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Effect of processing treatments on sensory quality and short-term microbial quality of flaxseed milk as a value-added plant-based beverage

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Abstract

The present study aimed to develop and evaluate flaxseed milk as a value-added plant-based beverage with improved sensory quality and short-term microbiological quality. Brown flaxseed (*Linum usitatissimum* L.) was subjected to five processing treatments-soaking, roasting, fermentation, germination and boiling to reduce undesirable mucilage-related characteristics and improve product acceptability. Flaxseed milk prepared from each treatment was evaluated for sensory attributes, including colour, flavour, consistency, taste and overall acceptability, using a 9-point hedonic scale administered by a 10-member semi-trained sensory panel. Microbiological quality was assessed by determining the total viable count (TVC) and enumerating coliforms during storage at different temperatures. Boiling produced the most acceptable product among the processing treatments evaluated. Microbial analysis demonstrated that TVC increased with increasing storage temperature and duration. Refrigeration at 4°C was associated with lower microbial counts, with TVC ranging from 48 CFU mL^{-1} to 4.78 × 103 CFU mL^{-1} over 96 h of storage. In contrast, storage at room temperature resulted in rapid microbial growth, with the TVC reaching 8.71 × 107 CFU mL^{-1} within 48 h. Coliforms were not detected in refrigerated samples but were detected and increased rapidly under ambient storage conditions. Similar microbial trends were observed in samples stored in polyethylene terephthalate (PET) bottles. The storage experiment was conducted using a single production batch; duplicate plate counts represented technical replicates only. Therefore, the microbiological observations are descriptive and do not represent biological replication. Product safety and commercial shelf life were not established. Overall, boiling improved the sensory quality of flaxseed milk, possibly by reducing undesirable mucilage-associated characteristics. Refrigeration at 4°C was associated with lower TVC levels and no detectable coliforms for up to 96 h under the experimental conditions. However, because the storage assessment was limited to TVC and coliform enumeration over 96 h, the findings indicate short-term microbiological quality rather than comprehensive shelf-life stability. The study highlights the potential of flaxseed as a value-added raw material for the development of functional plant-based beverages.

Key words: Flaxseed, dairy analogue, lactose-free beverage, plant-based beverage, sensory quality, microbiological quality.

Introduction

Plant-based milk substitutes (PBMS) are developed to mimic bovine milk and are mostly aqueous extracts of plant materials. These systems are colloidal in nature because they comprise an aqueous continuous phase and a dispersed phase comprising particles such as starch granules, protein complexes, fat globules, and other elements present in the plant matrix (Briviba *et al.,* 2016; Bocker and Silva, 2022). Plant-based dairy substitutes (PBDS) are non-dairy products made from plant ingredients as an alternative to traditional dairy products such as milk, cheese, and yoghurt (Khaliq *et al.,* 2023). Plant-based milk products have received considerable attention because of the health, ethical and environmental concerns associated with animal-derived dairy products. Demand for clean-label, minimally processed products that do not involve animal cruelty has increased the popularity of plant-based beverages. While plant-based beverages are inherently free of lactose, their nutritional adequacy and digestibility are influenced by a range of factors, including the composition of raw materials, processing methods, fortification strategies, allergen control measures, and the overall product formulation (Pandey and Poonia, 2020). Moreover, lifestyle choices such as veganism and vegetarianism have contributed to the growing consumption of plant-derived dairy alternatives. Due to the many benefits offered by these products, they have been accepted as potential alternatives to dairy products. Plants such as soybeans, almonds, coconuts, cashews, peanuts, among others, are used in the manufacture of PBMS. Among these plants, flaxseed (*Linum usitatissimum* L.) has gained prominence as a potential raw material for producing nutritious plant-based milk. The flaxseed is called "a nutritional powerhouse" because of its nutritional richness and many benefits. This seed contains substantial amounts of biologically active compounds, including fatty acids, proteins, fibre, lignans and trace elements, and is considered valuable for food, nutraceutical, cosmetic and biomaterial applications. It is recognized for its dense nutritional profile, comprising approximately 28-30% protein and 35% dietary fiber by weight, alongside a lipid fraction in which the omega-3 fatty acid alpha-linolenic acid (ALA) predominates, accounting for roughly 45% of its constituent fatty acids (Pramanik *et al.,* 2023). Flaxseed is often grown as an oilseed crop in India. It is widely available and well adapted to the agroclimatic conditions of these regions, including a variety of soil types and climates (Singh *et al.,* 2018). Brown flaxseed was chosen because it is commercially available in the study area and is widely grown as an oilseed crop in India. The growing demand for plant-based beverages presents an opportunity for diversification and value

addition of underutilized crops such as flaxseed. Development of flaxseed milk not only provides a nutritious dairy alternative but also promotes post-harvest utilization and commercialization of flaxseed beyond conventional oil extraction. Flaxseed-based milk may offer nutritional benefits because it contains macronutrients and micronutrients, including omega-3 fatty acids, lignans and soluble dietary fibre. Plant-based foods are intended to meet the needs of consumers seeking healthier products (Meng *et al., 2023).* However, the presence of mucilage, a soluble polysaccharide that imparts high viscosity and a characteristic slimy mouthfeel, is one of the major challenges in the development of flaxseed-based beverages, thereby limiting consumer acceptance (Fedeniuk and Biliaderis, 1994; Cui *et al.,* 1994). Flaxseed mucilage is primarily extracted through water extraction of whole seeds, seed meal, or hulls, and wet processing procedures are extensively employed to demucilify flaxseed (Puligundla and Lim, 2022).

Flaxseed milk, much like other plant-based emulsions, may undergo physical changes during storage, largely because of interactions within the protein-gum network that surrounds the oil droplets. Previous studies have identified temperature-dependent particle aggregation and subsequent sedimentation as factors capable of influencing emulsion stability and behaviour (Meng *et al., 2023).* Several extraction methodologies have been employed to isolate protein from flaxseed, including dehulling prior to extraction, defatting, alkaline extraction, enzymatic hydrolysis, and membrane-based separation techniques (Ye *et al*., 2022). Various processing interventions including soaking, thermal treatment, fermentation and germination have been reported to modify the physicochemical characteristics of plant-based beverages and improve their sensory quality and stability (Mäkinen *et al.,* 2016; Jeske *et al.*, 2018; Aydar *et al.,* 2020). Therefore, optimizing processing techniques is essential to improve sensory quality and product acceptability. In this context, the present study was undertaken to develop and evaluate the effect of different processing treatments on the sensory quality of flaxseed milk and to assess its short-term microbial quality during storage. The findings may contribute to the development of value-added flaxseed-based beverages, thereby supporting crop diversification, enhanced utilization and market expansion of flaxseed as an important agro-horticultural commodity.

Although various plant-based milk substitutes have been produced, research on flaxseed milk is still restricted. Previous studies on flaxseed milk have mainly focused on its physicochemical properties and storage stability. Meng and co-workers (2023) investigated the influence of storage temperature on the physical and oxidative stability of flaxseed milk over time. However, limited evidence is available regarding the impact of various pre-processing procedures on sensory quality and short-term microbial viability. As a result, the current study sought to assess the impact of various pre-processing methods on the sensory acceptability of flaxseed milk, as well as to conduct a preliminary assessment of its short-term microbiological quality.

Materials and methods

Selection of raw material: The study was conducted in the Department of Food and Nutrition, College of Community Science, Acharya Narendra Deva University of Agriculture and Technology (ANDUAT), Kumarganj, Ayodhya. Brown flaxseed (*Linum usitatissimum* L.) cultivar Garima, harvested in 2025 and widely used in Uttar Pradesh, was used to prepare flaxseed milk and was purchased from the local market of Kumarganj, Ayodhya. The seed moisture content was 5.4% (wet basis). The visible foreign matter, broken seeds, and other contaminants were removed manually. To ensure uniformity and reduce contamination, distilled water was utilised in all processing steps, including soaking, rinsing, extraction, and beverage preparation. The brown variety was selected due to its wide availability, affordability, nutritional richness and suitability for product development. Flaxseed is widely recognised as an oilseed rich in lipids, protein, dietary fibre, and lignans, which have sparked interest in food applications. Therefore, the utilization of flaxseed for development of flaxseed milk may provide an opportunity for value addition and enhance utilization of this underutilized crop while promoting diversification of plant-based beverages.

Processing treatments for flaxseed milk preparation: Prior to milk extraction, brown flaxseeds were subjected to one of five pretreatment methods—soaking (T1), boiling (T2), roasting (T3), spontaneous fermentation (T4), or germination (T5) — with milk made from untreated seeds serving as the control (T0). A standard seed quantity of 50 g was employed for every experimental batch, ensuring uniformity across all treatment groups. After the seeds had been pre-treated, milk extraction began by blending them with 250 mL of distilled water in a blender, keeping a constant seed-to-water ratio of 1:5 (w/v). The grinding procedure was carried out with a household mixer. The slurry was homogenized before being strained through a 250 µm pore diameter muslin cloth with two layers. The filtration procedure was repeated twice to remove solid fragments and yield the final milk product. Given the lack of preservative chemicals or heat-based stabilization processes such as pasteurisation, the milk was deemed highly perishable and was kept refrigerated before consumption.

Control (T_0): Cleaned flaxseeds were ground with water (1:5, w/v) using a domestic mixer grinder (Sumeet Kitchen Jet, 750 W, 220–240 V, 50 Hz, 18,000–20,000 rpm) and filtered through a double-folded muslin cloth to obtain flaxseed milk.

Soaking (T_1): Seeds were soaked in three times their volume of water for 12 h at room temperature, rinsed to remove released mucilage, and processed as described above.

Boiling (T_2): Flaxseed milk was prepared by boiling 50 g of flaxseed in distilled water at a seed-to-water ratio of 1:10 (w/v) for 10 minutes. Following boiling, the seeds were thoroughly rinsed with distilled water to eliminate the loosened mucilage layer. The rinsed seeds were then drained with the help of a sieve and they were ground with 250 mL of distilled water at a ratio of 1:5 (w/v), and the resulting slurry was filtered to yield flaxseed milk.

Roasting (T_3): Flaxseeds were dry roasted for 3 min at 150°C, followed by soaking and milk extraction as described for T1.

Fermentation (T_4): Flaxseeds were soaked and mixed with water (1:5, w/v) to create flaxseed milk. Fermentation took place naturally at 22 ± 2°C for 48 hours under aseptic laboratory conditions, without the intervention of starting cultures. Fermentation was considered complete when the pH fell below 4.6, resulting in the development of a distinct sour aroma and gas bubbles. Fermentation ended after 48 hours, with a final pH of 4.32 ± 0.08 (mean ± SD, n = 3), a distinct sour scent, and gas bubbles. Fermentation was carried out individually in three batches. As a result, the fermentation was viewed as a spontaneous laboratory fermentation rather than a standardized fermentation procedure. The fermented milk was then homogenized and filtered through muslin cloth to provide the finished product.

Germination (T_5): Soaked flaxseeds were sanitised before

germination by immersing them in 0.75% acetic acid (household vinegar) and then washing them with sterile distilled water. Soaked flaxseeds were germinated under dark conditions at 22 ± 2°C for 72 h. The seeds were spread on a tray, and moisture was maintained throughout the germination period by periodic spraying with water. The germinated seeds were subsequently processed into milk. All milk samples were prepared using a standardized seed-to-water ratio (1:5, w/v) and filtered through a double-folded muslin cloth (250 µm). The developed flaxseed milk was considered a fresh, non-pasteurized beverage and therefore required refrigerated storage. A schematic representation of the processing treatments is presented in Figure 1.

Acceptability assessment: The developed flaxseed milk analogue was analyzed for its preliminary sensory acceptability using a 9-point hedonic scale (Srilakshmi, 2024) by 10 semi-trained panel members from the Department of Food and Nutrition, College of Community Science, ANDUAT, Ayodhya. The same ten panelists assessed all six treatments (T0-T5) in each of three separately manufactured batches. Thus, sensory analysis was based on repeated observations from n = 10 panelists distributed across three distinct batches. The milk was evaluated for the following attributes: colour, flavour, consistency, taste and overall acceptability ranging from 1 (dislike extremely) to 9 (like extremely). Each sample contained approximately 30 mL and was delivered in identical glasses. The samples were coded as T0, T1, T2, T3, T4, and T5 and delivered in random order under daylight conditions in the Food and Nutrition laboratory. Sensory evaluation was performed at room temperature and under laboratory conditions. Three independent batches of flaxseed milk were prepared for each treatment and sensory scores were expressed as mean ± standard deviation. Plain drinking water was served between the consumption of two consecutive samples to rinse their palates. The panelists were kept unaware of the processing treatment applied. Prior to participation, all subjects willingly provided informed consent. The sensory evaluation required no invasive procedures and provided no risk to the subjects. The study was exempt from the formal Institutional Ethics Committee approval because it involves voluntary sensory evaluation of food products without the gathering of personal or clinical data. A one-way ANOVA was used to examine sensory scores, with treatment acting as the within-subject factor because all treatments were assessed by the same panelists. For pairwise comparisons, Tukey's honestly significant difference (HSD) test ($P < 0.05$) was employed.

Microbial analysis: The microbial quality of flaxseed milk was determined using the standard serial dilution and pour plate method on Plate Count Agar (PCA), as described by Harrigan (1998). Coliforms were presumptively identified through a two-step procedure involving lactose broth enrichment and selective plating on MacConkey agar, as described in standard microbiological methods (Cappuccino and Sherman, 2014). Serially diluted samples were aseptically plated. Plate count agar plates for TVC were incubated at 37°C for 48 hours, whereas MacConkey agar plates for coliform enumeration were incubated at 37°C for 24 hours. Freshly prepared samples were aseptically filled into sterilized PET bottles and stored at 4°C, 14°C, and room temperature. Microbial analyses were performed periodically during storage up to 96 h, and results were expressed as colony-forming units per millilitre (CFU mL^{-1}). The study was conducted to evaluate the short-term microbial quality of flaxseed milk under different storage conditions. The storage experiment was carried out on one separately prepared manufacturing batch, with each microbiological determination performed in duplicate plate counts. Duplicate plates represent solely technical replication and were averaged for descriptive purposes. There was no independent biological replication, hence no inferential statistical comparisons between storage temperatures were made. For each sample, plate counts were conducted in technical duplicate. The resulting values were averaged for the purpose of descriptive reporting; however, given that only two technical replicates originating from a single production batch were available, measures of variability (e.g., standard deviation) have not been reported. The study was not intended to assess batch-to-batch variability or to enable inferential statistical comparisons of storage temperatures

Results and discussion

Different pre-processing methods significantly influenced the sensory characteristics and overall acceptability of flaxseed milk. Table 1 summarises the processing methods used to formulate flaxseed milk and the selection of the final formulation.

Table 1. Results of processing technique implemented for flaxseed milk analogue formulation

Treatment	Processing Methods	Main sensory limitation	Overall acceptability	Selection status
T_0	Control (unprocessed)	Pronounced nutty flavour and undesirable taste characteristics.	6.35 ± 0.21	Not selected
T_1	Soaking	Residual nutty flavour and characteristic aftertaste.	7.40 ± 0.46	Not selected
T_2	Boiling	No major sensory limitation observed; highest acceptability.	8.13 ± 0.46	Selected
T_3	Roasting	Lower acceptability than boiled sample despite improved flavour.	7.55 ± 0.67	Not selected
T_4	Fermentation	Sour flavour and undesirable taste attributes during fermentation.	5.63 ± 0.74	Not selected
T_5	Germination	Undesirable flavour and aroma	6.27 ± 0.77	Not selected

Table 2. Sensory scores of flaxseed milk (Mean ± SD) prepared by different processing methods using a 9-point hedonic scale.

Treatments	Colour	Flavour	Consistency	Taste	Overall acceptability
Control (T_0)	7.0 ± 0.00^{bc}	5.4 ± 0.51^{c}	7.3 ± 0.48^{ab}	5.7 ± 0.48^{cd}	6.35 ± 0.21^{bc}
Soaking (T_1)	7.7 ± 0.48^{a}	7.6 ± 0.51^{a}	7.3 ± 0.67^{ab}	7 ± 0.94^{b}	7.40 ± 0.46^{ab}
Boiling(T_2)	8.1 ± 0.56^{a}	8 ± 0.47^{a}	8.1 ± 0.56^{a}	8.3 ± 0.67^{a}	8.13 ± 0.46^{a}
Roasting(T_3)	7.7 ± 0.82^{a}	7.6 ± 0.69^{a}	7.3 ± 1.05^{ab}	7.6 ± 0.69^{ab}	7.55 ± 0.67^{ab}
Fermentation (T_4)	6.9 ± 0.31^{b}	6.2 ± 1.03^{bc}	6.5 ± 1.08^{bc}	5.5 ± 0.97^{c}	5.63 ± 0.74^{c}
Germination (T_5)	6.7 ± 0.67^{c}	5.4 ± 1.07^{c}	6 ± 1.15^{c}	4.6 ± 1.07^{d}	6.27 ± 0.77^{bc}

Values are expressed as mean ± SD (n = 10 panelists). Data was analyzed using one-way repeated-measures ANOVA, followed by Tukey-adjusted pairwise comparisons. Means with different superscript letters within a column differ significantly at $p < 0.05$

The sensory scores of flaxseed milk analogue were significantly influenced by the processing treatments employed. One-way

ANOVA showed significant differences ($p < 0.05$) for color, flavor, consistency, taste, and overall acceptability scores across the treatment groups (Table 2). Treatment means for each sensory attribute were compared at $p < 0.05$ using Tukey's Honestly Significant Difference (HSD) test. The boiled flaxseed milk analogue (T_2) obtained the highest overall acceptability score (8.13 ± 0.46) among all treatments. The improved sensory acceptability of boiled flaxseed milk could be ascribed to changes that occur during thermal processing. Boiling may have reduced the perception of mucilage-related sliminess and disagreeable flavour notes, thereby improving mouthfeel and overall palatability. Earlier experimental studies have shown that the mucilage in flaxseed can impair mouthfeel, and that removing part of it through degumming can make the product more acceptable to consumers (Li et al., 2025). Moreover, Zhao et al. (2025) reported that processing methods, namely microwave treatment and soaking, modulated endogenous enzyme activities and the volatile aroma composition of flaxseed milk, resulting in a modified flavour profile. However, these explanations are offered as possible hypotheses based on past research, as mucilage content, volatile chemicals, bitterness, enzyme activity, protein denaturation, viscosity, particle size, and physical stability were not directly assessed in the current study. Future research should incorporate objective measurements of these parameters to validate the underlying mechanisms.

Standardization of flaxseed milk processing: The technology developed for the formulation of flaxseed milk was standardized for each unit operation. The brown flaxseeds were procured from the local market of Kumarganj, Ayodhya. The seeds were weighed (50 g), cleaned and washed to remove impurities. Filtered water was then heated in a pan until bubbles appeared. The flaxseeds were added and boiled for 10 minutes, until the water became slightly yellow and a thick, slimy layer appeared. The flaxseeds were promptly drained and rinsed with water to remove the visible slimy layer before grinding. The current study did not quantify the extent of mucilage clearance. The seeds were then ground in a domestic mixer grinder for 3–4 minutes with water at a ratio of 1:5 (w/v). The resulting slurry was filtered twice through a double-folded fine muslin cloth (250 µm mesh size) to obtain flaxseed milk. Thermal processing and pasteurization are widely employed in plant-based beverages to improve microbial safety, extend shelf life and enhance product stability (Aydar *et al*., 2020; Silva *et al*., 2020).

Sensory acceptability of the developed flaxseed milk: The sensory evaluation of the developed flaxseed milk showed high acceptability among the semi-trained panelists when assessed using a 9-point hedonic scale. Mean scores for colour (8.10), flavour (8.0), consistency (8.10), taste (8.30) and overall acceptability (8.13) indicated that the product was liked very much by the panelists. The beverage exhibited appealing colour, pleasant flavour, creamy consistency and good palatability, contributing to its favourable sensory acceptance among panel members. Boiling treatment resulted in higher sensory acceptance scores than other processing modalities. The increased sensory perception could be attributed to changes that occur during heat processing; however, the underlying mechanisms were not examined in the current investigation. In general, the flaxseed milk displayed good preliminary sensory acceptability among the semi-trained panelists indicating potential for further product development. A more recent study published in Frontiers in Food Science and Technology pointed out that milks derived from oats, almonds, soy, coconut, and flaxseeds were well accepted sensory-wise when optimized for viscosity, flavor and appearance. Consumer acceptance of plant-based milk alternatives is largely shaped by sensory attributes such as taste, texture, thickness, and visual appeal (Irondi *et al*., 2025). Their review also noted that various processing methods—including heat treatment, homogenization, enzymatic treatment, and fermentation—can help enhance these sensory qualities in plant-based beverages.

Microbial analysis: Total viable counts increased from 48 CFU mL^{-1} at 0 hours to 4.78×10^3 CFU mL^{-1} at 96 hours at 4°C. During the same period, counts increased from 51 CFU mL^{-1} to $6.31 \times$ ~~10^2~~ 10^4 CFU mL^{-1} at 14°C. At room temperature, the count was 8.71×10^7 CFU mL^{-1} at 48 hours, and after 60 h, counts were too numerous to count (TNTC). Microbial growth was observed to progress more slowly under refrigerated conditions (4°C) compared with storage at 14°C or ambient temperature within the tested parameters. As the storage experiment involved a single independently produced batch, with replication restricted to duplicate plate counts at the technical level, no statistical comparison across storage temperatures could be carried out. These findings should therefore be regarded as descriptive rather than reflecting statistically confirmed differences among the conditions. During the 96-hour observation period, refrigeration at 4°C lowered total viable counts while preventing detectable coliform growth. Before determining the storage stability or commercial shelf life of flaxseed milk, more research is needed employing multiple independently created production batches, as well as pathogen detection, yeast and mould enumeration, physicochemical analysis, and sensory evaluation.

Table 3. Total viable count (CFU mL^{-1}) of flaxseed milk at different temperatures

Duration (h)	4°C (CFU mL^{-1})	14°C (CFU mL^{-1})	Room Temperature (CFU mL^{-1})
0	48	51	55
12	97	129	2.55×10^3
24	1.32×10^2	172	9.88×10^3
36	1.48×10^2	2.85×10^2	8.66×10^4
48	1.82×10^2	7.31×10^2	8.71×10^7
60	3.81×10^2	2.96×10^3	TNTC
72	6.71×10^2	5.19×10^3	TNTC
84	9.71×10^2	9.72×10^3	TNTC
96	4.78×10^3	6.31×10^4	TNTC

TNTC = Too numerous to count.

Coliform count: Coliforms were not detected in samples maintained at 4°C throughout the 96-h observation period; however, measurable coliform growth was observed at 14°C and room temperature. Because only one production batch was examined, these findings should be viewed descriptively rather than as statistically significant differences. No detectable coliform count was observed in flaxseed milk stored at 4°C for up to 96 h, indicating good microbiological and hygienic quality under refrigerated conditions. At 14°C, coliforms remained absent during the initial 60 h of storage; however, slight microbial growth was detected after 72 h, increasing gradually from 4 CFU mL^{-1} to 41 CFU mL^{-1} by the end of the storage period. In contrast, samples stored at room temperature exhibited rapid coliform proliferation. Detectable coliform growth appeared after 12 h of storage (4 CFU mL^{-1}) and increased substantially to 1782 CFU mL^{-1} after 96 h as shown in Table 4.

Table 4. Coliform count of flaxseed milk at different storage conditions

Duration (h)	4°C (CFU mL^{-1})	14°C (CFU mL^{-1})	Room Temperature (CFU mL^{-1})
0	ND	ND	ND
12	ND	ND	4
24	ND	ND	18
36	ND	ND	49
48	ND	ND	92
60	ND	ND	201
72	ND	4	417
84	ND	12	938
96	ND	41	1782

ND = Not detected below the method detection limit of <3 CFU mL^{-1}.

These findings suggest that lower storage temperatures were linked to decreased coliform growth under the experimental settings. However, as only one production batch was evaluated, these observations should be considered descriptive rather than statistically confirmatory.

Short-term microbiological storage assessment: A short-term microbiological storage assessment of flaxseed milk was carried out to determine the effect of various storage temperatures on the measured microbial indicators after 96 hours of storage. The evaluation was limited to total viable count (TVC) and coliform enumeration and was designed to offer preliminary information on microbial alterations under the investigated storage conditions (Tables 3 and 4). The observations revealed that the measured microbiological indicators were influenced by storage temperature over the 96-hour storage period because only one production batch was evaluated, these data should be interpreted as descriptive rather than statistically significant changes between storage temperatures. Refrigerated storage at 4°C resulted in decreased total viable counts and no detectable coliforms during the 96-hour observation period under the test conditions. In contrast, samples stored at 14°C showed comparatively higher microbial proliferation and showed comparatively slower microbial growth for a limited duration. Flaxseed milk kept at room temperature exhibited rapid increases in total viable counts and coliforms after 48 hours under the investigated circumstances. However, microbiological deterioration and product acceptability cannot be determined conclusively because only a subset of microbial markers was tested. According to the International Commission on Microbiological Specifications for Foods (ICMSF, 2011), excessive microbial proliferation adversely affects the microbiological quality, sensory quality and shelf stability of dairy and plant-based beverages. Over the span of the 96-hour observation period, no coliforms were found, and total viable counts were low under refrigerated storage (4°C). These findings suggest that refrigeration restricted growth in the microbiological markers measured (TVC and coliforms) across the observation period. However, because only indicator species were assessed, the results should not be construed as proof of microbiological safety or commercial shelf life. However, because the study only tested for TVC and coliforms, the findings should be viewed as a short-term microbiological storage assessment rather than a determination of product safety or commercial shelf life because only one independently prepared production batch was tested with duplicate plate counts, the storage data are only technical replication and should be interpreted as preliminary descriptive observations rather than statistically validated estimates of shelf life or storage temperature effects.

Multiple independently produced batches will be required for a comprehensive shelf-life evaluation, as well as pathogen identification, yeast and mould enumeration, physicochemical measurements (including pH and water activity), sensory evaluation during storage, and packaging validation. A limitation of the present study is that the microbiological storage testing was performed on only one independently prepared production batch. As a result, the findings reflect technical rather than biological replication and do not account for batch-to-batch variability. The final flaxseed milk was not subjected to pasteurization after extraction. Since the beverage underwent rinsing, grinding and filtration steps following heat treatment, the possibility of post-processing microbial contamination cannot be completely excluded. Therefore, the developed product should be considered a fresh beverage intended for refrigerated storage and short-term consumption. Other limitations include a small sensory panel, a lack of consumer acceptance testing and the absence of extensive nutritional, physicochemical, microbiological and commercial shelf-life assessments. Future investigations should involve multiple independently prepared production batches in order to conduct statistical analysis of short-term microbial quality during storage. Furthermore, the mechanisms underlying the superior sensory quality of boiling flaxseed milk were not directly explored. Future research should measure mucilage content, volatile taste compounds, bitterness, enzyme activity, protein denaturation, viscosity, and particle size to confirm the mechanisms responsible for the observed sensory improvement.

The present study demonstrated that processing influenced the sensory quality of flaxseed milk. Among the treatments evaluated, boiling produced the highest overall sensory acceptability. Under the experimental conditions, refrigerated storage (4°C) was associated with lower total viable counts and no detectable coliforms during the 96-hour observation period compared with storage at 14°C or room temperature. However, these microbiological observations were obtained from a single independently prepared production batch with duplicate technical plate counts and should therefore be interpreted descriptively rather than as statistically validated estimates of storage performance. The current work should be regarded as a preliminary investigation. The sensory evaluation was carried out with a small panel of semi-trained assessors, and the sensory findings need to be validated in larger, independent consumer research. Furthermore, the storage assessment was confined to total viable count and coliform enumeration, with no pathogen identification, yeast and mould analysis, physicochemical stability, or sensory evaluation during storage. As a result, the current findings do not prove product shelf life, microbiological safety, or commercial suitability. Subsequent research should incorporate several independently manufactured production batches, expanded and more heterogeneous sensory panels, testing for pathogenic microorganisms, enumeration of yeasts and moulds, assessment of physicochemical stability parameters (including pH and water activity), and sensory evaluation conducted throughout the storage period, in order to substantiate these preliminary observations. Nonetheless, the findings suggest that boiling is a promising processing strategy for enhancing the sensory attributes of flaxseed milk, thereby warranting continued exploration of flaxseed as a value-added plant-based beverage.

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