



Evaluation of Storage Stability of Milk-flaxseed-based Probiotic Composite Beverage

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ABSTRACT

The study was conducted during September, 2022 to April, 2023 at National Dairy Research Institute and aimed to investigate the storage stability of a novel milk-flaxseed-based probiotic composite beverage under refrigerated conditions. The beverage was developed by combining skim milk with roasted flaxseed flour and fermenting it with *Lactiplantibacillus plantarum* NCDC-21. The product was evaluated for various quality attributes over a 14 days storage period at $4\pm 2^{\circ}\text{C}$, using both PET and glass packaging materials. Sensory analysis revealed a gradual decline in quality attributes such as color, consistency, and overall acceptability, with a significant changes observed after the 8th day of storage. Physicochemical analysis indicated a decrease in pH and an increase in titratable acidity over time, while viscosity initially increased followed by a declining trend after the 8th day of refrigerated storage. *L. plantarum* count decreased from $8.8 \log \text{cfu ml}^{-1}$ to $8.26 \log \text{cfu ml}^{-1}$ in PET bottles and $8.35 \log \text{cfu ml}^{-1}$ in glass bottles by the 10th day of refrigerated storage. The study concluded that the milk-flaxseed-based probiotic beverage maintained the acceptable quality up to 10 days under refrigerated storage conditions, with glass bottles offering slightly better preservation in terms of sensory and microbial quality as compared to PET bottles. Additionally, the study also highlighted the potential of flaxseed incorporation in fermented dairy beverages for enhancing functional and nutritional properties while maintaining probiotic viability during refrigerated storage.

KEYWORDS: Flaxseed, milk, probiotic, composite, storage study

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1. INTRODUCTION

Flaxseed (*Linum usitatissimum*), or linseed, is a member of the Linaceae family. The flaxseed plant has a height of 60 cm and is characterized by its slender, highly fibrous stems. Its fruit contains the valuable seed referred as flaxseed or linseed (Pradhan et al., 2010; Shahada et al., 2024; Duarte et al., 2025). Flaxseed is one of the most important oilseed crops used for food, feed, fibre, and industrial purposes. Flaxseed is crispy in texture and nutty in taste (Rubilar et al., 2010; Hu et al., 2020; Kauser et al., 2024). The average whole flaxseed contains 4–8% moisture, 3–4% ash, 20–35% dietary fiber, 20–30% protein, 30–41% fat, and 1% simple sugars. Flaxseed can be an excellent source of bioactive components like omega-3 fatty acids, dietary fibre, vitamins, minerals, etc. (Nowak and Jeziorek, 2023). Additionally, flaxseed contains minor components such as phytic acid, trypsin inhibitors, phenolics, minerals, cadmium, vitamins, and selenium. (Mueed et al., 2022). Flaxseed is the richest source of secoisolariciresinol diglycoside (SDG), which help to manage several health problems in females during estrogen-deficient conditions. (Singh et al., 2011; Singh and Sharma, 2025). Flaxseed oil is a rich source of alpha-linolenic acid (ALA), which comprises approximately 53% of the oil, as well as oleic acid, which comprises about 19%. The high ALA content in flaxseed oil results in a favorable ω -6: ω -3 fatty acid ratio. Alpha-linolenic acid (ALA) has anti-inflammatory properties and helps to protect the lining of the gastrointestinal tract. Research has shown that flaxseed can be beneficial for those who suffer from digestive disorders such as Crohn's disease (Plissonneau et al., 2022; Kauser et al., 2024). Among its many applications, flaxseed can be incorporated into dairy-based products, particularly probiotic beverages, to explore its nutritional and health beneficial property. Ursoniu et al. (2016) conducted a study that revealed the potential of flaxseed supplementation in reducing both systolic and diastolic blood pressure. Consuming 10 g of flaxseed daily can provide 1 g of soluble fiber and 3 g of insoluble fiber. Additionally, a serving of 30 g of flaxseed can meet 7 to 30% of the recommended dietary allowances (RDA) for minerals such as calcium, phosphorus, and magnesium (Yawale et al., 2022). Similarly, a barley-milk composite fermented beverage was developed with higher sensory acceptability, probiotic viability, and β -glucan content (Ahuja et al., 2017). In recent years, probiotics have gained an immense attention as a potential bio-therapeutic agent. Most common probiotic organisms are bacteria, specially *lactobacillus* and *bifidobacterial* spp. (Alard et al., 2018). Appropriate modifications in gut microbiota with probiotics can help individuals protect themselves from gastrointestinal diseases and improve their health (Markowiak and Slizewska, 2017). Probiotics also help to improve lactose

metabolism, manage life-style induced diseases like diabetes, CVD and cholesterol, stimulate the immune system, and can aid treating inflammatory bowel disease, and diarrhea (Shah, 2004; Sankarganesh et al., 2024). The combination of milk and flaxseed presents a unique opportunity to create a nutrient-rich, cost-effective functional beverage. Milk provides essential proteins, vitamins, and calcium, while flaxseed contributes dietary fiber, phytoestrogens, and omega-3 fatty acids. This synergy makes milk-flaxseed-based probiotic beverages particularly beneficial for women's health, addressing issues such as hormonal imbalances and osteoporosis. This study aims to investigate the storage stability of a novel milk-flaxseed-based probiotic beverage under refrigerated conditions, evaluating its sensory, physicochemical, and microbiological properties over a 14-day of refrigerated storage. The findings aimed to provide insights into the optimal storage conditions and shelf-life estimation of this functional beverage, which would contribute towards commercialization of this probiotic beverage.

2. MATERIALS AND METHODS

2.1. Materials

The study was conducted during September, 2022 to April, 2023 at National Dairy Research Institute. Fresh and good quality of skim milk was collected from the Experimental Dairy, ICAR-National Dairy Research Institute, Karnal. Good quality flaxseed grains of brown variety, commercial-grade refined iodized salt, cumin, and black pepper seeds were purchased in a single lot from the local market of Karnal, Haryana. Cumin and black pepper seeds were carefully roasted, finely ground into powder form followed by storage in sterilized containers for further experiment and use. All chemicals used throughout this study were AR grade and purchased from standard suppliers. The microbiological media supplied by HiMedia Laboratories Pvt. Ltd. (Mumbai) were used in the microbiological analysis of the samples. Probiotic strains namely, *Lactiplantibacillus plantarum* NCDC-21 was collected from the National Collection of Dairy Cultures (NCDC), ICAR-NDRI, Karnal, India and used for beverage preparation.

2.2. Beverage preparation

The skim milk was heated to 40°C, followed by addition of 3% roasted flaxseed flour. The mixture was then subjected to heating to 90°C for 15 min and cooled to 37°C. The probiotic organism *L. plantarum* NCDC-21 was added to the substrate at 3% level, and kept for incubation at 37°C for 12 h. The fermented substrate was converted into a spicy beverage by adding water (20%), pectin as a stabilizer (0.3%), salt (1.0%), roasted cumin powder (0.75%), and roasted black pepper powder (0.5%). The water was heated

to 70°C for 5 min before addition of pectin and the other spices. The mixture was then blended, heated to 80°C for 5 min and cooled to 4°C. Finally, the probiotic composite substrate was mixed with the spicy water mixture using a laboratory stirrer to produce the milk-flaxseed-based probiotic composite beverage and kept under refrigerated conditions till further study. The optimized beverage was filled into pre-sterilized clear PET and glass bottles and stored at a refrigeration temperature of 4± 2°C for 14 days. The beverage was evaluated for various quality parameters at 0, 4, 8, 10, 12, 14th day of refrigerated storage till spoilage.

2.3. Sensory analysis

For sensory analysis, 50 ml of sample was served for 14 days to sensory panel consisting of 40 panellists of scientists and researchers of ICAR-National Dairy Research Institute, Karnal. The beverage sample was served at 16°C and the judges were provided with a quantitative descriptive sensory score card to evaluate the quality of the product based on various parameters such as appearance (colour, free whey, particulate matters), texture (consistency, ropiness, granularity), flavor (acidic, salty, oxidized/rancid, bitter, unnatural/stale), and overall acceptability. A scale ranges from 0 (nil) to 10 (pronounced) indicating the minimal and maximal values for the measurement of intensity of each attribute.

2.4. Physicochemical analysis

The optimized beverage was investigated for various physicochemical parameters including pH, acidity, color, and viscosity measurement. The titratable acidity of optimized product was determined using back titration protocol (Anonymous, 2005). The pH values were measured at 20°C using a pH meter manufactured by Labindia, India. The surface colour of the milk-flaxseed-based probiotic composite beverage was measured using a ColorFlex® colorimeter supplied by Hunter Lab, a laboratory based in Reston, VA, USA, along with the software (version 4.10). A rheometer (model; MCR52; make: Anton Paar, Germany) equipped with measurement probe CC27 (cup and bob) was used to determine the rheological properties (apparent viscosity at shear rate ranging from 1–100 s⁻¹) of milk-flaxseed-based probiotic composite beverage at 20±0.1°C.

2.5. Microbiological analysis

The enumeration of lactobacillus was performed using MRS agar via pour plate technique, followed by incubation at 37°C for 48–72 h (Anonymous, 2015). Yeast and mold count was determined using Potato Dextrose Agar (PDA) adjusted to pH 3.5 with sterile 10% tartaric acid, incubated at 25°C for 3–5 days (Anonymous, 2008). Violet Red Bile Agar (VRBA) was used to determine coliforms, with incubation at 37°C for 24 h (Anonymous, 2006).

2.6. Statistical analysis

Data obtained from various experiments during the experimentation was subjected to suitable statistical analysis using IBM SPSS 25.0 software. Student's t-test and Analysis of variance (ANOVA) with Tukey's post hoc test was used for testing the significance of difference between two or more treatments respectively at $p=0.05$ level of significance. Results were expressed as mean±standard deviation (SD). Prism Graph-pad version 8.4.2 was used for graphical representation of the data.

3. RESULTS AND DISCUSSION

3.1. Effect of refrigerated storage on sensory quality of the product

The sensory scores of the beverages gradually and significantly decreased ($p<0.05$) over the 12-day of storage period, regardless of the packaging material used (PET and Glass bottles). However, no significant difference in beverage homogeneity was observed between the two packaging materials during the entire storage duration. The color value of the beverages showed a significant decrease ($p<0.05$) of 1.29 to 0.10 in PET bottles and from 1.29 to 0.60 in glass bottles. Initially, neither packaging material showed any presence of free whey. Nevertheless, on the 10th day of storage, a small amount of free whey was detected in PET bottles. Subsequently, by the 12th day, the free whey content increased to 2.5 in PET bottles and 1.5 in glass bottles. Particulate and saltiness levels showed no significant differences in both packaging materials during the 12-day storage period (Table 1). The beverages remained free from bitterness, astringency, unnatural flavors, and ropiness until the 8th day of storage. However, after that, these undesirable sensory attributes were detected in both PET and glass bottle. Overall acceptability of the beverages kept in PET and glass bottles decreased significantly from 7.79 to 5.3 and 6.25, respectively, during the end of storage periods (Table 2). The changes in sensory attributes of the milk-flaxseed-based probiotic composite beverage during storage might be because of an increased wheying off, alterations in viscosity, and darkening of color, leading to reduced color and appearance score, as well as consistency scores. Additionally, the decrease in pH during storage caused a reduction in the flavor and overall acceptability score.

During storage, sensory attributes score decreased in pearl millet-based *rabadi* (Modha and Pal, 2011) and sorghum malt-based fermented milk beverage (Hussain et al., 2014). The storage period of approximately 7 days had been reported for pearl millet *rabadi* and 14 days for the sorghum malt-based beverage at refrigeration. Ahuja (2017) observed a decline flavor score of the product which might be impacted by increased acidity during storage, which aligned with the finding of Modha and Pal (2011) for fermented

Table 1: Changes in sensory attributes of milk-flaxseed-based probiotic composite beverages during storage in different packaging materials

Day	Packaging Material	Homogeneity	Color	Free whey	Particulate	Acidic	Salty
0	PET bottle	6.22±1.015 ^{aAB}	1.29±0.776 ^{aA}	0±0 ^{aB}	6.86±0.769 ^{aA}	4.98±0.915 ^{aC}	5.2±0.919 ^{aA}
	Glass bottle	6.15±0.901 ^{aB}	1.29±0.776 ^{aAB}	0±0 ^{aB}	7.09±0.97 ^{aA}	4.87±0.629 ^{aC}	5.11±1.054 ^{aA}
4	PET bottle	6.11±0.601 ^{aAB}	1.65±0.944 ^{aA}	0±0 ^{aB}	6.73±0.517 ^{aA}	5.5±0.972 ^{aC}	5.67±1.118 ^{aA}
	Glass bottle	6.67±1.225 ^{aAB}	1.7±0.949 ^{aA}	0±0 ^{aB}	6.84±0.713 ^{aA}	5.1±0.568 ^{aC}	5.63±0.518 ^{aA}
8	PET bottle	6.5±0.756 ^{aAB}	1±0.667 ^{aAB}	0±0 ^{aB}	6.8±0.789 ^{aA}	6.1±1.174 ^{aBC}	5.85±0.818 ^{aA}
	Glass bottle	7.25±0.707 ^{aA}	1.3±0.483 ^{aAB}	0±0 ^{aB}	6.8±0.778 ^{aA}	5.45±0.832 ^{aBC}	5.7±0.949 ^{aA}
10	PET bottle	6.6±1.075 ^{aA}	0.2±0.422 ^{bBC}	0.8±1.033 ^{bB}	7±0.943 ^{aA}	7.1±0.568 ^{aB}	5.85±0.784 ^{aA}
	Glass bottle	7.3±0.675 ^{aA}	0.8±0.422 ^{aBC}	0±0 ^{aB}	6.9±0.994 ^{aA}	6.2±0.422 ^{bB}	5.75±0.92 ^{aA}
12	PET bottle	6.8±1.398 ^{aA}	0.1±0.316 ^{bC}	2.5±0.85 ^{aA}	6.95±0.762 ^{aA}	8.9±0.738 ^{aA}	6.2±1.033 ^{aA}
	Glass bottle	7.35±0.944 ^{aA}	0.6±0.516 ^{aBC}	1.5±0.707 ^{bA}	7±0.707 ^{aA}	7.6±0.699 ^{bA}	5.9±0.994 ^{aA}

Values were mean±SD (n=40); Means with different superscripts (a, b) differ significantly ($p<0.05$) within the same day for different packaging materials, Means with different superscripts (A, B) differ significantly ($p<0.05$) within the same packaging material for different days

Table 2: Changes in sensory attributes of milk-flaxseed-based probiotic composite beverages during storage in different packaging materials

Day	Packaging Material	Bitter/Astringency	Un natural	Consistency	Ropiness	Granularity	Overall acceptability
0	PET bottle	0±0 ^{aC}	0±0 ^{aC}	6.41±0.862 ^{aA}	0±0 ^{aC}	4.99±0.853 ^{aB}	7.79±0.594 ^{aA}
	Glass bottle	0±0 ^{aC}	0±0 ^{aC}	6.58±0.862 ^{aA}	0±0 ^{aB}	4.8±0.748 ^{aB}	7.86±0.741 ^{aA}
4	PET bottle	0±0 ^{aC}	0±0 ^{aC}	6.4±0.966 ^{aA}	0±0 ^{aC}	6±0.866 ^{aAB}	7.22±0.441 ^{aAB}
	Glass bottle	0±0 ^{aC}	0±0 ^{aC}	6.5±0.85 ^{aA}	0±0 ^{aB}	5.78±0.833 ^{aAB}	7.5±0.707 ^{aAB}
8	PET bottle	0±0 ^{aC}	0±0 ^{aC}	6.13±1.126 ^{aA}	0±0 ^{aC}	6.7±0.949 ^{aA}	6.56±0.726 ^{aBC}
	Glass bottle	0±0 ^{aC}	0±0 ^{aC}	6.33±0.866 ^{aA}	0±0 ^{aB}	6.6±0.966 ^{aA}	7.11±0.821 ^{aAB}
10	PET bottle	1.9±0.738 ^{aB}	2±0.943 ^{aB}	5.8±0.919 ^{aA}	0.9±0.876 ^{aB}	6.75±0.486 ^{aA}	6±0.333 ^{bCD}
	Glass bottle	0.9±0.738 ^{bB}	1.5±0.972 ^{aB}	6.2±0.789 ^{aA}	0±0 ^{bB}	6.6±0.81 ^{aA}	6.7±0.422 ^{aBC}
12	PET bottle	3.8±0.789 ^{aA}	4±1.155 ^{aA}	5.22±0.667 ^{bA}	3.3±0.823 ^{aA}	6.85±0.709 ^{aA}	5.3±0.422 ^{bD}
	Glass bottle	2.1±0.994 ^{bA}	2.7±0.949 ^{bA}	6.25±0.354 ^{aA}	2.25±0.354 ^{bA}	6.7±0.675 ^{aA}	6.25±0.486 ^{aC}

Values were mean±SD (n=40); Means with different superscripts (a, b) differ significantly ($p<0.05$) within the same day for different packaging materials, Means with different superscripts (A, B) differ significantly ($p<0.05$) within the same packaging material for different days

probiotic drink. Ganguly (2014) reported a declined score for both the consistency and the overall acceptability of probiotic beverage during 18 days of refrigerated storage.

3.2. Physicochemical changes

3.2.1. Viscosity

The initial viscosity of the fresh beverage was 251±2.12 cP in the PET bottle and 253±3.49 cP in the glass bottle. By the 8th day of storage, the viscosity increased to 292±1.87 cP in the PET bottle and 286±4.19 cP in the glass bottle.

However, after the 8th day, there was a subsequent decrease in viscosity. On the 14th day of refrigeration storage, the viscosity reached to 187±2.63 cP in the PET bottle and 205±5.06 cP in the glass bottle. The effects of storage on the viscosity of the product are presented in Figure 1 and was found to be statistically significant.

A similar trend in viscosity during storage was documented by multiple researchers. Ganguly (2014) observed for a whey-cereal-based probiotic beverage, the initial increment in viscosity upto the 8th day of refrigerated storage followed

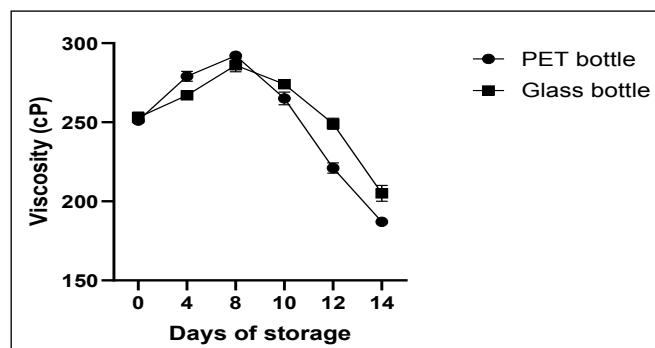


Figure 1: Changes in viscosity of milk-flaxseed-based composite beverage during storage

by a declining trend till the 18th day of storage. Similarly, Modha and Pal (2011) found the viscosity of a pearl millet-based *rabadi*-like product increased initially during storage followed by a decreasing trend, reaching to 53 cP on the 7th day of storage. Hussain et al. (2014) reported a similar pattern in sorghum malt-based fermented milk beverages, where an increment in viscosity of the stored samples was observed upto the 7th day of storage, followed by a gradual decline. The rise in viscosity was possibly due to the chemical reactions occurring between pectin and water molecules, which resulted in strengthening the gel structure (Oliveira et al., 2001). Further, this viscosity increase was potentially impacted by the development of a denser gel caused by the interactions occurred between hydrocolloids and milk proteins (Fernandez-Garcia et al., 1998). Subsequently, a decrease in viscosity might have occurred due to the hydrolysis of starch or the degradation of the casein network by organisms, resulting in lower water-retention capabilities. Consequently, bound water was released, leading to a reduction in viscosity (Gokavi et al., 2005).

3.2.2. pH and acidity

The initial titratable acidity of the milk-flaxseed-based probiotic composite product was $0.65 \pm 0.013\%$ LA in PET bottles and $0.64 \pm 0.009\%$ LA in glass bottles. On the 10th day of storage, the acidity increases to $0.87 \pm 0.007\%$ LA and $0.85 \pm 0.016\%$ LA, respectively in PET and glass bottles. After a storage period of 14 days, the titratable acidity increased to $1.06 \pm 0.020\%$ LA and $0.98 \pm 0.005\%$ LA, respectively in PET and glass bottles. A significant difference in acidity change was observed between the product packed in PET and glass packaging material. Additionally, the pH of samples in both PET and glass bottles decreased during storage. In PET bottles, the pH declined from 4.88 ± 0.012 to 4.37 ± 0.021 by the 10th day and further decreased to 3.93 ± 0.017 by the 14th day. Similarly, in glass bottles, the pH decreased from 4.89 ± 0.01 to 4.41 ± 0.021 on the 10th day and further dropped to 4.02 ± 0.01 by the 14th day. The trend of acidity can be observed in Figure 2.

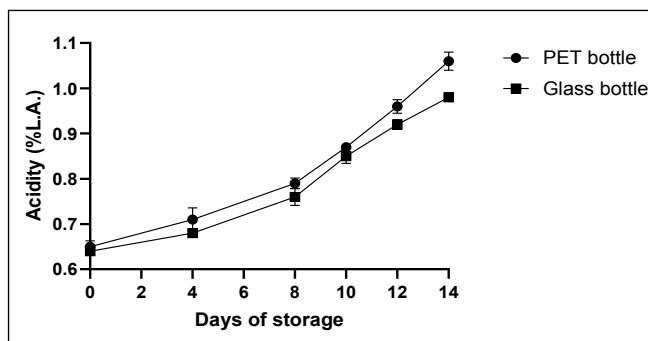


Figure 2: Changes in acidity of the milk-flaxseed-based beverage during storage in PET and glass packaging material

Ahuja (2017), reported an increase in titratable acidity (% lactic acid) from 0.69 to 1.16 in PET bottles and from 0.69 to 1.15 in LDPE pouches after 15 days of storage in oat-milk-based probiotic drink. The decrease might be attributed to the utilization of residual carbohydrate by viable microorganisms and production of lactic acid and small amount of formic acid from lactose (Panesar and Shinde, 2012). Vahedi et al. (2008) reported that the decrease in pH was due to activity of microorganisms. Kailasapathy (2006) stated that post-acidification during storage, was due to β -galactosidase activity, which remained active at low temperature.

3.2.3. Colour

Color is a crucial parameter influencing the acceptability of a product, and it undergoes changes during probiotic fermentation. The color profile is represented by the “L” value, indicating lightness (100) to blackness (0), the “a” value, indicating red (+ve) to green (-ve) hues, and the “b” value, indicating yellow (+ve) to blue (-ve) hues (Trias-Sanz et al., 2008). The changes in the colour of the beverage during storage at various packaging materials has shown in Table 3. Throughout the storage period, no significant difference was observed in the instrumental color parameters between the two packaging materials. Although there were slight changes in the L* value during the entire storage period, they were not statistically significant between the PET and glass bottles. Similar findings were reported by Girish (2015) during 7 days storage of *rabadi*-like wheat based fermented milk beverage.

3.3. Microbiological changes

3.3.1. Probiotic count

The initial probiotic count of beverage was 8.8 ± 0.019 and 8.84 ± 0.014 log cfu ml⁻¹, respectively in PET and Glass bottles. Over the course of storage, the counts of *L. plantarum* NCDC21 decreased. On the 10th day of storage, the counts reached to 8.26 ± 0.056 and 8.35 ± 0.026 log cfu ml⁻¹, respectively in PET and Glass packaging material.

Table 3: Effect of storage on colour parameters of milk-flaxseed-based probiotic beverage at different packaging materials

Days	L*		a*		b*	
	PET bottle	Glass bottle	PET bottle	Glass bottle	PET bottle	Glass bottle
0	60.44±0.391 ^{cdA}	60.94±0.19 ^{bcA}	3.07±0.116 ^{aA}	3.06±0.15 ^{aA}	16.53±0.261 ^{aA}	16.81±0.362 ^{aA}
4	60.92±0.172 ^{cA}	60.37±0.116 ^{cdA}	3.13±0.118 ^{aA}	3.18±0.37 ^{aA}	16.48±0.04 ^{aA}	16.81±0.596 ^{aA}
8	59.69±0.08 ^{dA}	59.75±0.006 ^{dA}	3.26±0.148 ^{aA}	3.29±0.035 ^{aA}	16.32±0.06 ^{aA}	16.83±0.05 ^{aA}
10	61.22±0.651 ^{cbA}	61.09±0.29 ^{bcA}	3.23±0.1 ^{aA}	3.2±0.086 ^{aA}	16.56±0.619 ^{aA}	16.47±0.23 ^{aA}
12	62.07±0.533 ^{abA}	61.82±0.078 ^{abA}	3.19±0.053 ^{aA}	3.03±0.417 ^{aA}	16.63±0.178 ^{aA}	16.85±0.286 ^{aA}
14	62.34±0.612 ^{aA}	62.13±0.161 ^{aA}	3.11±0.105 ^{aA}	2.92±0.101 ^{aA}	16.87±0.457 ^{aA}	16.89±0.125 ^{aA}

Values were mean±SD (n=3); Means with different superscripts (a, b, c, d, e, f) differ significantly ($p<0.05$) within the same column for different days, Means with different superscripts (A, B) differ significantly ($p<0.05$) within the same row for different packaging material

The probiotic count remained stable and decreased to 6.39 ± 0.012 and 6.43 ± 0.012 log cfu ml⁻¹, respectively in PET and Glass bottles during 24 days of refrigerated storage. Figure 3 indicated the viability of *L. plantarum* NCDC21 in the milk-flaxseed-based probiotic composite beverage during refrigeration storage. Similar declining trends of probiotic count was observed by various researcher

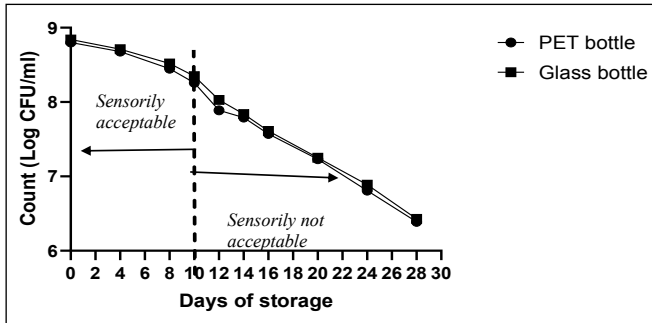


Figure 3: Effect of refrigerated storage on probiotic count of the beverage in PET and glass packaging material

during storage of various probiotic products. *L. acidophilus* survived at sufficient levels for about 4 weeks in oat-based non- dairy beverage (Gokavi et al., 2005). Only one log count reduction was observed for *L. plantarum* during 21 days of refrigerated storage for oat- based probiotic drinks (Angelov et al., 2006). Ganguly (2014) reported reduction of probiotic counts ($11\text{--}12$ log cfu ml⁻¹) to 6.36 ± 0.35 log cfu ml⁻¹ during 30 days of refrigerated storage in whey-cereal based beverage. Ahuja (2017) observed a reduction of probiotic count in oat-milk-based probiotic drink during 15 days of refrigerated storage, where greater reduction in probiotic count was observed for PET container as compared to LDPE pouches.

3.3.2. Effect of refrigerated storage on coliform and yeast and mold count of the product in different packaging material

During the storage of the beverage, no coliforms were

Table 4: Effect of refrigerated storage on coliform and yeast and mold count of the beverage in different packaging materials

Days	Coliform (log cfu ml ⁻¹)		Yeast and mold (log cfu ml ⁻¹)	
	PET bottle	Glass bottle	PET bottle	Glass bottle
0	ND*	ND*	ND*	ND*
4	ND*	ND*	ND*	ND*
8	ND*	ND*	ND*	ND*
10	ND*	ND*	ND*	ND*
12	ND*	ND*	1.32±0.021 ^{Ab}	1.25±0.014 ^{Bb}
14	ND*	ND*	2.25±0.135 ^{Aa}	2.01±0.024 ^{Ba}

Values were mean±SD (n=3); Means with different superscripts (a, b) differ significantly ($p<0.05$) within the same column for different days Means with different superscripts (A, B) differ significantly ($p<0.05$) within the same row for different packaging material ND*: Not detected at 1st dilution

detected in both fresh and stored samples until the 14th day of storage. Similarly, yeast and mold count was absent in the fresh product, but on the 12th day of storage, yeast and molds count was detected in both PET and glass bottles. However, the count of yeast and mold was significantly higher in PET bottles as compared to glass bottles on the 12th and 14th day of storage (Table 4). Similarly, Girish (2015) observed no coliforms, yeast, and mold count in a milk-wheat-based *rabadi*-like fermented beverage during 7 days of storage. Overall, the microbial analysis demonstrated the presence of yeast and molds in the beverage on 12th day of refrigerated storage, indicating shelf life of concerned product of 10 days at refrigerated temperature.

4. CONCLUSION

The beverage remained acceptable for 10 days under refrigerated condition ($4\pm 2^{\circ}\text{C}$) in both packaging materials. Probiotic count of beverage was reduced $8.8 \log \text{cfu ml}^{-1}$ to $6.39 \log \text{cfu ml}^{-1}$ in PET bottles and $6.43 \log \text{cfu ml}^{-1}$ in glass bottles by the 24th day. In PET bottles higher count of yeast and mold were detected on the 12th day. However, further research is needed to explore interventions and packaging conditions to extend shelf life of the beverage.

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6. REFERENCES

- Ahuja, K.K., Singh, A.K., Bala, K., Arora, S., Sabikhi, L., 2017. Optimisation of the formulation for barley-milk composite-based fermented drink. *International Journal of Dairy Technology* 70(2), 237–244. DOI: <https://doi.org/10.1111/1471-0307.12328>.
- Alard, J., Peucelle, V., Boutillier, D., Breton, J., Kuylle, S., Pot, B., Grangette, C., 2018. New probiotic strains for inflammatory bowel disease management identified by combining in vitro and in vivo approaches. *Beneficial Microbes* 9(2), 317–332. DOI: 10.3920/BM2017.0097.
- Angelov, A., Gotcheva, V., Kuncheva, R., Hristozova, T., 2006. Development of a new oat-based probiotic drink. *International Journal of Food Microbiology* 112(1), 75–80. DOI: <https://doi.org/10.1016/j.ijfoodmicro.2006.05.015>.
- Anonymous, 2005. Official methods of analysis (18th Edn.) Association of Official Analytical Chemists, USA. Available from: <https://www.scirp.org/reference/ReferencesPapers?ReferenceID=2033299>. Accessed on: January 5, 2023.
- Anonymous, 2015. Milk and milk products–Starter cultures, probiotics and fermented products–Quantification of lactic acid bacteria. Available from: <https://www.iso.org/standard/64658.html>. Accessed on: January 5, 2023.
- Anonymous, 2008. Microbiology of food and animal feeding stuffs–Horizontal method for the enumeration of yeasts and molds–Part 1: Colony count technique. Available from: <https://www.iso.org/standard/38275.html>. Accessed on: January 5, 2023.
- Anonymous, 2006. Microbiology of food and animal feeding stuffs–Horizontal method for the enumeration of coliforms–Colony count technique. Available from: <https://www.iso.org/standard/38282.html>. Accessed on: January 5, 2023.
- Duarte, S., Shah, M. A., Sanches Silva, A., 2025. Flaxseed in diet: a comprehensive look at pros and cons. *Molecules* 30(6), 1335. DOI: <https://doi.org/10.3390/molecules30061335>.
- Fernández-Garia, E., McGregor, J.U., Traylor, S., 1998. The addition of oat fiber and natural alternative sweeteners in the manufacture of plain yogurt. *Journal of Dairy Science* 81(3), 655–663. DOI: [https://doi.org/10.3168/jds.S0022-0302\(98\)75620-6](https://doi.org/10.3168/jds.S0022-0302(98)75620-6).
- Ganguly, S., Kumar, S., Singh, A. K., Sabikhi, L., 2014. Effect of fermentation by probiotic *Lactobacillus acidophilus* NCDC-13 on nutritional profile of a dairy-cereal based composite substrate. *Journal of Food and Nutritional Disorders* 59(8), 3073–3085. Available from: https://www.researchgate.net/publication/286245415_effect_of_fermentation_by_probiotic_lactobacillus_acidophilus_ncdc_13_on_nutritional_profile_of_a_dairycereal_based_composite_substrate.
- Girish, L., Rajunaik, B., Kumar, M.G., Singh, A.K., Naik, N.L., 2015. Development of a rabadi-like wheat based fermented milk beverage. *Frontiers in Veterinary Science* 4(1), 83–88. Available from: https://www.researchgate.net/profile/Laxmana-Naik/publication/279249821_development_of_a_Rabadi-like_wheat_based_fermented_milk_beverage/links/5abf7b650f7e9bfc045a7e91/Development-of-a-Rabadi-like-wheat-based-fermented-milk-beverage.pdf.
- Gokavi, S., Zhang, L., Huang, M.K., Zhao, X., Guo, M., 2005. Oat-based symbiotic beverage fermented by *Lactobacillus plantarum*, *Lactobacillus paracasei* ssp. *casei*, and *Lactobacillus acidophilus*. *Journal of Food Science* 70(4), M216–M223. DOI: <https://doi.org/10.1111/j.1365-2621.2005.tb07191.x>.
- Hu, Y., Shim, Y.Y., Reaney, M.J.T., 2020. Flaxseed gum solution functional properties. *Foods* 9, 681. DOI: <https://doi.org/10.3390/foods9050681>.
- Hussain, S.A., Garg, F.C., Pal, D., 2014. Effect of different preservative treatments on the shelf-life of sorghum malt based fermented milk beverage. *Journal of Food Science and Technology* 51(8), 1582–1587. Available from: https://www.researchgate.net/publication/257798501_effect_of_different_preservative_treatments_on_the_shelf-life_of_sorghum_malt_based_fermented_milk_beverage.
- Kailasapathy, K., 2006. Survival of free and encapsulated probiotic bacteria and their effect on the sensory properties of yoghurt. *LWT-Food Science and Technology* 39(10), 1221–1227. DOI: 10.1016/j.lwt.2005.07.013.
- Kausar, S., Hussain, A., Ashraf, S., Fatima, G., Javaria, S., Abideen, Z.U., Korma, S.A., 2024. Flaxseed (*Linum usitatissimum*); phytochemistry, pharmacological

- characteristics and functional food applications. Food Chemistry Advances 4, 100573. DOI: <https://doi.org/10.1016/j.focha.2023.100573>.
- Markowiak, P., Slizewska, K., 2017. Effects of probiotics, prebiotics, and synbiotics on human health. Nutrients 9(9), 1021. DOI: 10.3390/nu9091021.
- Modha, H., Pal, D., 2011. Optimization of rabadi-like fermented milk beverage using pearl millet. Journal of Food Science and Technology 48(2), 190–196. DOI: 10.1007/s13197-010-0146-6.
- Mueed, A., Shibli, S., Jahangir, M., Jabbar, S., Deng, Z., 2023. A comprehensive review of flaxseed (*Linum usitatissimum* L.): health-affecting compounds, mechanism of toxicity, detoxification, anticancer and potential risk. Critical Reviews in Food Science and Nutrition 63(32), 11081–11104. DOI: <https://doi.org/10.1080/10408398.2022.2092718>.
- Nowak, W., Jeziorek, M., 2023. The role of flaxseed in improving human health. : Healthcare 11(3), 395. MDPI. DOI: <https://doi.org/10.3390/healthcare11030395>.
- Oliveira, M.N.D., Sodini, I., Remeuf, F., Corrieu, G., 2001. Effect of milk supplementation and culture composition on acidification, textural properties and microbiological stability of fermented milks containing probiotic bacteria. International Dairy Journal 11(11–12), 935–942. Available from: https://www.researchgate.net/publication/235819213_Effect_of_milk_supplementation_andculture_composition_on_acidification_textural_properties_and_microbiological_stabilityof_fermentedmilks_containing_probiotic_bacteria.
- Panesar, P.S., Shinde, C., 2012. Effect of storage on syneresis, pH, *Lactobacillus acidophilus* count, Bifidobacterium bifidum count of aloe vera fortified probiotic yoghurt. Current Research in Dairy Sciences 4(1), 17–23. Available from: <https://www.scirp.org/reference/referencespapers?referenceid=2753597>.
- Plissonneau, C., Sivignon, A., Chassaing, B., Capel, F., Martin, V., Etienne, M., Barnich, N., 2022. Beneficial effects of linseed supplementation on gut mucosa-associated microbiota in a physically active mouse model of Crohn's disease. International Journal of Molecular Sciences 23(11), 5891. DOI: <https://doi.org/10.3390/ijms23115891>.
- Pradhan, R.C., Meda, V., Rout, P.K., Naik, S., Dalai, A.K., 2010. Supercritical CO₂ extraction of fatty oil from flaxseed and comparison with screw press expression and solvent extraction processes. Journal of Food Engineering 98(4), 393–397. DOI: 10.1016/j.jfoodeng.2009.11.021.
- Rubilar, M., Gutierrez, C., Verdugo, M., Shene, C., Sineiro, J., 2010. Flaxseed as a source of functional ingredients. Journal of Soil Science and Plant Nutrition 10(3), 373–377. DOI: <http://dx.doi.org/10.4067/S0718-95162010000100010>.
- Sankarganesh, P., Adeyeye, S.A.O., Chakka, A.K., Babu, A.S., 2024. Prebiotics and probiotics food: future aspects. Food Coatings and Preservation Technologies 551–583. DOI: <https://doi.org/10.1002/9781394237623.ch16>.
- Shah N.P., 2004. Probiotics and prebiotics. Agro Food Industry Hi Tech 15(1), 13–16. Available from: <https://hub.hku.hk/handle/10722/144462>.
- Shahada, C., Morya, S., Awuchi, C.G., 2024. A narrative review on nutraceutical, food and industrial applications of flaxseed (*Linum usitatissimum* L). Cogent Food & Agriculture 10(1), 2306017. Available from: <https://www.tandfonline.com/doi/full/10.1080/23311932.2024.2306017>.
- Singh, K.K., Mridula, D., Rehal, J., Barnwal, P., 2011. Flaxseed: a potential source of food, feed and fiber. Critical Reviews in Food Science and Nutrition 51(3), 210–222. DOI: 10.1080/10408390903537241.
- Singh, S., Sharma, S., 2025. Lignans: advances in biosynthesis, bioavailability, and pharmacological activity. Combinatorial Chemistry & High Throughput Screening 28(8), 1331–1344. DOI: <https://doi.org/10.2174/0113862073316439240515072946>.
- Trias-Sanz, R., Stamon, G., Louchet, J., 2008. Using colour, texture, and hierarchical segmentation for high-resolution remote sensing. ISPRS Journal of Photogrammetry and Remote Sensing 63(2), 156–168. Available from: https://www.researchgate.net/publication/222677164_Using_colour_texture_and_hierarchical_segmentation_for_high-resolution_remote_sensing_ISPRS_Journal_of_Photogrammetry_and_Remote_Sensing_632_156-168.
- Ursioniu, S., Sahebkar, A., Andrica, F., Serban, C., Banach, M., 2016. Effects of flaxseed supplements on blood pressure: A systematic review and meta-analysis of controlled clinical trial. Clinical Nutrition 35(3), 615–625. DOI: <https://doi.org/10.1016/j.clnu.2015.05.012>.
- Vahedi, N., Tehrani, M.M., Shahidi, F., 2008. Optimizing of fruit yoghurt formulation and evaluating its quality during storage. American-Eurasian Journal of Agricultural & Environmental Science 3(6), 922–927. Available from: https://www.researchgate.net/publication/242304779_optimizing_of_fruit_yoghurt_formulation_and_evaluating_its_quality_during_storage.
- Yawale, P., 2022. A comprehensive review on recent novel food and industrial applications of flaxseed: 2014 onwards. Food and Feed Research 49(1), 67–95. DOI: 10.5937/ffr49-35420.