Jurnal Media Penelitian dan Pengembangan Kesehatan

Vol 36 No 1, Maret 2026
e-ISSN: 2579-8103
p-ISSN:1979-8253

Microbial safety and stability of avocado–turmeric oil-based herbal milk as a basis for food safety education in public health promotion

Stabilitas dan Keamanan Mikroba Susu Herbal Minyak Alpukat - Kunyit sebagai Dasar Edukasi Keamanan Pangan dalam Promosi Kesehatan Masyarakat

Sri Winarni[1*], Annasari Mustafa[2], Atik Kurniawati[3]
[1]Jurusan Promosi Kesehatan, Poltekkes Kemenkes Malang, Malang, Indonesia
[2]Jurusan Gizi, Poltekkes Kemenkes Malang, Malang, Indonesia
[3]Jurusan Analisis Farmasi dan Makanan, Poltekkes Kemenkes Malang, Malang, Indonesia
*Email: sri_winarni@poltekkes-malang.ac.id

ABSTRACT

Background: *Herbal milk formulated with avocado oil and turmeric is a functional beverage with potential health benefits, but its microbiological safety requires evaluation to determine appropriate storage conditions.*
Objective: *This study aimed to analyze the effects of storage temperature and duration on total bacterial count (Total Plate Count/TPC) as well as the presence of Escherichia coli, coliforms, Staphylococcus aureus, and Enterobacteriaceae.*
Methods*: A laboratory experimental design using a factorial Completely Randomized Design (CRD) was applied, involving two factors: storage temperature (4°C, 25°C, and 40°C) and storage duration (0, 3, and 7 days).*
Results: *The results showed that storage at 4°C effectively affected microbial growth, indicated by a decrease in TPC from 5.51 log CFU/mL to 2.57 log CFU/mL on day 7. In contrast, storage at room temperature (25°C) and elevated temperature (40°C) led to increased microbial activity during the first three days.*
Conclusion: *Overall, storage at 4°C is recommended as the safest condition, with a maximum storage duration of seven days to maintain the microbiological safety and stability of the avocado–turmeric herbal milk.*

Keywords: *avocado–turmeric oil, food safety, herbal milk, microbial stability, storage temperature.*

ABSTRAK

Latar Belakang: Susu herbal berbasis minyak alpukat dan kunyit merupakan minuman fungsional yang berpotensi memberikan manfaat kesehatan, namun memerlukan evaluasi keamanan mikrobiologis untuk menentukan kondisi penyimpanan yang tepat.
Tujuan: Penelitian ini bertujuan menganalisis pengaruh suhu penyimpanan dan lama simpan terhadap jumlah total bakteri (Total Plate Count/TPC) serta jumlah bakteri Escherichia coli, Coliform, Staphylococcus aureus, dan Enterobacteriaceae.
Metode: Penelitian menggunakan metode eksperimen laboratorik dengan rancangan Rancangan Acak Lengkap (RAL) faktorial, melibatkan dua faktor suhu penyimpanan (4°C, 25°C, dan 40°C) dan lama penyimpanan (0, 3, dan 7 hari).
Hasil: Hasil menunjukkan bahwa penyimpanan pada suhu dingin 4°C berpengaruh terhadap pertumbuhan mikroba total, ditandai dengan penurunan nilai TPC dari 5,51 log CFU/mL menjadi 2,57 log CFU/mL pada hari ke-7. Sebaliknya, pada suhu ruang (25°C) dan tinggi (40°C) terjadi peningkatan aktivitas mikroba pada tiga hari pertama.
Kesimpulan: Berdasarkan keseluruhan hasil, suhu 4°C dinyatakan sebagai kondisi penyimpanan paling aman, dengan batas penyimpanan maksimal tujuh hari untuk mempertahankan keamanan dan stabilitas mikrobiologis susu herbal minyak alpukat-kunyit.

https://doi.org/10.34011/jmp2k.v36i1.4076

Vol 36 No 1, Maret 2026
e-ISSN: 2579-8103
p-ISSN:1979-8253

Kata kunci: keamanan pangan, minyak alpukat-kunyit, susu herbal, stabilitas mikroba, suhu penyimpanan

INTRODUCTION

A healthy diet plays a crucial role in preventing various diseases, particularly in addressing metabolic disorders such as hyperlipidemia. Consuming functional foods rich in bioactive compounds has become a strategy for managing cholesterol levels and improving heart health[1], [2]. One food ingredient that has the potential to act as an antihypercholesterol agent is avocado oil (Persea americana), which is rich in oleic acid and phytosterols. This oil is known to lower bad cholesterol (LDL) and increase good cholesterol (HDL)[3]Besides avocado, there are other herbal plants such as turmeric (Curcuma longa), which contain curcuminoids, which have antioxidant and anti-inflammatory properties and also play a role in reducing the risk of cardiovascular disease[4]Our previous research showed that avocado oil in turmeric herbal milk (milk supplemented with avocado oil and turmeric) had a lowering effect on LDL cholesterol levels in animal models. Therefore, avocado oil-supplemented herbal milk has the potential to become a functional food ingredient to prevent non-communicable diseases, particularly lipid-related diseases and heart health problems[5].

A high consumption of saturated fat is a major factor that can be controlled through improved diet and the consumption of natural, functional foods. In Indonesia, the trend of herbal beverage consumption is increasing due to the growing interest in healthy lifestyles, making the development of bioactive herbal milk increasingly relevant. Based on the changing trend in healthy lifestyles, demand for traditional herbal beverages in Indonesia has increased sharply during the pandemic, driven by public confidence in their health benefits[6]. With increasing public awareness of health and a balanced diet, the development of herbal milk products rich in combined bioactive compounds, such as avocado oil and turmeric, has great potential to be developed as innovative food products based on local wisdom that support healthy lifestyle trends in Indonesia.

The development of herbal milk based on avocado oil and turmeric is indeed an interesting prospect, but there is no data yet on its microbiological safety. The safety of food and beverage products is a critical issue worldwide, especially the safety of herbal milk. Temperature and storage duration also play a crucial role in determining the quality and safety of a beverage. At room temperature, bacteria can multiply rapidly and accelerate the spoilage process, while cold storage only slows, not stops, the growth of microorganisms. If products like herbal milk are not stored under proper conditions, the number of germs can increase far beyond safety thresholds in a short time. Bacteria such as Escherichia coli, Staphylococcus, and Enterobacter can contaminate beverages and cause health problems, ranging from stomach upset to more serious infections[7].

Cases of poisoning from herbal milk-based drinks have never been reported. Usually, cases of poisoning related to food or drinks arise due to the production process, dosage, interactions between ingredients, and storage errors, especially in milk-based products that have high nutritional value[8]. This situation highlights the need for in-depth studies on the microbiological stability of avocado and turmeric herbal milk under various storage conditions. This is to prevent the risk of contamination and poisoning. The nutrient-rich ingredients of milk and oil make it a breeding ground for bacteria, which can occur if improperly processed and stored[9].Therefore, it is important to conduct microbial stability and safety tests to ensure that beverages remain safe during storage.

The purpose of this study was to analyze the effect of storage temperature treatment (4°C, 25°C, and 40°C) and storage duration (days 0, 3, and 7) on the total microbial count (Total Plate Count/TPC) and to determine the number of microbial indicators of food contamination (Coliform, Escherichia coli (E. coli), Staphylococcus aureus (SA),

https://doi.org/10.34011/jmp2k.v36i1.4076

Vol 36 No 1, Maret 2026
e-ISSN: 2579-8103
p-ISSN:1979-8253

and Enterobacteriaceae) in avocado-turmeric oil-based herbal milk. The results of this study can be the basis for food safety education for the public, namely about storage methods, safe storage duration, and the importance of cleanliness when producing herbal drinks. That way, the public not only gets the health benefits of avocado and turmeric content, but also is protected from the risk of disease due to contaminated food and drinks. In addition to ensuring that the product remains suitable for consumption, information regarding microbial safety can also be used as educational material for the public.

METHODS

Study design

This study was a laboratory experimental study using a factorial Completely Randomized Design (CRD) to evaluate the effect of storage temperature and storage time on microbial quality in avocado–turmeric oil herbal milk. Two factors were applied: storage temperature (4°C, 25°C, and 40°C) and storage time (days 0, 3, and 7). Each treatment combination was performed in triplicate. The study was conducted at the Integrated Microbiology Laboratory, Poltekkes Kemenkes Malang, from March to August 2025.

Data source and sampling procedure

The study samples consisted of avocado–turmeric oil herbal milk produced under controlled laboratory conditions based on a standardized formulation, with 2% turmeric extract added to milk and avocado oil. The samples were aseptically distributed into sterile containers and stored under three temperature conditions: cold (±4°C), room temperature (±25°C), and accelerated temperature (±40°C). Sampling was conducted at predetermined intervals (days 0, 3, and 7) to evaluate microbial changes during storage.

Variables of the Study

The independent variables were storage temperature and storage time. The dependent variables included total microbial count (TPC) and the presence of indicator microorganisms, namely Coliform, Escherichia coli, Staphylococcus aureus, and Enterobacteriaceae.

Data collection

Samples were collected aseptically at each storage condition and time point. Each sample was labeled according to batch, temperature, and storage duration, then immediately processed for microbiological analysis. All procedures were conducted under sterile conditions using laminar air flow to prevent contamination.

Measurement and instruments

Microbiological analysis was performed using EasyPlate ready-to-use media (Kikkoman Biochemifa, Japan). Total Plate Count (TPC) was measured using EasyPlate AC and incubated at 35 ± 1°C for 24–48 hours. Identification of E. coli and Coliform was conducted using EasyPlate EC based on chromogenic color reactions after 24 hours of incubation. Detection of Staphylococcus aureus was performed using EasyPlate SA, with colony identification based on color characteristics according to the manufacturer's protocol. All tests followed validated AOAC Performance Tested Methods (PTM). Results were expressed as colony-forming units per milliliter (CFU/mL). The materials used included low-fat milk, avocado oil, turmeric extract, and distilled water. Laboratory equipment included an autoclave, incubator, laminar air flow cabinet, micropipettes, sterile tubes, sample bottles, and an analytical balance.

Data Analysis

Microbial counts were recorded in CFU/mL and transformed into log10 values for analysis. Descriptive statistics (mean and standard deviation) were calculated for each

https://doi.org/10.34011/jmp2k.v36i1.4076

Vol 36 No 1, Maret 2026
e-ISSN: 2579-8103
p-ISSN:1979-8253

treatment group. Differences between storage temperatures and times were analyzed using two-way ANOVA, followed by Tukey HSD post hoc test to identify significant differences. A significance level of $p < 0.05$ was applied.

RESULTS

Based on the research results, it is known that bacteria grow on easyplate media by showing specific colony characteristics used for identification. In EasyPlate EC media, in all dilutions, there was no appearance of purplish-blue colonies, which are characteristic of E. coli. However, there were pink-purple colonies, which indicate the presence of non-E. Coli coli coliform bacteria (Figure 1a). Based on the research results, it was found that in EasyPlate AC media, there were characteristics of aerobic bacterial growth, where red colonies were formed on the media (Figure 1b). Based on the research results, it was known that there was growth of Staphylococcus aureus bacteria growing on EasyPlate SA media, which was identified based on the formation of blue colonies (Figure 1c).

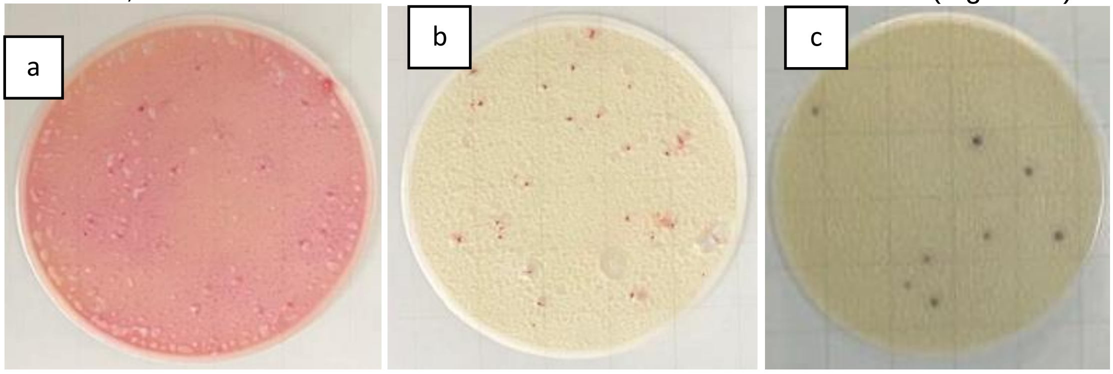

Figure 1. Visual depiction of bacterial growth on EasyPlate media for E. coli and Coliform (a), EasyPlate AC for aerobic bacteria (b), and EasyPlate SA for S. aureus

Based on the results of the study, it was shown that on day 0 of storage, the number of microbes in all treatments was at a level (6 log CFU/ml). On the 3rd day, there was a gradual increase at 4°C, a higher increase at 25°C, and the largest spike at 40°C, reaching around 10 log CFU/ml. Entering the 7th day, this pattern changed: the number of microbes at 4°C decreased to around 3 log CFU/ml, at 25°C it remained high, approaching 9 log CFU/ml, while at 40°C it dropped drastically to near zero.

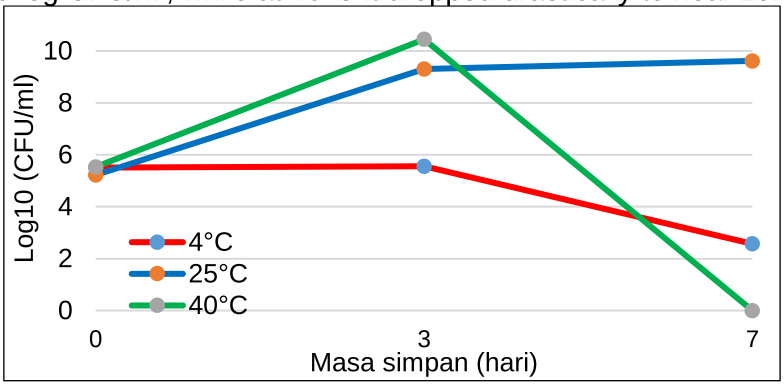

Figure 2. Graph of total aerobic bacteria based on the TPC test of avocado-turmeric-based herbal milk at storage times (0, 3, and 7 days) and temperatures (4 ˚C, 25 ˚C, and 40 ˚C)

https://doi.org/10.34011/jmp2k.v36i1.4076

Vol 36 No 1, Maret 2026
e-ISSN: 2579-8103
p-ISSN:1979-8253

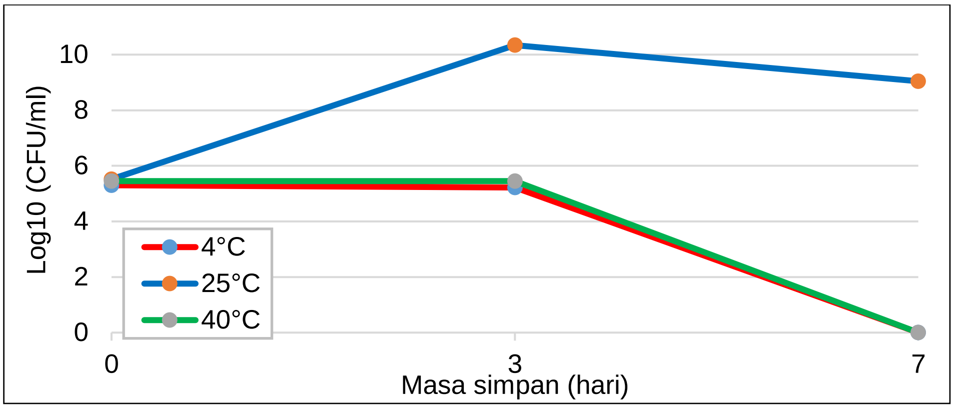

Figure 3. Graph of herbal milk coliform counts for storage duration (0, 3, and 7 days) and temperature (4 ˚C, 25 ˚C, and 40 °C)

Figure 3 shows the growth of coliform groups in the samples. The results showed that the number of coliforms tended to decrease with the length of storage. At 4°C storage temperature, microbial growth showed a very low initial number (0.19 log CFU/mL), then increased sharply on day 3 (6.13 log CFU/mL), then decreased again on day 7 (2.57 log CFU/mL). At 25°C storage temperature, microbial growth clearly increased consistently from 5.22 log CFU/mL on day 0 to 10.14 log CFU/mL on day 7. At 40°C storage temperature, the initial microbial number was quite high (6.06 log CFU/mL) and increased drastically on day 3 (11.14 log CFU/mL), but then decreased to 0 log CFU/mL on day 7. In this study, E. coli bacteria did not show any growth on days 0, 3, and 7 for all storage temperatures.

Figure 4 shows the results of the Staphylococcus aureus bacterial count test against shelf life, showing an increasing trend in bacterial counts with storage duration. At a storage temperature of 4°C, no bacterial growth was found at all storage durations (0, 3, and 7 days). At a storage temperature of 25°C, a different growth pattern was seen, where on days 0 and 3, the microbial count was still at 0 log CFU/mL, but increased significantly to 3.75 log CFU/mL on day 7. At a storage temperature of 40°C, there was no microbial growth on days 0 and 3, but there was an increase to 3.45 log CFU/mL on day 7.

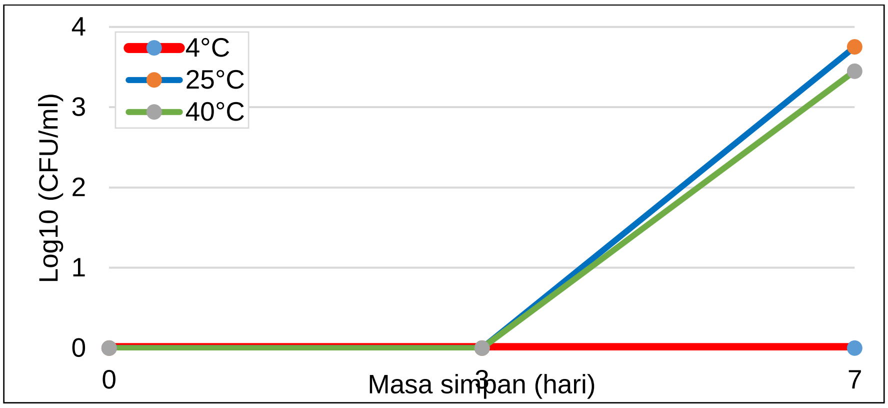

Figure 4. Graph of the number of S.aureus bacteria in herbal milk at storage times (0, 3, and 7 days) and temperatures (4 ˚C, 25 ˚C, and 40 ˚C)

https://doi.org/10.34011/jmp2k.v36i1.4076

Vol 36 No 1, Maret 2026
e-ISSN: 2579-8103
p-ISSN:1979-8253

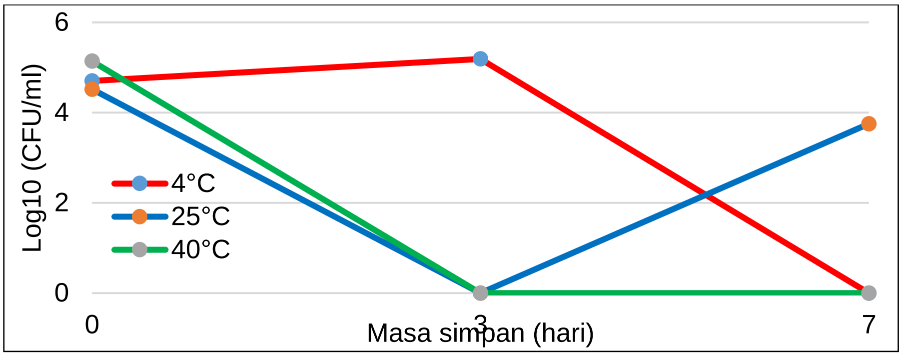

Figure 5. **Graph of the number of Enterobacteriaceae bacteria in herbal milk at storage times (0, 3, and 7 days) and temperatures (4 ˚C, 25 ˚C, and 40 ˚C)**

Figure 5 shows that Enterobacter counts in avocado oil–turmeric herbal milk generally decreased over time and were influenced by storage temperature. At 4°C, counts rose slightly from 4.7 to 5.19 log CFU/mL on day 3, then dropped to 0 log CFU/mL by day 7. Microbiological results also showed variations in Total Plate Count (TPC) across temperatures and storage times. At 4°C, TPC remained relatively stable from day 0 (3.2×10^5 CFU/mL; log 5.51) to day 3, then decreased sharply by day 7 (3.7×10^2 CFU/mL; log 2.57). At 25°C, TPC was initially high (2.5×10^9 CFU/mL; log 9.31), slightly increased on day 3, and decreased by day 7 (1.6×10^5 CFU/mL; log 5.22). At 40°C, TPC rose markedly by day 3 (2.8×10^{10} CFU/mL; log 10.45) before becoming undetectable on day 7, likely due to heat-induced cell death and pH reduction. Statistical analysis showed that temperature had no significant effect on TPC (p=0.204), while storage time had a significant effect (p=0.012). The interaction between temperature and time was not significant (p=0.261).

Table 1. **Results of Total Microbial Tests (TPC), Coliform, E. coli, S. aureus, and Enterobacteriaceae in Herbal Milk Supplemented with Avocado Oil**

Temperature	Day	TPC (CFU/mL)	log10 TPC	Coliform (CFU/mL)	log10 Coliform	*E. coli*(CFU/mL)	log10 *E. coli*	*S. aureus*(CFU/mL)	log10 *S. aureus*	Enterobacter (CFU/mL)	log10 Enterobacter
4°C	0	3.2×10^5	5.51	2.0×10^6	5.30	0	–	0	–	5.0×10^4	4.70
	3	3.6×10^5	5.56	2.3×10^5	5.22	0	–	0	–	1.6×10^5	5.19
	7	3.7×10^2	2.57	0	–	0	–	0	–	0	–
25°C	0	2.5×10^9	9.40	3.3×10^5	5.52	0	–	0	–	3.3×10^4	4.52
	3	4.2×10^9	9.62	2.2×10^{10}	10:36	0	–	0	–	0	–
	7	1.6×10^5	5.20	1.1×10^9	9.06	0	–	5.6×10^3	3.75	5.6×10^3	3.75
40°C	0	3.3×10^5	5.52	2.8×10^5	5.45	0	–	0	–	1.3×10^5	5.14
	3	2.8×10^{10}	10.45	2.0 × 109	9.30	0	–	0	–	0	–
	7	0	–	0	–	0	–	2.8×10^3	3.45	0	–

Based on the results of the Tukey HSD further test on the Total Plate Count (TPC) variable based on the storage day factor, significant differences were obtained between several pairs of storage times. The significance value between day 0 and day 7 of 0.042 (<0.05) indicates that there is a statistically significant difference between the number of microbes at the beginning of storage and the end of storage. This means that there is a significant decrease in the number of microbes from the first day to the seventh day. Conversely, the comparison between day 0 and day 3 (Sig. = 1,000) and day 3 and day 7 (Sig. = 0.059) shows that the difference between the two is not statistically significant.

https://doi.org/10.34011/jmp2k.v36i1.4076

Jurnal Media Penelitian dan Pengembangan Kesehatan
Vol 36 No 1, Maret 2026
e-ISSN: 2579-8103
p-ISSN:1979-8253

This indicates that the change in the number of microbes between days 0 and 3 is still relatively stable, while a significant decrease only occurs after day 7.

Table 1 shows that the number of Coliforms at a cold temperature of 4°C, which was initially 2.0 × 10^6 CFU/mL (log 0.3), also decreased to undetectable on day 7. At a room temperature of 25°C, the Coliforms were initially 3.3 × 10^5 CFU/mL (log 5.52) and jumped to 2.2 × 10^{10} CFU/mL (log 10.36) on day 3, then returned to a high of 1.1 × 10^9 CFU/mL (log 9.06) on day 7. At a high temperature of 40°, the initial Coliforms were 2.8 × 10^5 CFU/mL (log 5.45) and increased rapidly to log 9.3 on day 3, then disappeared on day 7. Based on the results of the hypothesis test in the Tests of Between-Subjects Effects table for the coliform variable, information was obtained that the significance value (Sig.) for the Temperature factor was 0.105 (> 0.05), which means that the difference in storage temperature (4°C, 25°C, and 40°C) did not have a significant effect on the number of coliform bacteria. The Day factor had a significance value of 0.010 (< 0.05), so it can be concluded that the storage period had a significant effect on changes in the number of coliforms in avocado-turmeric oil herbal milk. Meanwhile, the interaction between Temperature × Day had a significance value of 0.143 (> 0.05), which indicates that the combination of temperature and storage period did not have a significant interactive effect on the number of coliforms.

Based on the results of the Tukey HSD further test on the coliform variable based on storage days, it appears that there are significant differences between several pairs of observation times. The significance value between day 0 and day 7 of 0.027 (<0.05) indicates that there is a statistically significant difference in the number of coliforms between the beginning and end of the storage period. This means that the number of coliforms decreased significantly from the first day to the seventh day, indicating that longer storage can suppress the coliform bacterial population. Meanwhile, the comparison between day 0 and day 3 (Sig. = 0.824) and between day 3 and day 7 (Sig. = 0.117) shows a significance value greater than 0.05, which means there is no significant difference in that period. Thus, it can be concluded that changes in the number of coliforms were not yet visible until day 3, and a significant decrease only occurred after day 7 of storage.

Table 1 shows the number of Enterobacteriaceae at 4 °C. The Enterobacter population was still found since day 0 at 5.0 × 10^4 CFU/mL (log 4.70), increased on day 3 to 1.6 × 10^5 CFU/mL (log 5.20), then decreased to undetectable on day 7. This shows that at cold temperatures, these bacteria are still able to survive and grow slightly at the beginning, but eventually decline due to low temperature stress that inhibits metabolism. At 25 °C, growth was seen to fluctuate on day 0 at 3.3 × 10^4 CFU/mL, then decreased to undetectable on day 3, and appeared again on day 7 at 5.6 × 10^3 CFU/mL (log 3.75). This pattern illustrates the adaptation phase and partial death due to competition with other microbes. Meanwhile, at a temperature of 40 °C, the initial number was 1.3 × 10^5 CFU/mL (log 5.11), but it decreased drastically and disappeared on days 3 and 7 because high temperatures damage gram-negative bacterial cells such as Enterobacteriaceae.

Based on the results of the hypothesis test in the Tests of Between-Subjects Effects table for the Enterobacteriaceae variable, it is known that the significance value (Sig.) for the Temperature factor is 0.065, slightly above the significance limit of 0.05. This indicates that differences in storage temperatures (4°C, 25°C, and 40°C) have a significant effect on the number of Enterobacteriaceae, but are not statistically strong enough to be declared significantly different. The Day factor has a significance value of 0.010 (<0.05), which means that the storage period has a significant effect on changes in the number of Enterobacteriaceae. In other words, storage duration is the main factor

https://doi.org/10.34011/jmp2k.v36i1.4076

Vol 36 No 1, Maret 2026
e-ISSN: 2579-8103
p-ISSN:1979-8253

that influences the dynamics of bacterial growth or decline. Meanwhile, the interaction between Temperature × Day has a significance value of 0.026 <0.05, which means there is a significant difference. This indicates that the combination of temperature and storage period has a significant joint effect on the number of Enterobacteriaceae.

Based on the results of the Tukey HSD further test on the Enterobacteriaceae variable based on the storage day factor, it appears that there is a significant difference between several observation periods. The significance value (Sig.) between day 0 and day 7 of 0.005 (<0.05) indicates that there is a statistically significant difference in the number of Enterobacteriaceae between the beginning and end of the storage period. This means that the number of Enterobacteriaceae decreased significantly from day 0 to day 7, indicating that the longer the product is stored, the population of these bacteria decreases. Conversely, the comparison between day 0 and day 3 (Sig. = 0.109) and between day 3 and day 7 (Sig. = 0.290) shows no significant difference. This indicates that changes in the number of Enterobacteriaceae in the initial period (days 0 to 3) are still relatively stable, and a significant decrease only occurs after longer storage up to day 7.

Table 1 shows that Staphylococcus aureus bacteria were also not found (0 CFU/mL) at 4°C and 40°C throughout the entire storage period. However, at 25°C for 7 days, growth of 5.6×10^3 CFU/mL (log 3.75) appeared. This indicates that room temperature provides optimal conditions for the growth of S. aureus, although it is still relatively low compared to the total number of other microbes. This growth could originate from secondary contamination or the activity of bacteria that were initially dormant and reactivated when the temperature increased. Based on the results of the hypothesis test in the Tests of Between-Subjects Effects table for the Staphylococcus aureus (S. aureus) variable, a significance value (Sig.) was obtained for the Temperature factor of 0.292 (> 0.05), which means that the difference in storage temperature (4°C, 25°C, and 40°C) did not have a significant effect on the number of S. aureus bacteria. The Day factor has a significance value of 0.061 > 0.05, so it can be interpreted that the storage period does not affect the number of S. aureus, in other words, the effect is not statistically significant. The interaction between Temperature × Day shows a significance value of 0.301 (> 0.05), which indicates that the combination of temperature and storage period does not provide a significant interactive effect on changes in the S. aureus population.

Table 1 shows that during storage at all three temperatures (4°C, 25°C, and 40°C), the number of E. coli was undetectable or zero (0 CFU/mL) on almost all observation days. This indicates that E. coli bacteria did not grow or contaminate the herbal milk. Even up to day 7, there was no measurable growth. These results indicate that the initial pasteurization or sanitation process was likely quite effective, thus completely preventing E. coli contamination throughout the shelf life.

DISCUSSION

The results showed that storage time was the most influential factor affecting changes in microbial counts, while temperature had a limited effect except for the Enterobacteriaceae group, which is sensitive to the combination of heat and time. At cold temperatures (4°C), microbial growth was very slow. This was evident from the TPC and coliform values, which were stable at the beginning of storage and then decreased drastically after 7 days. Low temperature conditions inhibit microbial enzymatic activity and reduce metabolic rates, preventing bacteria from multiplying rapidly. Therefore, storage at 4°C for 7 days is the best condition for suppressing microbial growth without damaging milk quality. This finding is in line with the basic principle of food microbiology that low temperatures slow the metabolism and replication of mesophilic bacteria[11].

https://doi.org/10.34011/jmp2k.v36i1.4076

Vol 36 No 1, Maret 2026
e-ISSN: 2579-8103
p-ISSN:1979-8253

Storage at room temperature (25°C) causes rapid growth of microorganisms on the 3rd day, especially for coliforms and *S. aureus*, which then decreased due to the possible formation of natural antimicrobial compounds from turmeric and avocado oil components. This condition indicates that room temperature storage is less safe for long shelf life. This confirms that room temperature provides optimal conditions for mesophilic bacteria and opportunistic pathogens. Although there was a decrease in TPC on day 7, most likely due to nutrient limitations and the accumulation of natural antimicrobial compounds, the initial high growth phase still indicates a food safety risk[12].

Based on the results, storage at 40°C accelerates the growth of TPC and coliforms initially, but leads to microbial death by day 7 due to heat stress and decreased pH. Although this temperature can inactivate Enterobacteriaceae more rapidly, it is not recommended because it may damage the nutritional content and organoleptic quality of the milk[13].

The absence of Escherichia coli under all storage conditions indicates that processing, pasteurization, and sanitation were well maintained. However, the presence of high coliform counts at room, and elevated temperatures suggests possible contamination from the environment or raw materials, as coliforms include bacteria other than E. coli that are more resistant to moderate conditions[14]. Meanwhile, the presence of S. aureus only at room temperature on day 7 indicates the possibility of secondary contamination or dormant bacterial activity[15].

Apart from temperature and storage time, the bioactive compounds in avocado and turmeric have been shown to help suppress microbial growth, thus enhancing the effects of this herbal milk, making it a functional, particularly health-promoting beverage. Avocado oil contains phytosterols and monounsaturated fatty acids, such as oleic acid, which have antibacterial properties, particularly against Gram-positive bacteria. Meanwhile, curcuminoids from turmeric have broad antimicrobial effects and also function as antioxidants, which can inhibit the formation of protective layers (biofilms) on microbial surfaces[16], [17]. The combination of these two ingredients creates a synergistic effect, working together to inhibit the growth of spoilage-causing and pathogenic microbes. This is thought to be one of the reasons why the Total Plate Count (TPC) and Enterobacteriaceae counts decrease during storage, especially at low temperatures (4°C). Thus, the combination of cold storage and the bioactive compounds of avocado and turmeric helps maintain product safety and shelf life naturally[12], [18].

Comparison with food safety standards (BPOM Regulation No. 13 of 2019) shows that TPC values at 4°C on days 0 and 3 were slightly above the SNI limit (1×10^5 CFU/mL). Although coliform and Enterobacteriaceae counts exceeded thresholds at certain points, pathogenic indicators such as Escherichia coli and Staphylococcus aureus were negative or very low. These findings suggest that the product remains safe for consumption for up to 7 days when stored at 4°C. Despite some initial values exceeding standards, the decreasing microbial trend indicates the potential of cold storage combined with bioactive compounds from avocado oil and turmeric to maintain product safety[19], [20].

Based on the decreasing trend in TPC and the absence of pathogenic microbes growth until the 7th day, the avocado oil-turmeric herbal milk product is still suitable for consumption for up to 7 days at 4°C. However, at room temperature and high temperatures, microbial activity increases drastically in the first 3 days, so unrefrigerated storage is not recommended. From a food technology perspective, these results can be the basis for developing natural herbal milk products that do not require synthetic preservatives, provided they are stored at low temperatures. This approach is in line with the trend of the modern food industry that prioritizes clean labels. This means products

https://doi.org/10.34011/jmp2k.v36i1.4076

Vol 36 No 1, Maret 2026
e-ISSN: 2579-8103
p-ISSN:1979-8253

without chemical additives, but remain microbiologically safe through temperature control and the use of natural bioactive compounds. Therefore, the combination of cooling technology and the phytosterol, oleic acid, and curcuminoid content of natural ingredients is a potential strategy for extending shelf life while maintaining the functional quality of products[21].

The important educational value of this research is that it can increase public and small industry players' understanding of the safety of natural food without synthetic preservatives. The finding that storing avocado oil-turmeric herbal milk at 4°C for seven days can suppress microbial growth to below the safe threshold indicates that temperature control is a key factor in maintaining the microbiological quality of dairy and herbal-based food products. This is in line with other research that states that Indonesians tend to consume high-fat products and pay less attention to the health of their products. Food storage aspects, in addition, there is a phenomenon of increasing trends in consumption of traditional herbal drinks in Indonesia, requiring the implementation of the principles of Good Manufacturing Practices (GMP) and Good Storage Practices (GSP) to ensure product safety[6]. Therefore, the results of this study can be used as a basis for public food safety and nutrition education, both through counseling and vocational learning in the fields of health, nutrition, and food technology, with an emphasis on the importance of sanitation, pasteurization, and cold storage in the processing of natural herbal milk. With the implementation of this education, it is hoped that the community will be able to develop a conscious behavior of safe food while supporting the national clean-label food movement based on local bioactive ingredients that are healthy and safe for consumption.

CONCLUSION

This study shows that storage temperature and storage duration affect the total microbial count (TPC) and the number of food contamination indicator microbes such as E. coli, Coliform, S. aureus, and Enterobacteriaceae in avocado-turmeric oil-based herbal milk. Storage at low temperature (4°C) proved to be the most effective in suppressing microbial growth during the shelf life, while room temperature (25°C) and high temperature (40°C) caused an increase in the number of microbes, so that it is not recommended for long-term storage. Storage at low temperature (4°C) was most effective in suppressing the growth of microbes such as total bacteria, Coliform, and Enterobacteriaceae, with the results showing a significant decrease until the 7th day. Low storage temperature also did not detect pathogenic microbes such as E. coli and S. aureus. In contrast, storage at room temperature (25°C) and high temperature (40°C) accelerated microbial growth at the beginning of storage and reduced product quality. This is due to the bioactive compounds in avocado oil (phytosterols and oleic acid) and turmeric (curcuminoids), which play a crucial role as natural antimicrobial agents that help maintain the product's microbiological stability. The combination of cold temperatures and these bioactive compounds works synergistically to maintain the safety, quality, and shelf life of herbal milk.

Further research is recommended to optimize the preheating and sanitation processes to ensure compliance with all SNI parameters from the first day of storage. Furthermore, organoleptic testing (color, aroma, and taste) is necessary to assess product quality and consumer acceptance of herbal milk after storage at various temperatures.

ACKNOWLEDGMENT

Thanks are extended to the Indonesian Ministry of Health and the Director of the Poltekkes Kemenkes Makant for their support in financing this research.

https://doi.org/10.34011/jmp2k.v36i1.4076

Vol 36 No 1, Maret 2026
e-ISSN: 2579-8103
p-ISSN:1979-8253

REFERENCES

[1] R. Alfahel, T. Sawicki, M. Jabłońska, and K. E. Przybyłowicz, "Anti-Hyperglycemic Effects of Bioactive Compounds in the Context of the Prevention of Diet-Related Diseases," *Foods*, vol. 12, no. 19, pp. 3698–3698, 2023, doi: 10.3390/foods12193698.

[2] S. Asgary, A. Rastqar, and M. Keshvari, "Functional Food and Cardiovascular Disease Prevention and Treatment: A Review," *J. Am. Coll. Nutr.*, vol. 37, no. 5, pp. 429–455, 2018, doi: 10.1080/07315724.2017.1410867.

[3] S. Winarni, A. Mustafa, and A. Kurniawati, " Kajian Pembuatan & Karakterisasi Minyak Alpukat Sebagai Sumber Asam Oleat & Linoleat Serta Potensi Sebagai Antioksidan & Antihiperlipidemia Sebagai Bahan Dasar Susu Herbal Tinggi HDL.," Laporan Penelitian SIMLITABKES, Poltekkes Kemenkes Malang, Malang, 2023.

[4] S. Sukamto, "Metode Formulasi Kurkumin Kunyit Susu dan Asam Organik Asam Jawa yang Dipasteurisasi untuk Penyiapan Bahan Minuman Fungsional IN Patent P00,200,900,281," 2009

[5] S. Winarni, A. Mustafa, and A. Kurniawati, "Analisis Profil Senyawa Kimia Minyak Alpukat Dari Dua Varietas Buah Alpukat (Persea Americana) Dan Potensinya Untuk Kesehatan.," *Media Penelitian dan Pengembangan Kesehatan*, vol. 34, no. 4, pp. 879–892, Dec. 2024, doi: 10.34011/jmp2k.v34i4.2039.

[6] T. Estiasih *et al.*, "Indonesian traditional herbal drinks: diversity, processing, and health benefits," *Journal of Ethnic Foods*, vol. 12, no. 1, pp. 7–7, Feb. 2025, doi: 10.1186/s42779-025-00267-5.

[7] W. Nopitasari, M. Anggraini, and L. Advinda, "Pengendalian Cemaran Mikroba Pada Bahan," *Jurnal Lidbang Pertanian (Prosiding SEMNAS BIO)*, vol. 28, no. 3, 2021.

[8] J. Kenyon *et al.*, "Campylobacter outbreak associated with raw drinking milk, North West England, 2016," *Epidemiol. Infect.*, vol. 148, 2020, doi: 10.1017/S0950268820000096.

[9] A. A. Adine, E. Wulandari, and D. T. Utama, "Karakteristik Mikrobiologi (Total Bakteri, Total Yeast) dan pH Produk Susu Kurma Selama Penyimpanan Suhu Rendah (4-6°C)," *Jurnal Teknologi Hasil Peternakan*, vol. 4, no. 1, 2023, doi: 10.24198/jthp.v4i1.46258.

[10] A. C. Simpson, T. Suzuki, D. R. Miller, and K. Venkateswaran, "Microbial Burden Estimation of Food Items, Built Environments, and the International Space Station Using Film Media," *Microorganisms*, vol. 10, no. 9, 2022, doi: 10.3390/microorganisms10091714.

[11] K. M. Edwards, A. Badiger, D. R. Heldman, and M. S. Klein, "Metabolomic markers of storage temperature and time in pasteurized milk," *Metabolites*, vol. 11, no. 7, pp. 410–419, 2021, doi: 10.3390/metabo11070419.

[12] T. M. Ilesanmi, O. O. Oladipo, A. C. Olaleye, and O. D. Osasona, "Antimicrobial Activity of Essential Oil from Avocado (Persea americana) Seed and Pulp on Some Pathogenic Organisms," *South Asian Journal of Research in Microbiology*, vol. 12, no. 3, pp. 61–68, May 2022, doi: 10.9734/sajrm/2022/v12i330276.

[13] N. Zeaki, S. Johler, P. N. Skandamis, and J. Schelin, "The role of regulatory mechanisms and environmental parameters in staphylococcal food poisoning and resulting challenges to risk assessment," *Front. Microbiol.*, vol. 10, no. JUN, pp. 1306–1307, 2019, doi: 10.3389/fmicb.2019.01307.

[14] P. N. Tempini, S. S. Aly, B. M. Karle, and R. V. Pereira, "Multidrug residues and antimicrobial resistance patterns in waste milk from dairy farms in Central California," *J. Dairy Sci.*, vol. 101, no. 9, pp. 8110–8122, 2018, doi: 10.3168/jds.2018-14398.

https://doi.org/10.34011/jmp2k.v36i1.4076

Vol 36 No 1, Maret 2026
e-ISSN: 2579-8103
p-ISSN:1979-8253

[15] G. Lv *et al.*, "Molecular Characteristics of Staphylococcus aureus From Food Samples and Food Poisoning Outbreaks in Shijiazhuang, China," *Front. Microbiol.*, vol. 12, pp. 652236–652276, 2021, doi: 10.3389/fmicb.2021.652276.
[16] Sanjana Seth, Kashmeera Agarwal, and Abdul Rahman, "Curcumin: A Review of Its' Effects on Human Health," *International Healthcare Research Journal*, vol. 5, no. 10, pp. RV1–RV4, 2022, doi: 10.26440/ihrj/0510.01486.
[17] S. J. Hewlings and D. S. Kalman, "Curcumin: A review of its effects on human health," *Foods*, vol. 6, no. 10, pp. 90–92, 2017, doi: 10.3390/foods6100092.
[18] M. Rai, A. P. Ingle, R. Pandit, P. Paralikar, N. Anasane, and C. A. Dos Santos, "Curcumin and curcumin-loaded nanoparticles: antipathogenic and antiparasitic activities," *Expert Rev. Anti. Infect. Ther.*, vol. 18, no. 4, pp. 367–79, 2020, doi: 10.1080/14787210.2020.1730815.
[19] C. Dai *et al.*, "The Natural Product Curcumin as an Antibacterial Agent: Current Achievements and Problems," 2022. doi: 10.3390/antiox11030459.
[20] A. Marra *et al.*, "Avocado and Its By-Products as Natural Sources of Valuable Anti-Inflammatory and Antioxidant Bioactives for Functional Foods and Cosmetics with Health-Promoting Properties," *Applied Sciences*, vol. 14, no. 14, pp. 5978–5978, Jul. 2024, doi: 10.3390/app14145978.
[21] M. E. M. Soutelino, A. C. de O. Silva, and R. da S. Rocha, "Natural Antimicrobials in Dairy Products: Benefits, Challenges, and Future Trends," *Antibiotics*, vol. 13, no. 5, pp. 415–415, May 2024, doi: 10.3390/antibiotics13050415.

https://doi.org/10.34011/jmp2k.v36i1.4076