

Impact of Storage Time on Microbial and Milk Quality in Tropical Regions of Indonesia

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Abstract

Agriculture plays a crucial role in Indonesia's economy, and milk is recognized as a vital component of the human diet due to its high nutritional value. In Indonesia, some farmers do not immediately transport their milk to Village Unit Cooperatives, which serve as Milk Collection Centres, leading to delays that can span several hours. Delays of this type may result in the raw milk becoming low quality and tend to generate a lower farmer payout price. The objective of this research was to determine the dynamics of milk quality immediately post-milking after 0, 2, and 4 h of storage. Samples from seven milk cans were collected in May 2025 at Lucky Farm, Medowo, Kediri Regency, East Java. The samples were further separated into two groups for TPC and Lactoscan analysis. Three treatments were tested: T0 (baseline) at 0 hours; T1, stored in an incubator at 28 °C for 2 hours; and T2, stored under the same conditions for 4 hours. Statistical analysis showed that TPC results differed significantly between treatments 4.93, 5.14 and 5.65 CFU/mL of milk for T0, T1 and T2, respectively. The Lactoscan analysis revealed significant changes in fat, density, and salt content. These data highlight the importance of delivering milk to collection centers as quickly as possible to minimize bacterial growth and maintain optimal quality.

Keywords: Zero Hunger, Storage Time, Total Plate Count, Milk Quality, Tropical Regions, Indonesia

INTRODUCTION

Agriculture plays a crucial role in Indonesia's economy, with as many as 87.31% of the population relying on it for their livelihood.¹ The progress of this sector is typically measured by several factors, including land size, production levels, livestock numbers, and the farmers' terms of trade.² Cattle farming is a widespread agricultural practice, particularly in rural communities.³ Beyond providing food, this sector generates employment and serves as a vital source of household income.⁴ Additionally, it contributes significantly to local government revenue, underlining the importance of optimising its potential.⁵

The benefits of livestock farming are diverse, ranging from income generation to the utilisation of animal waste through processing, as well as the production of meat and milk.⁶ Within this sector, dairy cattle farming stands out as a promising driver of growth in agriculture, essential for meeting the rising demand for animal-derived protein, specifically milk.⁷ As of 2024, Indonesia recorded a dairy cattle population of 485,809 heads. Although milk production in 2023 accounted for 20% of demand, it experienced a 6.19% decline in 2024 compared to the same period in the previous year. This reduction has been attributed to the government's large-scale milk imports. Milk is recognised as a vital

component of the human diet due to its high nutritional value.⁸

According to the Food and Agriculture Organization of the United Nations, over 80% of the global population regularly consumes dairy products.⁹ The raw milk, as the primary input for dairy products, has a direct impact on the quality and safety of the final dairy products.¹⁰ Therefore, determining the quality of raw milk is very important to guarantee the quality of the final product and is essential to the consumers' health.¹¹ The nutritional quality of raw milk consists mainly of fat and protein, which influences its physical and chemical properties.^{12,13}

Fat affects the colour, consistency, taste, and texture of milk, and the texture of protein plays a crucial role in forming gel, viscosity, and milk acidity.¹⁴ The total plate count (TPC) is one of the standard methods for calculating the microbial quality of milk. High TPC levels might indicate infection of the udder or other health problems, or inadequate hygiene practices during milking.¹⁵ Therefore, factors including fat percent, protein percent, and TPC are pivotal factors for the evaluation of raw milk quality. This happens because some farmers in Indonesia do not deliver milk to Village Unit Cooperatives as Milk Collection Centres immediately, and it can take a few hours. This can affect the quality of raw milk and its price for farmers, especially when the cold chain

Table 1. Total Plate Count (TPC) results

Sample	0 hour (CFU/ml)	2 hours (CFU/ml)	4 hours (CFU/ml)
1	1.7 x 10 ⁵	3.7 x 10 ⁵	5.7 x 10 ⁵
2	2.5 x 10 ⁴	2.2 x 10 ⁵	3.7 x 10 ⁵
3	1.9 x 10 ⁵	2.0 x 10 ⁵	5.0 x 10 ⁵
4	2.5 x 10 ⁴	2.5 x 10 ⁴	3.0 x 10 ⁵
5	2.5 x 10 ⁴	5.5 x 10 ⁴	6.5 x 10 ⁵
6	2.5 x 10 ⁴	5.5 x 10 ⁴	4.0 x 10 ⁵
7	1.4 x 10 ⁵	4.5 x 10 ⁴	3.4 x 10 ⁵

Note: The term sample indicates the number of milk cans from which the raw milk was collected

is not guaranteed. To address this question, we investigate milk quality within hours 0, 2, and 4 post-milking in the present study.

The objective of the study was to provide farmers with information concerning the potential risks of long milk storage at the farm prior to delivery. The expected results increased farmers' understanding of the need to deliver fresh milk to collection points after milking, in turn protecting its quality and supporting food security and Zero Hunger.

MATERIALS AND METHODS

Research design

The samples were obtained in May 2025 from seven milk cans at Lucky Farm (7°45'50"S, 112°22'09"E), Medowo (7°46'00"S, 112°22'43"E), Kediri Regency (7°48'05"S, 112°06'34"E), East Java (7°50'14"S, 112°54'09"E). Milk from each milk can was decanted into six sterilized milk pouches, with each sachet having about 100 ml of milk. The samples were delivered in a cool box to the Faculty of Veterinary Medicine, Universitas Airlangga, the same day for TPC examination and analysis by Lactoscan Analysis. The samples were separated into two groups of 42 aseptic milk pouches each, 21 for TPC and 21 for Lactoscan. Each group was then divided into three treatment groups: T0, immediately analysed at 0 h; T1, incubated at 28 °C to mimic tropical room temperature for 2 h; and T2, stored under the same conditions for 4 h.

Total plate count

The level of bacterial contamination in milk samples was quantified by the Total Plate

Table 2. Average of Total Plate Count (TPC)

Storage Time	Mean ± SD (log CFU/mL)
0 hour	4.93 ± 0.46 ^a
2 hours	5.14 ± 0.37 ^b
4 hours	5.65 ± 0.12 ^c

Note: Different superscripts indicate significant differences between treatments ($p < 0.05$)

Count method. The milk was serially diluted in a 0.9% NaCl solution to concentrations of 10⁻¹, 10⁻², 10⁻³, 10⁻⁴, 10⁻⁵, and 10⁻⁶ using a 0.9% NaCl solution as the diluent. A volume of 0.1 ml from each dilution was cultured in triplicate on Nutrient Agar. The TPC results in this research follow the guidelines from the Standard Plate Count (SPC).¹⁶

Lactoscan analysis

The analysis was carried out using the Lactoscan SAP Standard Milk Analyser - Basic Model (Milkotester Ltd., Nova Zagora, Bulgaria), measuring fat (%), protein (%), SNF (%), density (kg/m³), lactose (%), salts (%), Freezing Point (°C), and added water (%). To obtain representative results, the milk was homogenised by stirring for at least 5 min to achieve a homogeneous fat and solid distribution. For each analysis, 20 ml was needed, which was conducted in triplicate to ensure precision.¹⁷

Data analysis

The obtained data were tabulated and subjected to descriptive and One-way ANOVA followed by post hoc Duncan's test using IBM SPSS Statistics 2023. The significance level was set at 0.05 and the results are presented as mean±sd.

RESULTS

After 24 hours of incubation, the TPC plates were counted, and the results can be seen in Table 1. Analysis of the results using one-way ANOVA followed by Duncan's test showed significant differences between each treatment. The results of the analysis can be seen in Table 2.

Based on the statistical analysis shown in Table 3, the Lactoscan results indicate significant changes in fat, density, and salt content of raw milk after four hours of storage. However, no

Table 3. Average results from the Lactoscan analysis for each treatment

Parameter	0 hour	2 hours	4 hours
Fat (%)	5.04 ± 0.42 ^a	3.23 ± 0.67 ^b	2.74 ± 0.24 ^b
Protein (%)	2.91 ± 0.14 ^a	2.95 ± 0.11 ^a	3.04 ± 0.05 ^a
SNF (%)	8.36 ± 0.22 ^a	8.23 ± 0.15 ^a	8.15 ± 0.06 ^a
Density (kg/m ³)	27.20 ± 1.13 ^a	28.33 ± 1.18 ^{ab}	30.05 ± 0.69 ^b
Lactose (%)	4.59 ± 0.07 ^a	4.64 ± 0.12 ^a	4.75 ± 0.02 ^a
Salts (%)	0.56 ± 0.03 ^a	0.66 ± 0.20 ^{ab}	0.72 ± 0.00 ^b
Freezing Point (°C)	-0.528 ± 0.01 ^a	-0.537 ± 0.01 ^a	-0.545 ± 0.01 ^a
Added Water (%)	0	0	0

Note: Different superscript letters indicate significant differences between treatments ($p < 0.05$)

significant differences were observed for protein, SNF, lactose content, or freezing point in the raw milk. Additionally, there was no indication of added water in any of the samples.

DISCUSSION

Based on the results in this study, there were significant differences between each treatment in the TPC. The analysis showed that the bacterial count increased over time when stored at room temperature, from 8.6×10^4 CFU/mL (4.93 log CFU/mL) at 0 hours, to 1.4×10^5 CFU/mL (5.14 log CFU/mL) after 2 hours, and increased to 4.5×10^5 CFU/mL (5.65 log CFU/mL) after 4 hours. If we follow the Indonesian standard, the results from this research are still considered safe because they are below the maximum bacterial count based on Badan Standardisasi Nasional (SNI 3141.1:2011).¹⁸ However, to reduce bacterial development and preserve optimal quality, these results emphasize the importance of bringing milk to collection centres as soon as possible and storing it in cooling equipment.

Another finding of this study is that fat content in raw milk decreases significantly following two hours of storage at 28 °C, indicating poor quality in milk fat when milk is not refrigerated promptly. Findings indicate that fat content decreases significantly from approximately 5.0% at 0 hours to approximately 3.2% after 2 hours of storage and, subsequently, falls to approximately 2.7% after 4 hours. These results might be affected by enzymatic action, such as lipases, which might help in fat degradation when stored at room temperature.¹⁹ Another significant change in density and salt composition might also be

affected because of the enzymatic action. As for density, the fat separation might also affect the results.

On the other hand, protein levels changed from 2.91% to 3.04%, SNF from 8.36% to 8.15%, lactose from 4.59% to 4.75%, and freezing point from -0.528 °C to -0.545 °C. These variables showed some notable changes, but they were statistically not significant. It can be concluded that four hours at room temperature is not enough to make a significant difference for these variables. This result aligns with Khan,¹⁹ who reported that during short-term storage, the lactose content stays stable. These results also show that there was no additional water in the milk or any sign of milk adulteration in the sample, as indicated by freezing point measurements.²⁰

Another study by Vigolo et al.²¹ and Dorte et al.²² found that temperature, storage time, and preservatives significantly affect the protein composition in milk, which differs slightly from the results of this research. This might happen because the observation in this study was focused only on the storage time, and four hours might not be enough for the proteolytic enzyme to degrade the milk proteins until they show significant changes. Many factors can affect milk quality. A research project by Vithanage et al.²³ also demonstrated that microbial and milk quality can be influenced by temperature variations. These findings correspond with the study by Larsen et al.,²⁴ which demonstrated the impact of geographical location on milk quality. The difference between room temperature in the lowland and highland might also impact the implementation of these findings in Indonesia. Farmers from the highlands may have more time

to store their milk at room temperature before its quality decreases, compared to those from the lowlands.

This research has shown that storage time affects microbial and milk quality. However, there are still many limitations in this study, such as the limitation of storage time and the use of different temperatures. It's important to study further and consider various perspectives to enhance the application of this study and help achieve zero hunger in food security.

CONCLUSION

This study showed that the storage time has an impact on microbial and milk quality, especially for TPC, fat, density, and salt component of raw milk in Indonesia. These results highlight how crucial it is to get milk to collection facilities as soon as possible in order to reduce bacterial growth and preserve the best possible milk quality.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

WT conceived and designed the study. YP, HMR, and DAP conducted sample collection and laboratory analyses. MHE, WW, EML, and RNZA contributed to data analysis and interpretation. MRNM, CNZ, ARK, NBA, IBM, and ANMA wrote, reviewed and edited the manuscript. All authors read and approved the final manuscript for publication.

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DATA AVAILABILITY

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

This article does not contain any studies on human participants or animals performed by any of the authors.

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