



Microbiological quality of zabady (yogurt) produced by small-scale industries and households in Khartoum and Gezira state, Sudan

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ABSTRACT

Background: Zabady (yogurt) is widely produced in Sudan by households and small-scale dairies, often without standardized hygiene or temperature control. Such informal production systems increase the risk of microbial contamination and compromise product safety.

Objectives: This study aimed to assess the microbiological quality and safety of traditionally produced Zabady and the raw milk used in its preparation across different seasons and locations in Khartoum and Gezira States, Sudan.

Methods: An analytical study was conducted during the summer and winter of 2018–2019. Samples of Zabady and raw cow's milk were collected from four localities. Microbiological analyses included total viable counts, lactic acid bacteria, coliforms, *E. coli*, *Staphylococcus aureus*, yeasts, moulds, and *Salmonella* spp. Yeasts and lactic acid bacteria were identified using classical biochemical and morphological methods. Sensory evaluation was performed using a semi-trained panel.

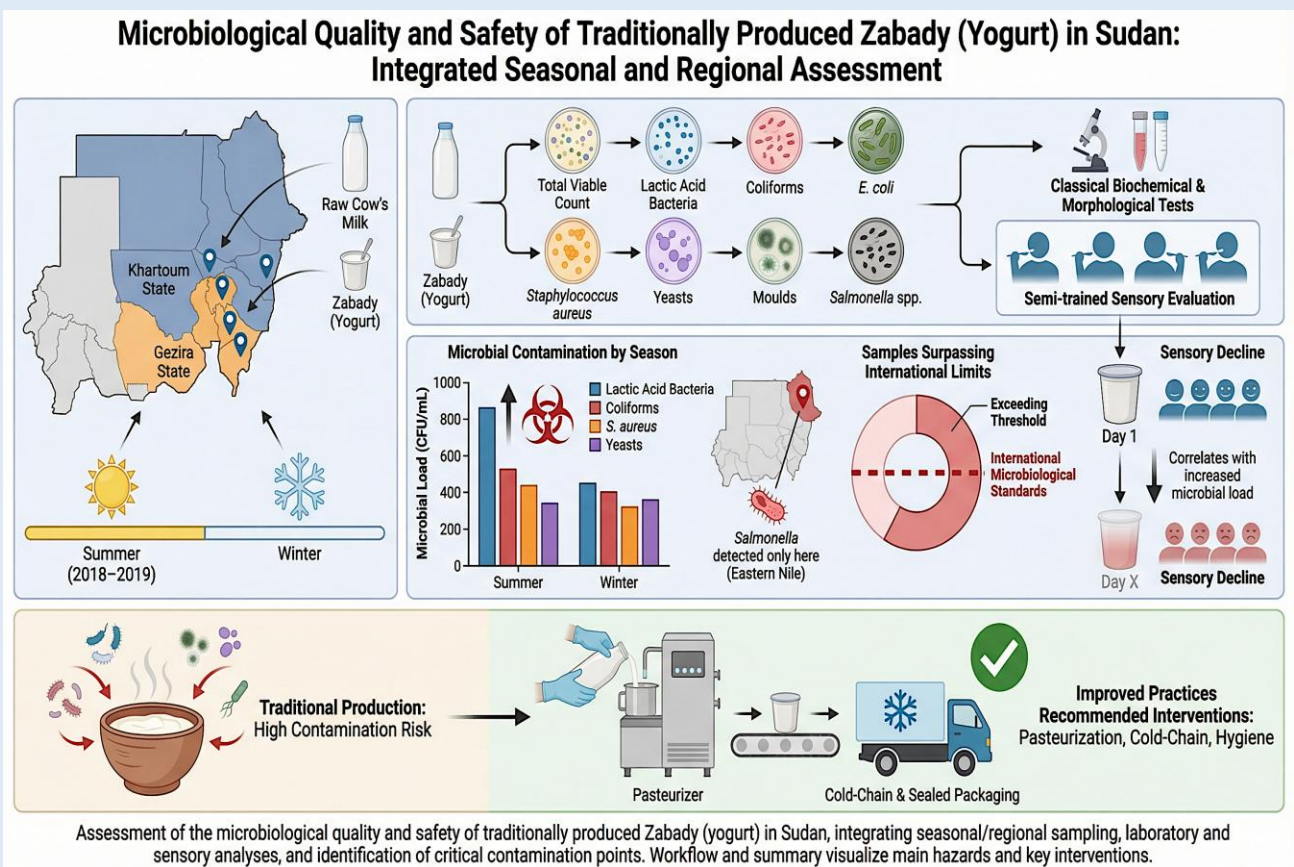
Results: Microbial contamination varied significantly by season and location. Summer samples showed markedly higher microbial loads, including elevated counts of coliforms, *S. aureus*, and spoilage yeasts. *Salmonella* spp. was detected only

in samples from Eastern Nile. Many samples exceeded international microbiological limits. Sensory quality declined significantly during storage and correlated with higher microbial loads.

Conclusions: Traditionally produced Zabady in Sudan presents substantial microbiological hazards, particularly during summer, due to inadequate hygiene and lack of temperature control. Improved manufacturing practices, mandatory pasteurization, and strengthened cold-chain systems are essential to ensure product safety.

Novelty: This study provides the first integrated seasonal and regional assessment of the microbiological quality of traditionally produced Zabady in Sudan. It uniquely combines pathogen detection, spoilage yeast profiling, and sensory evaluation, identifying contamination hotspots and demonstrating clear links between microbial load and sensory deterioration. The findings offer practical, evidence-based guidance for improving safety in informal dairy production systems.

Keywords: Zabady; yogurt; Sudan; microbiological quality; lactic acid bacteria; spoilage yeasts; food safety.



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INTRODUCTION

Sudan is one of Africa's largest countries in terms of land area and livestock population, ranking second in Africa for total livestock numbers. Livestock plays a critical role in the national economy, providing meat, milk, and other animal products. Cows are the primary source of milk, followed by goats and sheep, while camels also contribute significantly in some regions. According to the Ministry of Animal Resources, Fisheries and Range [1], Sudan's livestock population includes approximately 29.6 million cattle, 39.3 million sheep, 30.6 million goats, and 40.7 million camels.

Milk is one of the most complete natural foods, supplying high-quality proteins, essential amino acids, carbohydrates, vitamins, minerals, and bioactive compounds. However, it is highly perishable and prone to contamination if not handled under strict hygienic conditions. To ensure safety, milk must come from healthy, well-nourished animals and be stored at $\leq 4^{\circ}\text{C}$ during collection, transportation, and distribution [2].

Fermented milk products, such as yogurt (locally known as Zabady), have been consumed for thousands of years as a means of preserving milk and enhancing its digestibility and nutritional value. Zabady production typically involves the fermentation of milk by starter cultures containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, producing a tangy flavor, thick texture, and extended shelf life. Modern Zabady varieties include plain, flavored, drinkable, low-fat, and probiotic-enriched products [3,4], valued for their sensory qualities and health benefits [5, 6].

Globally, yogurt consumption is associated with improved gut health, enhanced lactose digestion, immune modulation, and potential protection against gastrointestinal disorders and other chronic diseases [7-9]. However, the safety and quality of yogurt depend heavily on hygienic handling, pasteurization of milk, and

sanitary conditions during fermentation, packaging, and storage.

In Sudan, commercial Zabady is often produced by small-scale dairies and households, sometimes using reconstituted powdered milk, without consistent application of good manufacturing practices (GMP). This creates potential risks of microbial contamination, including pathogenic bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., and *Listeria monocytogenes*. Such contamination can lead to foodborne illnesses and pose a serious public health concern [10].

This study provides novel baseline data on the microbiological quality of traditionally produced Zabady and its raw materials across seasonal and regional contexts in Sudan. Unlike previous studies that focused on isolated locations or single seasons, this work integrates seasonal variation, pathogen detection, spoilage microbiota profiling, and sensory quality correlations. The findings highlight region-specific contamination hotspots and demonstrate the link between microbial load and sensory deterioration, offering practical insights for improving safety and quality in informal dairy production systems

MATERIALS AND METHODS

Study Design and Sample Collection: An observational, analytical study was conducted during the summer and winter of 2018–2019. Zabady and raw cow's milk samples were aseptically collected from four localities in Khartoum State (Bahri, Omdurman, Khartoum, Eastern Nile) and Gezira State. Samples were transported in sterile containers within insulated iceboxes ($0-4^{\circ}\text{C}$) to the laboratory at the Faculty of Agriculture, University of Khartoum, for immediate analysis.

Microbiological Analysis: Serial decimal dilutions of each sample were prepared in 0.1% peptone water.

Microbiological analyses were performed according to standard methods and ISO protocols:

- **Total Viable Count (TVC):** Pour plate method using Plate Count Agar (PCA), incubated at 37°C for 48h [7].
- **Lactic Acid Bacteria (LAB):** Spread plate method on de Man, Rogosa and Sharpe (MRS) agar, incubated anaerobically at 37°C for 48h [8].
- **Yeasts and Moulds:** Spread plate method on acidified Potato Dextrose Agar (PDA), incubated at 28°C for 72h [9].
- ***Staphylococcus aureus*:** Surface plating on Baird-Parker agar, incubated at 37°C for 36h [10].
- **Coliforms and *E. coli*:** Determined using a 3-tube Most Probable Number (MPN) technique in MacConkey Broth, Brilliant Green Bile Broth, and EC Broth, with confirmation on Eosin Methylene Blue agar [11].
- ***Salmonella* spp.:** Pre-enriched in peptone water, selectively enriched in Selenite Cystine broth, and streaked onto Bismuth Sulphite agar [12].

Identification of Microbial Isolates

Lactic Acid Bacteria: Pure colonies from MRS agar were subjected to Gram staining, catalase test, spore staining, motility test, growth under anaerobic conditions, oxidase test, oxidation/fermentation (O/F) test, gas from glucose, and arginine hydrolysis [13].

Yeasts: Isolates from PDA were purified and identified based on morphological characteristics (cell shape, pseudohyphae, ascospore formation) and biochemical profiles, including carbohydrate fermentation and assimilation patterns, following the methodologies of [14] and [15].

Sensory Evaluation: A panel of 15-20 semi-trained assessors evaluated fresh and stored Zabady samples for color, flavor, texture, and overall acceptability using a 9-

point hedonic scale (1=dislike extremely, 9=like extremely) under controlled conditions, following ISO 22935-2:2009 guidelines [16]. All sensory evaluations were conducted only on microbiologically safe samples. Samples that tested positive for *Salmonella* spp. or exceeded internationally recommended safety limits for pathogenic microorganisms were excluded from sensory analysis to protect panelists. Sensory evaluation was performed under controlled laboratory conditions following ISO 22935-2:2009 guidelines, and panelists provided informed consent.

Statistical Analysis: Data were analyzed using SPSS version 25.0. Differences among means were assessed using one-way ANOVA followed by Duncan's Multiple Range Test at a significance level of $*p* \leq 0.05$.

Yogurt Production Context: Zabady samples were produced using traditional household and small-scale industrial methods, typically involving spontaneous or back-slopping fermentation of raw or reconstituted milk without standardized starter cultures or pasteurization. Variations in fermentation time, temperature, and hygiene practices were expected and reflect real-world informal production conditions.

This study relied on classical microbiological and biochemical identification techniques, which may not provide species-level resolution for bacterial isolates. Additionally, the cross-sectional sampling design limits causal inference regarding contamination sources. Future studies should incorporate molecular identification and longitudinal sampling to improve reproducibility and traceability.

Results and Discussion

Microbiological Quality of Zabady: The microbiological analysis of Zabady samples showed clear seasonal and regional variations (Tables 1 and 2). During winter, total viable counts (TVC) ranged from 16.27×10^4 to 42.00×10^4 CFU/g, while yeast and mould contamination was

absent in Bahri and Khartoum but present in Omdurman and Eastern Nile. In summer, microbial loads increased sharply, with TVC reaching 373.33×10^4 CFU/g in Eastern Nile. These findings are consistent with previous reports from tropical regions, where high ambient temperatures accelerate microbial growth in fermented dairy products [17,18].

Sudan's summer temperatures often exceed 40 °C, and small-scale producers frequently lack adequate refrigeration, creating ideal conditions for microbial proliferation. This highlights the vulnerability of informal

dairy systems to environmental temperature fluctuations.

Recent FFHDJ ecosystem publications emphasize the importance of controlled fermentation conditions and standardized starter cultures in maintaining microbial stability in fermented dairy products. Several studies highlight that uncontrolled temperature fluctuations—common in informal dairy systems—promote rapid increases in total viable counts and spoilage organisms, consistent with the patterns observed in the present study.

Table 1. Microbiological Analysis of Zabady in Winter Season.

Location	TVC ($\times 10^4$ CFU/g)	Yeast & Mould ($\times 10^3$ CFU/g)	LAB ($\times 10^2$ CFU/g)	S. aureus ($\times 10^4$ CFU/g)	Total Coliform (MPN/g)	E. coli (MPN/g)	Salmonella
Bahri	31.67 \pm 1.5 ^a	0.00 \pm 0.0 ^c	3.43 \pm 4.8 ^b	00	4.00 \pm 1.7 ^c	00	–
Omdurman	42.00 \pm 2.0 ^a	63.00 \pm 2.7 ^a	3.23 \pm 0.2 ^b	4.00 \pm 1.0 ^a	9.00 \pm 2.0 ^b	00	–
Khartoum	16.27 \pm 20.6 ^a	0.00 \pm 0.0 ^c	4.47 \pm 0.4 ^a	00	00	0.006 \pm 0.0	–
Eastern Nile	42.00 \pm 20.0 ^a	52.67 \pm 3.1 ^b	3.40 \pm 0.4 ^b	4.00 \pm 1.0 ^a	21.00 \pm 1.0 ^a	2.00 \pm 1.73 ^a	+

Table 2. Microbiological Analysis of Zabady in Summer Season.

Location	TVC ($\times 10^4$ CFU/g)	Yeast & Mould ($\times 10^3$ CFU/g)	LAB ($\times 10^3$ CFU/g)	S. aureus ($\times 10^2$ CFU/g)	Total Coliform (MPN/g)	E. coli (MPN/g)	Salmonella
Bahri	49.67 \pm 4.5 ^{bc}	0.00 \pm 0.0 ^c	3.57 \pm 0.2 ^b	0.00 \pm 0.0 ^b	7.33 \pm 1.5 ^c	0.00 \pm 0.0 ^b	–
Omdurman	76.00 \pm 2.0 ^b	5.20 \pm 0.0 ^a	4.53 \pm 0.3 ^b	5.00 \pm 1.0 ^a	13.00 \pm 2.0 ^b	0.006 \pm 0.0	–
Khartoum	8.53 \pm 0.3 ^c	0.00 \pm 0.0 ^c	5.47 \pm 0.7 ^b	0.00 \pm 0.0 ^b	0.00 \pm 0.0 ^d	0.006 \pm 0.0	–
Eastern Nile	373.33 \pm 30.6 ^a	4.27 \pm 0.3 ^b	52.00 \pm 2.0 ^a	7.00 \pm 1.0 ^a	28.00 \pm 1.0 ^a	5.33 \pm 2.01 ^a	+

Pathogenic Contamination and Public Health

Implications: Pathogenic microorganisms—including *Staphylococcus aureus*, coliforms, *E. coli*, and *Salmonella* spp.—were detected in several samples. *S. aureus* counts exceeded 10^2 CFU/g in both seasons, raising concerns about enterotoxin production, which can occur even when bacterial cells are later destroyed [19]. *Salmonella* spp. was consistently detected only in Eastern Nile samples, suggesting a localized contamination source.

The presence of fecal indicators such as coliforms and *E. coli* reflects poor hygiene during milking,

contaminated water, and inadequate equipment sanitation. Similar contamination patterns have been reported in locally produced dairy products in Sudan [17].

Spoilage Microbiota and Fermentation Dynamics:

Lactic acid bacteria (LAB) counts varied across locations and seasons, with the highest levels recorded in Eastern Nile during summer (52.00×10^5 CFU/g). LAB are essential for fermentation; however, their dominance was overshadowed in summer by spoilage yeasts and moulds. Yeast counts increased markedly, with *Debaryomyces*

hansenii and *Saccharomyces rouxii* identified as the predominant spoilage species.

This microbial shift reflects the natural succession in fermented dairy products: LAB initiate acidification, but high temperatures and extended storage favor acid-tolerant yeasts. These yeasts cause gas formation, off-flavors, and texture defects, consistent with spoilage patterns reported in tropical dairy systems [20].

Table 3. Microbiological Analysis of Milk in Winter Season.

Location	TVC ($\times 10^3$ CFU/g)	Yeast & Mould	LAB	<i>S. aureus</i> ($\times 10^2$ CFU/g)	Total Coliform (MPN/g)	<i>E. coli</i> (MPN/g)	Salmonella
Bahri	37.67 \pm 2.5 ^a	0.00 \pm 0.0	0.00 \pm 0.0	0.00 \pm 0.0 ^b	14.67 \pm 1.5 ^b	2.00 \pm 1.7 ^a	–
Omdurman	3.23 \pm 0.3 ^b	0.00 \pm 0.0	0.00 \pm 0.0	0.00 \pm 0.0 ^b	6.33 \pm 3.1 ^c	0.00 \pm 0.0	–
Khartoum	32.00 \pm 2.0 ^a	0.00 \pm 0.0	0.00 \pm 0.0	4.00 \pm 1.0 ^a	12.00 \pm 1.0 ^b	0.00 \pm 0.0	–
Eastern Nile	36.33 \pm 3.2 ^a	0.00 \pm 0.0	0.00 \pm 0.0	2.67 \pm 2.5 ^{ab}	20.00 \pm 1.0 ^a	0.00 \pm 0.0	–

Microbiological Quality of Raw Milk: Raw milk samples also exhibited higher contamination during summer (Table 4). TVC reached 82.67×10^3 CFU/g in Bahri, and yeasts/moulds—absent in winter—were detected in summer. *S. aureus*, coliforms, and *E. coli* were more prevalent and at higher levels in summer, mirroring the Zabady results. *Salmonella* spp. was detected only in

Table 4. Microbiological Analysis of Milk in Summer Season.

Location	TVC ($\times 10^3$ CFU/g)	Yeast & Mould ($\times 10^2$ CFU/g)	LAB	<i>S. aureus</i> ($\times 10^2$ CFU/g)	Total Coliform (MPN/g)	<i>E. coli</i> (MPN/g)	Salmonella
Bahri	82.67 \pm 2.5 ^a	1.00 \pm 1.7 ^a	0.00 \pm 0.0	4.00 \pm 1.0 ^{bc}	20.00 \pm 1.0 ^c	5.33 \pm 2.1 ^a	Not detected
Omdurman	5.40 \pm 0.4 ^d	0.00 \pm 0.0	0.00 \pm 0.0	0.00 \pm 0.0 ^c	12.00 \pm 1.0 ^d	0.00 \pm 0.0 ^b	Not detected
Khartoum	47.67 \pm 2.5 ^c	0.00 \pm 0.0	0.00 \pm 0.0	43.33 \pm 3.1 ^a	36.00 \pm 2.7 ^b	0.00 \pm 0.0 ^b	Not detected
Eastern Nile	74.67 \pm 3.1 ^b	2.33 \pm 2.1 ^a	0.00 \pm 0.0	7.00 \pm 1.0 ^b	37.00 \pm 2.1 ^a	9.00 \pm 2.0 ^a	Detected

Identification of Microbial Isolates: Phenotypic characterization of LAB isolates suggested the presence of *Pediococcus*, *Streptococcus*, and *Lactobacillus* spp., although species-level identification was limited by the constraints of classical biochemical methods. Yeast identification was more definitive, with *D. hansenii* and *S.*

FFHDJ ecosystem articles on fermented foods report that *Debaryomyces* and *Saccharomyces* species frequently dominate spoilage communities in traditional dairy systems lacking refrigeration. These findings align with our identification of *D. hansenii* and *S. rouxii* as the predominant spoilage yeasts, reinforcing the role of high ambient temperatures and extended storage in shaping yeast succession.

Eastern Nile milk, reinforcing the likelihood of a localized contamination source.

These findings confirm that the microbiological quality of raw milk directly influences Zabady safety, especially when pasteurization is not practiced. Similar observations have been reported in studies on raw milk safety in Africa [19].

rouxii dominating across samples. The limitations of phenotypic identification highlight the need for molecular techniques such as 16S rRNA and ITS sequencing to improve accuracy and traceability in future studies [21].

Table 5. Identification of lactic acid bacteria isolates in yogurt in winter season.

Sample	Gram staining	Shape	Endospore Stain	Motility test	Anaerobic growth	Catalase test	Oxidase test	O/F test	Glucose test	Tetrad formation	Genus
Y1	Detected	Sphere/ Rod	Not detected	Not detected	Detected	Not detected	Not detected	F	Detected	+/-	<i>Pediococcus</i> / <i>Streptococcus</i> / <i>Lactobacillus</i>
Y2	Detected	Sphere/ Rod	Not detected	Not detected	Detected	Not detected	Not detected	F	Detected	+/-	<i>Pediococcus</i> / <i>Streptococcus</i> / <i>Lactobacillus</i>
Y3	Detected	Sphere/ Rod	Not detected	Not detected	Detected	Not detected	Not detected	F	Detected	Not detected	<i>Streptococcus</i> / <i>Lactobacillus</i>
Y4	Detected	Sphere/ Rod	Not detected	Not detected	Detected	Not detected	Not detected	F	Detected	Not detected	<i>Streptococcus</i> / <i>Lactobacillus</i>

* + and - mean positive and not negative, respectively.

** F means.

Yeasts (Table 6): Summer isolates were identified to the species level. *Debaryomyces hansenii* was the dominant spoilage yeast (55–99% in various samples), followed

by *Saccharomyces rouxii* (up to 72%). *Kluyveromyces lactis* was also identified in one sample (New Halfa, 16%).

Table 6. Identification of lactic acid bacteria isolates in yogurt in winter season.

Sample	Gram staining	Shape	Endospore staining	Motility test	Growth Anaerobes	Catalase test	Oxidase test	O/F test	Glucose test	Tetrad formation	Genus
Y1	+	Sphere/ Rod	Not detected	Not detected	Detected	Not detected	Not detected	F	Detected	+/-	<i>Pediococcus</i> / <i>Streptococcus</i> / <i>Lactobacillus</i>
Y2	+	Sphere/ Rod	Not detected	Not detected	Detected	Not detected	Not detected	F	Detected	+/-	<i>Pediococcus</i> / <i>Streptococcus</i> / <i>Lactobacillus</i>
Y3	+	Sphere/ Rod	Not detected	Not detected	Detected	Not detected	Not detected	F	Detected	Not detected	<i>Streptococcus</i> / <i>Lactobacillus</i>
Y4	+	Sphere/ Rod	Not detected	Not detected	Detected	Not detected	Not detected	F	Detected	Not detected	<i>Streptococcus</i> / <i>Lactobacillus</i>

* + and - mean positive and not negative, respectively.

** F means .

Sensory Quality and Its Relationship to Microbial Load:

Sensory evaluation showed a significant decline in color, flavor, texture, and overall acceptability over a 15-day storage period. Samples with higher initial microbial loads deteriorated more rapidly, particularly those with

elevated yeast and mould counts. The most pronounced decline occurred between Day 7 and Day 15, consistent with spoilage progression in fermented dairy products stored under suboptimal conditions [22]. The strong correlation between microbial load and sensory

deterioration has economic implications for small-scale producers, as reduced shelf-life limits marketability and consumer acceptance.

Recent FFHDJ ecosystem research on fermented dairy products demonstrates a strong association

between elevated yeast counts and accelerated sensory deterioration, particularly in flavor and texture attributes. This supports our observation that samples with higher initial yeast loads exhibited the most rapid decline in sensory acceptability during storage.

Sensory Evaluation of Zabady Samples Over Storage Time

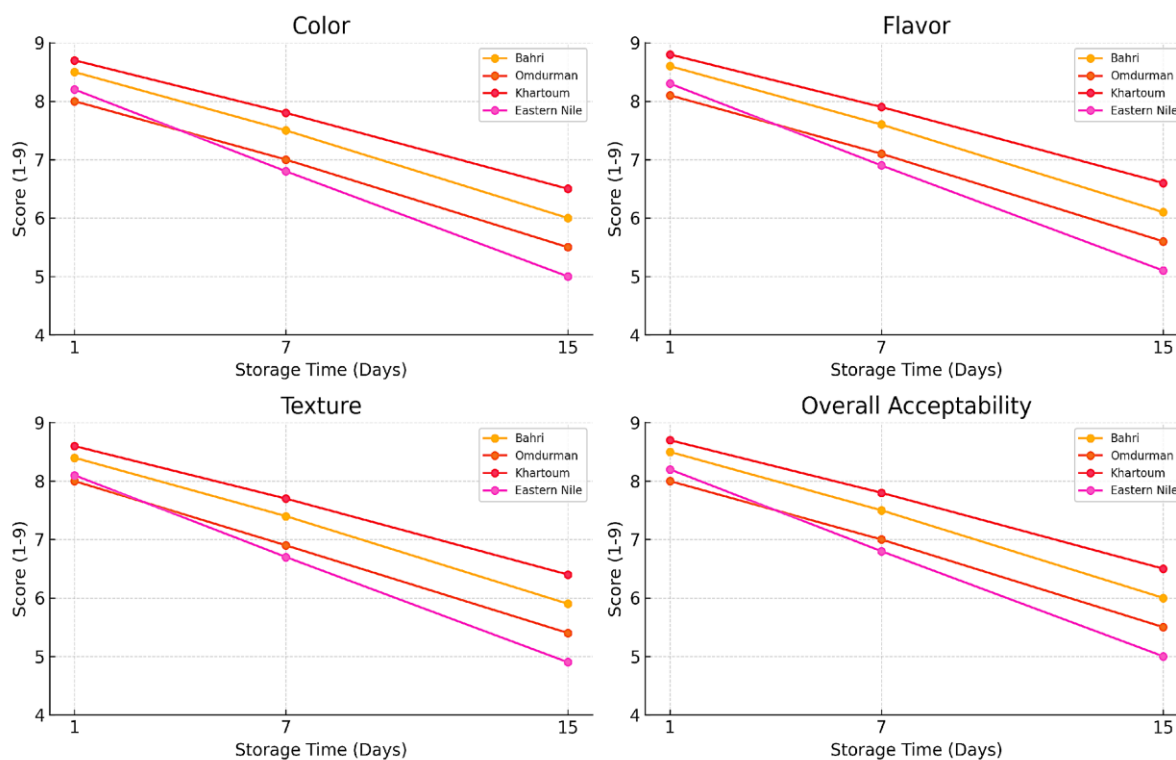


Figure 1. Results of sensory analysis for Zabady samples. A semi-trained panel (n=15–20) assessed color, appearance, flavor, texture, and overall acceptability using a 9-point hedonic scale (1 = dislike extremely, 9 = like extremely). Scores significantly decreased with storage duration, with the most pronounced drop occurring between Day 7 and Day 15.

CONCLUSION

This study demonstrates that traditionally produced Zabady in Sudan is frequently contaminated with pathogenic and spoilage microorganisms, particularly during the summer season, posing significant public health risks. The findings highlight that microbiological safety is a prerequisite for maintaining the functional and nutraceutical benefits associated with fermented dairy products. Seasonal temperature variations and inadequate hygienic practices were identified as major determinants of microbial contamination and sensory deterioration.

Ensuring the safety and functional quality of Zabady requires mandatory milk pasteurization, implementation of Good Manufacturing Practices, cold-chain infrastructure, and routine microbiological monitoring. These interventions are essential not only to protect consumers but also to preserve the probiotic and bioactive potential of traditional fermented dairy products in Sudan

List of Abbreviations: TVC, Total viable count; LAB, Lactic acid bacteria; CFU, Colony forming units; MPN, Most probable number; GMP, Good manufacturing practices

Competing Interests: The author declares no conflict of interest.

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Authors' Contributions: AES designed the study, conducted data analysis, and drafted the manuscript. HO and MA performed laboratory experiments and data collection. SMI contributed to manuscript editing and interpretation of results. All authors approved the final manuscript.

Data Availability: The datasets generated during the current study are available from the corresponding author upon reasonable request.

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