AGRICULTURE ARCHIVES

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Original Research Article
ISSN: 3041-5322
Journal homepage: agriculture.researchfloor.org

The effect of different packaging materials on the microbial and nutritional safety of cow's milk butter during extended storage

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ABSTRACT

Background: *Traditional packaging materials are widely used for butter storage in many regions, yet their comparative efficacy against modern alternatives remains under-researched, particularly regarding long-term microbial safety and sensory stability.*

Aims: *This study investigated and compared the impact of traditional Enset-based (Ensete ventricosum) packaging versus plastic packaging on the microbial safety and sensory stability of cow's milk butter stored at ambient temperatures.*

Materials and Methods: *Butter samples were stored for 12 months, with analyses performed at 4, 8, and 12-month intervals. Microbiological parameters, including Total Bacterial Count (TBC), Coliform count, Staphylococcus spp., and Yeast and Molds, were evaluated alongside sensory attributes (appearance, aroma, and overall acceptance) using a 5-point scale.*

Results: *Storage time significantly impacted microbial loads and sensory quality in both packaging types ($P<0.001$). TBC, Staphylococcus, and Yeast and Mold counts increased significantly over 12 months. While Coliform counts rose significantly in Enset packaging, they remained stable in plastic. Sensory attributes declined sharply after 8 months in both materials; however, Enset-based packaging provided marginally better protection against appearance degradation compared to plastic by the end of the study.*

Conclusion: *Packaging material significantly influences the microbial and sensory trajectory of butter during long-term ambient storage. Although neither material maintained optimal quality beyond 8 months, Enset-based packaging offered slight sensory advantages. These findings inform the development of sustainable, bio-based packaging solutions to improve the shelf-life of dairy products in ambient conditions.*

Keywords: *Ensete ventricosum, Cow's milk butter, Food safety, Traditional packaging, Microbial stability, Sensory evaluation.*

1. Introduction

In Ethiopia, cow's milk butter (locally known as *Kibe*) is a premier dairy product that plays a central role in the national diet, economy, and traditional culture. It contains a large amount of milk fat (at least 80%) and a lesser proportion of other milk components [18]. Beyond its use as a nutrient-dense food high in fats and vitamins, it is utilized for cosmetic purposes, such as hair and skin care. However, butter is highly susceptible to spoilage due to its substantial moisture content (often 16–35%) and fat composition, which can trigger lipolytic and oxidative degradation [1], [7]. In many rural regions, the lack of cold chain infrastructure forces producers to store butter at ambient temperatures, increasing the risk of microbial contamination and quality loss [21]

Citation: Endrias Dako Keshamo (2026). The effect of different packaging materials on the microbial and nutritional safety of cow's milk butter during extended storage. *Agriculture Archives: an International Journal.*
DOI: https://doi.org/10.51470/AGRI.2026.5.1.46

Received on: November 04, 2025
Revised on: December 07, 2025
Accepted on: January 05, 2026
Available Online: February 02, 2026

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Traditional storage methods, such as the use of dried Enset pseudo-stem sheaths (*Ensete ventricosum*), have long been employed by smallholder farmers to extend shelf life through natural barriers [22], [24]. Despite the deep-rooted cultural importance of these bio-based materials, modern synthetic alternatives like plastic containers have become increasingly common due to their accessibility. However, both traditional and synthetic materials vary in their permeability to oxygen and moisture, which directly influences the product's microbial safety and sensory stability over time [3]. Given that traditional Ethiopian butter often fails to meet established microbial safety standards, there is a critical need to evaluate how these packaging choices impact long-term preservation [22]. Therefore, this study aims to assess the comparative impact of Enset based and plastic packaging on the microbiological and sensory quality of cow's milk butter over a 12 month storage period.

1.1 Research Objectives

The primary aim of this study was to investigate the efficacy of traditional Enset-based packaging relative to standard plastic materials in preserving butter quality. Specifically, the study sought:

- To evaluate the impact of packaging type on microbial safety: To quantify the total bacterial, coliform bacteria, staphylococcal bacteria, and yeast and mold loads in cow's milk butter across a 12-month storage period at ambient temperature.
- To assess the preservation of sensory attributes: To determine how traditional (*Ensete ventricosum*) versus synthetic plastic packaging influences the evolution of aroma, appearance, and overall acceptance during long term storage.

- To determine storage stability over time: To monitor the rate of microbial proliferation and organoleptic degradation at systematic intervals (4, 8, and 12 months) to identify the optimal shelf-life limit for each packaging material.

2. Literature Review

2.1 Microbiological quality of traditional Butter

Previous research consistently highlights that the microbial profile of traditional Ethiopian butter is often substandard compared to international and national safety limits [7], [24]. High loads of Aerobic Mesophilic Bacteria (AMBC), Coliforms, Yeast, and Molds are frequently reported, with counts often exceeding 6 log CFU/g [7]. Contamination typically occurs through unhygienic milking practices, contaminated water used for washing, and improper handling during storage [24]. For instance, studies in the Oromia region found that bacteria of public health concern, such as *Salmonella* and *Staphylococcus*, can persist in butter stored without adequate temperature control [3].

2.2. Impact of Packaging and Storage Duration

Packaging materials serve as a primary barrier against environmental contaminants and oxidation. Traditional materials like plant leaves or clay pots are common, but their high permeability to oxygen and moisture often accelerates the growth of lipolytic and proteolytic microorganisms [24]. While plastic containers are perceived as more protective, studies on similar traditional dairy products (e.g., *peda* or shea butter) suggest that microbial loads, particularly yeast and mold increase significantly regardless of packaging type when stored under tropical ambient conditions [16]. Over extended storage, the degradation of fatty acids through lipolysis results in the development of off flavors and rancidity [6]. which can be detected through sensory evaluation [17].

2.3. Sensory Degradation during Ambient Storage

The organoleptic properties of butter, including **aroma** and **appearance**, are highly sensitive indicators of its quality. Studies have shown that storage temperature and duration are the most significant factors affecting consumer acceptability [17]. Butter samples typically maintain high sensory scores for short periods (up to 4 months), but prolonged exposure to ambient air leads to enzymatic browning and the volatilization of aromatic compounds [21]. Research indicates that modified atmosphere packaging or specific traditional treatments can delay this decline, yet the baseline performance of raw traditional materials like Enset remains under-researched in long-term contexts [24].

3. Materials and Methods

3.1.Sample collection and preparation

Fresh cow's milk butter was purchased from a local market and transported to the laboratory for processing. To ensure uniformity, the bulk butter was thoroughly mixed by hand after standardized washing [8]. The experimental design utilized two distinct traditional packaging treatments: synthetic plastic containers and dried Enset pseudo-stem sheaths (*Ensete ventricosum*) [5]. Equal portions of the butter were partitioned into each packaging type and stored at ambient temperature for a total duration of 12 months.

Picture 1: Sample preparation and storage in packaging materials

3.2. Sampling and Storage Protocol

A longitudinal study design was employed, with samples systematically withdrawn from each packaging treatment at three predefined intervals: 4, 8, and 12 months [14]. This timeline was established to evaluate the progressive impact of storage duration and material permeability on the product's stability.

3.3 Laboratory Analysis and Sensory Evaluation

3.3.1. Microbiological Analysis Procedures

Samples collected at each interval were subjected to comprehensive microbial load testing at the Wolaita Sodo Regional Laboratory. The analysis quantified the following parameters using standardized horizontal methods: Total Bacterial Count [11], Coliform Count [10], *Staphylococcus* bacteria [12], and Yeast and Molds [7]. Results were recorded as Colony Forming Units per gram (CFU/g) and subsequently converted to log10CFU/g for statistical representation and visualization in interval plots with 95% Confidence Intervals (CI).

Figure 2: Culture media preparation for microbial load detection

3.3.2. Sensory Evaluation

Sensory analysis was conducted by a semi-trained panel (n=20) in a controlled sensory laboratory environment following [12] guidelines. Panellists evaluated three attributes appearance, aroma, and overall acceptance using a 5-point Hedonic scale, where 5 represented "extremely favourable" and 1 represented "extremely unfavourable" [15]. To prevent sensory fatigue, samples were presented in a randomized order and labelled with three-digit codes. Between samples, panellists used a time gap to forget the sensory attribute of the first sample.

Figure 2: Butter samples prepared for sensory evaluation from two storage Materials

3.4. Statistical Analysis

All experiments were performed in triplicate. Data were analysed using One-Way Analysis of Variance (ANOVA) to determine the effect of storage duration on sensory and microbiological parameters. Post-hoc comparisons were performed and statistical significance was defined at p<0.001.

4. Results & Discussion

4.1. Microbial Loads on Plastic Packaging Material over Storage Time

The results in Table 1 illustrate the microbiological changes in a product over a 12-month storage period, with results measured in log CFU/g. Overall, the storage time had a statistically significant impact (P<0.05) on the growth of Total Bacteria, Staphylococcus Bacteria, and Yeasts and Molds, aligning with previous findings on traditional butter stability [7],[19]. In contrast, the changes in Coliform counts were not statistically significant (P<0.397), a phenomenon often attributed to the inhibitory nature of the butter matrix on enteric bacteria during extended storage, a similar finding observed by [8].

The Total Bacterial Count (TBC) significantly increased over 12 months (P=0.001), rising from 2.37 ± 0.031 to 2.48 ± 0.031 log CFU/g, reflecting a typical spoilage trajectory for ambient storage, aligning with previous findings by [4]. While *Staphylococcus*, Yeast, and Molds showed significant proliferation from non-detectable initial levels (P<0.001), Coliform counts remained statistically stable (P<0.397), likely due to the inhibitory effect of the butter's acidic environment on Gram-negative enteric bacteria; similar findings were reported by [7].

Table 1: Effect of storage time on the microbiological quality of butter samples packaged in plastic material

Storage time	Total bacteria count (Log CFU/g)	Coliform (Log CFU/g)	Staphylococcus (Log CFU/g)	Yeast and molds (Log CFU/G)
Initial	2.37 ± 0.032^{a}	2.08 ± 0.015^{a}	0.00 ± 0.000^{a}	0.00 ± 0.000^{a}
After 4 month	2.40 ± 0.017^{b}	2.11 ± 0.036^{a}	2.39 ± 0.002^{b}	2.22 ± 0.045^{b}
After 8 month	2.46 ± 0.047^{c}	2.17 ± 0.024^{a}	2.42 ± 0.015^{c}	2.29 ± 0.002^{c}
After 12 month	2.48 ± 0.031^{d}	2.23 ± 0.213^{a}	2.47 ± 0.037^{d}	2.47 ± 0.023^{d}
P value	0.001	0.397	0.001	0.001

Values are expressed as Mean ± Standard Deviation. Means within a column followed by different superscript letters are significantly different (P < 0.05).

4.2.Microbial Loads on Dried Enset pseudo-stem sheaths (*Ensete ventricosum*) Packaging Material over Storage Time

The longitudinal analysis revealed a highly significant impact of storage duration on the microbial profile of butter packaged in dried Enset pseudo-stem sheaths (P<0.001) across all evaluated microbial groups table 2. The data indicates a clear transition from a relatively stable initial state to a state of substantial proliferation by the end of the 12-month period. The Total Bacterial Count (TBC) exhibited a progressive and statistically significant increase, rising from an initial mean of 2.37± 0.032 log CFU/g to 2.48 ± 0.015 log CFU/g after 12 months of ambient storage. Interestingly, Coliform bacteria showed a slight, non-significant decline at the 4-month interval (2.08 ± 0.015 logs CFU/g) relative to the baseline, suggesting a transient inhibitory phase, before rising markedly to reach 2.48 ± 0.022 log CFU/g at the 12-month interval. Notably, both *Staphylococcus* and Yeast and Molds were not detected in the initial fresh samples (0.00 ± 0.000 log CFU/g); however, both groups demonstrated consistent and significant growth over the study period, attaining final loads of 2.44 ± 0.007 log CFU/g and 2.40 ± 0.002 log CFU/g, respectively, after 12 months. Post-hoc analyses confirmed statistically significant differences between all storage time points for each microbial category, underscoring that while Enset packaging is a culturally deep-rooted material, extended storage beyond 8 months under ambient conditions leads to substantial microbial proliferation that may compromise the product's safety and sensory integrity, similar funding was observe by [4].

Table 2: Effect of storage time on the microbiological quality of butter samples packaged in Dried Enset pseudo-stem sheaths (Ensete ventricosum)

Storage time	Total Bacteria (Log CFU/g)	Coliform Bacteria (Log CFU/g)	Staphylococcus (Log CFU/g)	Yeast and molds (Log CFU/g)
Initial	2.37 ± 0.032^{a}	2.08 ± 0.015^{a}	0.00 ± 0.000^{a}	0.00 ± 0.000^{a}
After 4 month	2.38 ± 0.003^{b}	2.04 ± 0.026^{b}	2.30 ± 0.003^{b}	2.08 ± 0.006^{b}
After 8 month	2.43 ± 0.012^{c}	2.13 ± 0.012^{c}	2.41 ± 0.013^{c}	2.17 ± 0.003^{c}
After 12 month	2.48 ± 0.015^{d}	2.48 ± 0.022^{d}	2.44 ± 0.007^{d}	2.40 ± 0.002^{d}
P value	0.001	0.001	0.001	0.001

Values are expressed as Mean ± Standard Deviation. Means within a column followed by different superscript letters are significantly different (P < 0.05).

4.3. Sensory quality of dried Enset pseudo-stem sheaths (*Ensete ventricosum*) Packaged Butter

A one-way ANOVA was conducted to evaluate the impact of storage duration (Initial, 4, 8, and 12 months) on the sensory profile of butter stored in Enset pseudo-stem sheaths. The analysis revealed a highly significant effect of storage time on appearance, aroma, and overall acceptance (p<0.001) for all parameters evaluated table 3. Post-hoc comparisons using the Tukey HSD test indicated that the12-month storage group yielded significantly lower scores across all sensory attributes compared to all other intervals (p<0.001). Notably, the Initial and 4-month samples exhibited statistically similar scores for appearance (P<0.397) and aroma (P< 0.999), suggesting a period of relative organoleptic stability. However, a significant decline in sensory quality was observed by the 8-month mark (P<0.001), characterized by the onset of enzymatic browning and a reduction in fresh aromatic compounds. The highest overall acceptability was maintained within 4-months followed by a drastic decline thereafter indicating that the protective efficacy of the Enset material significantly diminishes after two-thirds of the yearlong storage period, similar to what was observed by [17].

Table 3: Sensory Attributes of Enset Materials as a Function of Storage Duration (N=20)

Sensory attributes	Initial (n=20)	After 4-month (n=20)	After 8-month (n=20)	After 12-month (n=20)	p-value
Appearance	4.96 ± 0.12	4.90± 0.40	4.20 ± 0.09	3.06± 0.17	0.001
Aroma	4.91 ± 0.02	4.90 ± 0.05	4.53 ± 0.10	3.52 ± 0.10	0.001
Overall acceptance	4.99 ± 0.01	5.00 ± 0.00	4.26 ± 0.10	2.52± 0.15	0.001

Note: *Date is represented as mean ± SE. Within rows, all groups differ significantly from the12-month sample (p<0.001)*

4.4. Sensory quality of Plastic Material Packaged Butter

Sensory evaluation results indicated a statistically significant decline across all measured attributes over the 12-month storage period (p<0.001), with initial scores for appearance (4.96± 0.01), aroma (4.91± 0.02), and overall acceptance (4.95 ± 0.01) reflecting high consumer appeal (Table 4. While quality remained stable through the 4-month mark, a precipitous reduction in scores was observed by 8 months, ultimately reaching their lowest values by 12 months for appearance (2.20± 0.19), aroma 3.00± 0.16), and overall acceptance (1.80± 0.13), indicating a reduction in consumer preference over time. This downward trend suggests that the product undergoes substantial quality degradation, likely driven by enzymatic browning, similar research findings were obtained by [19] and the volatilization of aromatic compounds [24], rendering it unfavourable for consumption by the end of the year. According to a marked decrease in overall acceptance, it signals that the product has exceeded its viable sensory shelf-life [2].

Table 4: Sensory Attributes of Plastic Materials as a Function of Storage Duration (N=20)

Sensory attributes	Initial	4months	8 month	12-month	p-value
Appearance	4.96± 0.01	4.80± 0.08	3.60± 0.10	2.20± 0.19	0.001
Aroma	4.91± 0.02	4.40± 0.22	4.00± 0.06	3.00± 0.16	0.001
Overall acceptance	4.95 ± 0.01	4.50 ± 0.08	3.50 ± 0.12	1.80± 0.13	0.001

Note: *All values are expressed as Mean Standard Deviation (SD). Statistical significance was determined at P<0.001*

4.5. Discussion

The significant rise in *Staphylococcus*, Yeast, and Molds across both treatments (P<0.001) suggests that ambient storage overcomes the protective barriers of both materials. However, the fibrous nature of Enset sheaths may offer a different spoilage trajectory compared to the moisture-trapping properties of plastic, which can facilitate a 'micro-greenhouse' effect favourable to bacterial proliferation. A similar idea was reported by [16]. The stability of Coliform counts (P<0.397) further indicates that the increasing acidity of the butter matrix during storage effectively inhibits Gram-negative enteric bacteria regardless of packaging type, aligning with previous findings [4].

Sensory analysis revealed a significant decline in all measured attributes across both packaging types over the 12-month period (P<0.001). Initially, all samples received excellent scores (approx. 4.9 out of 5.0), which remained stable up to 4 months. However, a significant reduction in quality was observed after 8 months in both materials. For Enset packaging, overall acceptance dropped from initially 4.99± 0.01 to 2.52± 0.15 by 12 months. The lowest final scores were for appearance (3.06± 0.17). Quality attributes remained statistically stable through the 4-month interval. However, a "marked decline" was observed by month 8, culminating in critical failure by month 12. At the end of the study, overall acceptance dropped to 1.80± 0.13, falling below the threshold for commercial viability. These findings indicate that while both materials fail to maintain optimal sensory quality past 8 months under ambient storage, the Enset-based material seemed to provide marginally better protection against severe aesthetic degradation compared to plastic in the final period of storage.

5. Conclusion

The present study demonstrates that both microbiological and sensory stability are significantly compromised over a 12 month storage period, defining a clear shelf-life threshold for the product. Initially, the material exhibited high consumer appeal across all attributes (appearance, aroma, and overall acceptance (approximately 4.90/5.00) and a controlled bacterial load (approximately 2.37 log CFU/g). However, a critical inflection point was observed between 4 and 8 months of storage.

The sensory degradation was most pronounced in appearance and overall acceptance, falling to 2.20± 0.19 and 1.80± 0.13, respectively, by month 12. This decline is likely driven by enzymatic browning and the volatilization of aromatic compounds [20]. These sensory changes were further exacerbated by a significant escalation in bacterial load, which reached its maximum at approximately 12 months, approximately 2.48 logCFU/g. The correlation between increased microbial activity and diminished sensory quality suggests that metabolic by-products may contribute to off-flavors and visual deterioration [24].

Ultimately, while the product remains stable for the first trimester, it becomes commercially unfavorable and microbiologically less stable beyond 8 months. To ensure optimal quality and consumer safety, it is scientifically recommended to cap the sensory shelf-life at six months. Future work should investigate active packaging solutions, such as oxygen scavengers or antimicrobial coatings, to extend this stability window, which was reported by [23].

6. Recommendations

Based on the findings of this12-month longitudinal study, the following recommendations are made to improve the safety and quality of cow's milk butter during long-term storage time in Ethiopia:

- **Optimal Storage Duration:** Producers and consumers should be advised that while butter remains highly acceptable for up to 4 months, its quality and safety significantly degrade after 8 months at ambient temperatures. It is recommended to prioritize consumption or secondary processing (such as conversion to traditional spiced ghee/ *niter kibbeh*) before this threshold.
- **Hygienic Handling Protocols:** Due to the significant rise in *Staphylococcus* and Coliform counts over time (P<0.001), standardized hand washing and equipment sterilization protocols must be strictly followed during the initial manual mixing and washing stages to minimize initial inoculation.

- **Enset Packaging Enhancement:** Since dried Enset pseudo-stem sheath materials (*Ensete ventricosum*) demonstrated slightly better sensory preservation than plastic at the 12-month mark (2.52± 0.15 vs. 1.80± 0.13), further research should investigate treating these traditional sheaths with natural antimicrobial agents (e.g., essential oils or smoke) to further inhibit microbial proliferation.
- **Cold Chain Integration:** Where feasible, local government and dairy cooperatives should invest in low-cost cooling technologies. Ambient storage, even with traditional packaging, allows for lipolytic and proteolytic activities that compromise consumer safety.
- **Standardization of Bio-Packaging:** Regulatory bodies should develop quality standards for traditional bio-based packaging materials to ensure they are properly dried and free of contaminants before being used for long-term dairy storage.

7. Acknowledgments

The author would like to express his sincere gratitude to the South Ethiopia Agricultural Research Institute and Areka Agricultural Research Centre for their financial and material support. He also extends his thanks to Wolaita Sodo Regional Laboratory for providing the facilities and technical support necessary for the microbiological analyses.

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