportation, and storage [20]. Optimally, the quantity of bacteria present in unpasteurized milk acquired from dairy cows that are in a state of well-being and maintained in hygienic surroundings should not surpass 104 to 105 colony-forming units per milliliter (cfu/ml). However, the Pasteurized Milk Ordinance (PMO) specifies that Grade A pasteurized milk must have a bacterial count of at least 20,000 cfu/ml and a coliform concentration of less than 10 cfu/ml [21].

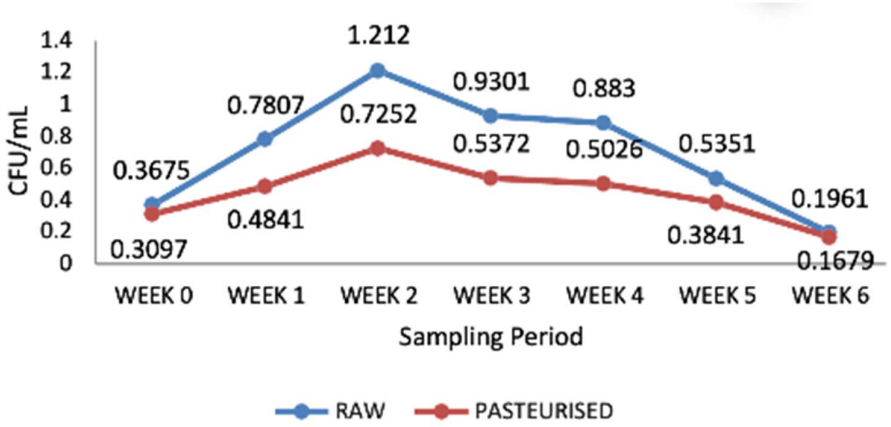


Figure 1. Average CFU of Raw and Pasteurized Goat Milk in PCA

The findings of the current investigation demonstrated that pasteurized milk exhibited reduced microbial populations in comparison to raw milk. Pasteurization is a reliable method for preserving goat milk, which helps to prolong its storage duration. While it is possible for thermophilic bacteria to survive the pasteurization process, thorough pasteurization guarantees the elimination of all harmful non-spore-producing bacteria [11]. During the second week of the trial, the microbiological examination of both raw and pasteurized goat milk showed significantly higher levels of microorganisms compared to the preceding weeks. The principal component analysis (PCA) revealed that the microbial counts in raw goat milk were 1.212 cfu/ml, whereas in pasteurized goat milk they were 0.7252 cfu/ml. Both samples were taken at a chilling temperature of 4°C (Figure 1).

The results of this study suggest that there are more amounts of microorganisms in both raw and pasteurized milk compared to prior reports. It was noted that pasteurized milk had lower levels of bacteria during storage durations of up to 9 days at temperatures of 5°C and 10°C [22]. The present investigation elucidated the existence of statistically significant disparities in log/cfu/ml values for both total bacterial count (TBC) and coliform bacterial count in pasteurized milk. These variations were found to be associated with fluctuations in temperature and the time of storage.

However, an intriguing observation emerged from this study, revealing a declining trend in the average cfu/ml of PCA starting from week 3. The microbial counts in raw goat milk decreased from 0.9301 cfu/ml in week 3 to 0.1961 cfu/ml in week 6, while in pasteurized goat milk, they declined from0.5372 cfu/ml in week 3 to 0.1679 cfu/ml in week 6. These results indicate a progressive reduction in the microbial load during the six-week storage period at 4°C.

The statistical analysis of this investigation revealed significant variations ($p<0.15$) in the total number of plate agar of PCA counts between raw and pasteurized goat milk samples over the whole 6-week storage period. Moreover, there was a decrease in the overall bacterial population in both raw and pasteurized goat milk samples from weeks 3 to 6. This decline can be related to the reduction of nutrients available in the milk.

Bacteria require nutrients for their development and metabolism, and when stored substances exhaust these essential nutrients, the bacterial population tends to decrease. This stage is referred to as the death phase, characterized by nutrient depletion and the accumulation of waste products that cells perish in response to this unfavorable condition [23]. Additionally, the limited availability of nutrients from the milk may lead to bacterial competition for survival, as they strive to obtain essential nutrients from the environment. This competitive behavior among bacteria for essential nutrients may impact their survival and population dynamics [24].

3.3.2 Analysis of EMB

The findings derived from the study of Eosine Methylene Blue (EMB) revealed that pasteurized goat milk exhibited a reduced concentration of Escherichia coli (E. coli) in comparison to raw goat milk. Overall, there was an observed increase in the concentration of E. coli during the second week. The raw goat milk samples had EMB counts of 0.802 cfu/ml, whereas the pasteurized goat milk samples had EMB counts of 0.363 cfu/ml (Figure 2). The statistical study employed the p-value variable to assess the influence of storage time on the E. coli count during a 6-week storage period at 4°C. The analysis revealed that the p-value was less than 0.15 (p-value < 0.15), indicating a substantial impact of storage time on the E. coli count. The initial enumeration of bacteria yielded significant findings on the level of contamination, as well as the overall quality and freshness of the milk samples obtained from the farm. Elevated levels of bacteria in the milk samples generally suggest the presence of contamination that occurred during the milking process. Multiple factors contribute to the contamination of milk, which include fluctuations in storage temperature, the level of cleanliness of storage equipment, the techniques employed in milk handling, and the health of the udder [12].

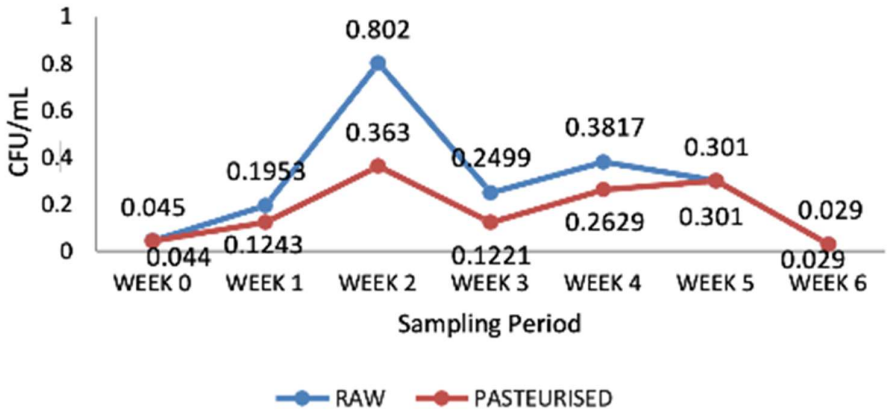


Figure 2. Average CFU of Raw and Pasteurized Goat Milk in EMB

A recent study investigated the bacteriological integrity of milk at various collection sites, such as farms and marketplaces. The study identified substantial disparities in the mean quantity of E. coli bacteria. The investigation recorded a concentration of E. coli at 3.92 log10 cfu/ml in the raw goat milk that was contaminated. On the other hand, the highest average count achieved was 6.62

log10 cfu/ml in goat milk that had been subjected to pasteurization [25]. Based on a specific study, goat milk samples collected from dairy farms on Penang Island, Malaysia showed an E. coli concentration of 4.6 log10 cfu/ml. [8]. The prevalence of E. coli in the collected goat milk samples from the dairy farms was linked to the presence of fecal matter.

The presence of contamination suggests the potential existence of other intestinal pathogens, such as K. pneumonia [24]. However, it is imperative to recognize that additional factors, such as insufficient hygiene and sanitary measures at the agricultural level, can also have a significant impact on milk contamination and should not be disregarded [26]. The present observations are consistent with this view, highlighting the complex nature of milk contamination and the necessity for comprehensive strategies to tackle the problem. According to the available dataset, there was a significant drop in the overall bacterial count during week 6. The decrease in microbial content was observed in both unprocessed and processed goat milk samples, as evidenced by an EMB value of 0.029 cfu/ml. (Figure 2). This observation is consistent with the pattern observed in the TPC counts during week 6. The decrease in bacterial counts observed during the sixth week might be ascribed to the inherent decline or death phase of bacteria, which is impacted by various variables including nutrition constraints and bacterial competition. These factors all contribute to a steady reduction in bacterial populations over time [22]. Therefore, it is imperative to closely monitor and uphold the bacteriological integrity of milk products to protect public health and mitigate the risk of foodborne disease outbreaks.

4. Conclusion and Recommendation

After the inquiry concluded, a thorough analysis was conducted on the physical characteristics and chemical makeup of both raw and pasteurized goat milk. The results indicated a gradual deterioration in the quality of the milk over a period of 6 weeks when it was kept in a refrigerator at a temperature of 4°C. The storage-induced cooling time has a notable influence on the chemical composition of milk, particularly its fat, solid non-fat, protein, and lactose constituents. The microbiological analysis revealed that raw goat milk had significantly higher bacterial levels compared to pasteurized goat milk. A study was performed to analyze the bacterial populations present in both raw and pasteurized goat milk. Both the raw and pasteurized samples exhibited evidence of decomposition starting in the second week of refrigerated storage, as indicated by the results. During this period, the microbial counts displayed elevated levels compared to the counts observed in the subsequent weeks. Moreover, the microbiological examination of both unpasteurized and pasteurized milk had notable effects on the overall bacterial plate count, coliform count, and aerobic spore count as a result of the duration of milk storage under refrigeration. The results of this study hold great significance for the sustainable future of dairy farming and the safety of human food consumption. Having an up-to-date understanding of the structure and microbial properties of goat milk obtained from local sources is of utmost

importance. The obtained data yields valuable knowledge on the characteristics of locally obtained raw and pasteurized goat milk during the storage duration. This data is an essential asset for enhancing the quality of dairy products and ensuring their fitness for human consumption.

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