Journal of Human Environment and Health Promotion
Print ISSN: 2476-5481 Online ISSN: 2476-549X

Evaluation of Microbiological, Chemical, and Sensory Attributes of Milk-Based Beverage Containing Herbal Extracts

Nesa Azimzadeh [a], Adel Mirza Alizadeh [a*], Majid Aminzare [b], Hassan Hassanzadazar [c], Fataneh Hashempour-Baltork [d], Majid Shirmohammadi [e]

a. Department of Food Safety and Hygiene, School of Public Health, Zanjan University of Medical Sciences, Zanjan, Iran.
b. Health and Environment Research Center, Zanjan University of Medical Sciences, Zanjan, Iran.
c. Nutrition and Food Security Research Center, Zanjan University of Medical Sciences, Zanjan, Iran.
d. Halal Research Center of IRI, Iran Food and Drug Administration, Ministry of Health and Medical Education, Tehran, Iran.
e. Research and Development Department, Zanjan Pegah Dairy Co., Iran Dairy Industries Co., Zanjan, Iran.

***Corresponding author:** Department of Food Safety and Hygiene, School of Public Health, Zanjan University of Medical Sciences, Zanjan, Iran. Postal Code: 4513956184. E-mail: a.alizadeh@zums.ac.ir

ARTICLE INFO

Article type:
Original article

Article history:
Received: 22 July 2026
Revised: 2 September 2026
Accepted: 17 September 2026
Available online: 29 September 2026

© The Author(s)

https://doi.org/10.66224/jhehp.774

Keywords:

Milk-based beverage
Herbal extracts
Stevia
Physicochemical properties
Food safety

ABSTRACT

Background: The growing demand for reduced-sugar functional dairy drinks has increased interest in natural sweeteners. This study investigated the effect of partially replacing sucrose with stevia in milk-based beverages containing black tea, cinnamon, ginger, cardamom, and star anise extracts on physicochemical properties, microbiological quality, and sensory characteristics during storage.
Methods: All sucrose-replacement formulations contained the selected herbal extracts and were evaluated on days 1, 14, and 30 of storage. Physicochemical analyses included pH, titratable acidity, fat, total solids, and sucrose content. Microbiological quality was assessed by total bacterial and coliform counts. Sensory attributes were evaluated by trained panelists using a 5-point hedonic scale under blinded conditions.
Results: Physicochemical properties changed during storage among the formulations. Microbial counts increased over the storage period; however, no consistent significant differences were observed among the sucrose-stevia replacement treatments. Sensory evaluation indicated that the formulation containing 100% sucrose generally received the highest scores for taste and overall acceptability. Increasing the level of sucrose replacement with stevia, particularly at higher substitution levels, negatively affected taste and overall acceptance.
Conclusion: Partial sucrose replacement with stevia is feasible, but high levels reduce sensory acceptability. No consistent negative effect on microbiological quality was observed during storage.

1. Introduction

Milk-based beverages are among the most important products in the dairy industry because of their high nutritional value, good digestibility, and key bioactive constituents such as proteins, calcium, vitamins, and minerals. Increasing consumer awareness of the role of nutrition in maintaining health has driven growing demand for functional and health-oriented foods. In this context, dairy beverages account for a considerable share of the functional food market (Sani et al., 2019). Moreover, the shift in consumer perspectives from extending lifespan to improving quality of life and promoting healthy aging has further emphasized the need to develop fortified dairy products (Özer & Kirmaci, 2010).

Milk and milk-based products play an essential role in the human diet because they provide high-quality proteins, essential fatty acids, lactose, vitamins, and minerals

How to cite: Azimzadeh, N., Mirza Alizadeh, A., Aminzare, M., Hassanzadazar, H., Hashempour-Baltork, F., & Shirmohammadi, M. (2026). Evaluation of Microbiological, Chemical, and Sensory Attributes of Milk-Based Beverage Containing Herbal Extracts. *J Hum Environ Health Promot*, *12*(4), 261-270.

(Shahbazi et al., 2016). In addition to their nutritional benefits, milk-based beverages are widely accepted due to their desirable sensory attributes and favorable functional properties (Granato et al., 2010). These products are generally classified into fermented and non-fermented categories, each characterized by distinct physicochemical and microbiological features (Jelen, 2009). Among them, flavored and ultra-high temperature (UHT) beverages are of particular interest in the food industry because they can be formulated using natural ingredients (Gürakan et al., 2009).

In recent years, the incorporation of medicinal plants and aromatic spices into functional dairy beverages has increased. According to the World Health Organization (WHO), approximately 80% of the global population uses herbal preparations for primary health care (Abdolmaleki et al., 2020; Khajebishak & Payahoo, 2015). The addition of herbal extracts to dairy beverages not only contributes desirable flavors and aromas but may also enhance antioxidant, anti-inflammatory, and antimicrobial properties. Common botanicals used in such products include ginger, cardamom, cinnamon, star anise, and black tea.

Ginger (*Zingiber officinale*), primarily due to the presence of gingerols, exhibits antioxidant, antibacterial, and anti-inflammatory activities and has been associated with improved gastrointestinal function (Mashhadi et al., 2013; Prasad & Tyagi, 2015). Cardamom (*Elettaria cardamomum*) also demonstrates high antioxidant activity as a result of its phenolic compounds and aromatic essential oils (Baghy Nia et al., 2008; Hinneburg et al., 2006). Cinnamon (*Cinnamomum verum*) contains bioactive constituents such as cinnamaldehyde and has been reported to have antibacterial, antifungal, and antidiabetic properties (Firmino et al., 2018). Similarly, star anise (*Illicium verum*), due to compounds such as anethole and shikimic acid, shows notable antimicrobial and antioxidant potential (Alias & Shafie, 2024). Black tea, derived from *Camellia sinensis* is a rich source of polyphenols and catechins and has received considerable attention because of its antioxidant activity (Robinson et al., 1997).

The combination of milk with black tea and spices such as ginger, cinnamon, cardamom, and star anise results in a well-known traditional beverage consumed in many Asian and Middle Eastern countries, commonly referred to as "Karak milk" or "Karak tea". In addition to its favorable sensory properties, this beverage contains multiple bioactive compounds. However, conventional formulations typically contain high levels of sugar, which may increase the risk of obesity and metabolic disorders.

In recent years, the use of low-calorie natural sweeteners in food product development has increased (Mahdian et al., 2011). Stevia (*Stevia rebaudiana*) has attracted substantial attention as a natural sweetener with high sweetening power. Its major constituents include steviol glycosides such as stevioside and rebaudioside, which, in addition to providing sweetness, have been associated with antioxidant properties (Dyduch-Siemińska et al., 2020; Sakr & Massoud, 2021). Stevia has also been reported to exhibit anti-inflammatory and antimicrobial effects (Kinghorn, 2001; Ozdemir & Ozcan, 2020).

Although numerous studies have investigated the use of herbal extracts or natural sweeteners in food products, limited information is available regarding the combined effects of herbal spices, black tea, and stevia in dairy beverages. Several studies have explored partial combinations, such as the use of stevia in dairy products (Alizadeh et al., 2014), the incorporation of herbal extracts in milk-based beverages (Sukhikh et al., 2019), and the development of functional drinks based on black tea (Tewari et al., 2018). Therefore, the present study aimed to develop and evaluate a novel milk-based beverage similar to Karak milk, containing extracts of ginger, cinnamon, cardamom, star anise, black tea, and stevia. In this work, the effects of these ingredients on physicochemical characteristics, microbiological quality, and sensory attributes during storage were investigated to assess the feasibility of developing a functional dairy beverage with reduced sugar content and enhanced nutritional value.

2. Materials and Methods

2.1 Materials

The raw materials used in this study included sterilized milk, herbal extracts, and sweeteners. Standard sterilized milk (sterilized low-fat milk by the UHT method), used as the main beverage base, was purchased from Pegah Co. The herbal extracts included cinnamon (*Cinnamomum verum*), ginger (*Zingiber officinale*), cardamom (*Elettaria cardamomum*), and star anise (*Illicium verum*), which were incorporated to impart functional properties and improve the sensory attributes of the beverage. These extracts were obtained from Giah Kala Co., Tehran, Iran. To provide sweetness in the beverage formulation, sucrose and the natural sweetener stevia (*Stevia rebaudiana*) were used; both were purchased from Giah Kala Co.

2.2 Preparation of Natural Plant Extracts

To prepare the natural extracts, the plant materials, including cinnamon, ginger, cardamom, and star anise (50 g each), as well as black tea (30 g), were first washed with cold water to reduce surface contaminants. 1000 mL of boiling water was then added, and extraction was performed at 85-95 °C for 15 min. Subsequently, 1000 mL of boiling water was added again, and extraction was continued at 90 °C for an additional 15 min. After cooling the mixture to approximately 70 °C, the extract was passed through a mesh filter. Finally, the primary and secondary extracts were combined to obtain the final extract.

2.3 Purification of Extracts

To remove coarse particles and reduce microbial load, the final extract was first passed through a mesh filter and then vacuum-filtered to minimize potential contaminants and microbial spores.

CC BY NC

2.4 Preparation of Hydroalcoholic Extracts

Hydroalcoholic extracts were included in the experimental design to obtain extracts with higher purity, lower microbial load, and improved functional performance compared with natural aqueous extracts. In another part of the experiment, ready-to-use hydroalcoholic extracts supplied by Giah Kala Co. were used. For preparation of the milk-based beverage, a specified amount of each herbal extract (cinnamon, ginger, cardamom, and star anise) was added to the milk base, and the remaining processing steps were identical to those used for beverages prepared with natural extracts.

2.5 Beverage Formulation

For beverage preparation, sterilized milk was heated to 80 °C. The herbal extracts, along with specified amounts of sucrose and stevia, were added to the milk. The mixture was then heated for 5 min at 80-85 °C to ensure homogeneous dispersion of the ingredients in the milk base.

2.6 Experimental Treatments

Four treatments were prepared based on the percentage replacement of sucrose with stevia in the beverage formulation:

- SUC100: 100% sucrose and 0% stevia
- SUC75: 75% sucrose + 25% stevia
- SUC50: 50% sucrose + 50% stevia
- SUC25: 25% sucrose + 75% stevia

In all treatments, the herbal extracts (cinnamon, ginger, cardamom, and star anise) were added to the milk base, and the effect of sucrose replacement with stevia on the physicochemical and sensory properties of the beverage was evaluated.

2.7 Physicochemical Analysis

Physicochemical properties of the samples, including pH, titratable acidity, total solids, fat content, and sucrose percentage, were determined. These analyses were performed according to the Iranian National Standards (INSO, 2014; INSO, 2016).

2.8 Microbiological Analysis

To evaluate microbiological quality, microbial tests including total plate count (TPC) and coliform enumeration were conducted according to Iranian National Standard No. 2406 (microbiology of milk and dairy products) to assess product microbial safety.

2.9 Sensory Evaluation

Sensory evaluation of the milk-based beverage samples was conducted by a trained panel consisting of 10 professional panelists from Pegah Company. Panelists were selected based on their sensory evaluation experience and were screened to ensure that they had no common cold symptoms at the time of testing, which could interfere with olfactory and gustatory perception. The sensory attributes, including color, aroma, taste, texture (mouthfeel), and overall acceptability, were evaluated using a five-point hedonic scale, where 1 represented dislike very much and 5 represented like very much. The sensory tests were conducted in the sensory laboratory of Pegah Company under controlled conditions. Samples were presented in identical containers and labeled with random three-digit codes to ensure blind evaluation of the treatments. The sensory assessments were carried out over several sessions. All panelists received appropriate instructions before the evaluation, and informed consent was obtained from all participants before their participation in the sensory test. The resulting sensory scores were used for subsequent statistical analysis.

2.10 Shelf-Life Evaluation

To assess product stability during storage, physicochemical and microbiological analyses were performed at three time points: days 1, 14, and 30 after production. These analyses were conducted based on the Iranian National Standard for sterilized milk.

2.11 Statistical Analysis

All experiments were carried out in three independent replicates, and results were reported as mean ± standard deviation. Statistical analysis was performed using GraphPad Prism (version 10). Differences among treatments were evaluated by one-way analysis of variance (one-way ANOVA), and mean comparisons were performed at a significance level of $p < 0.05$.

3. Results and Discussion

3.1 Physicochemical results

Physicochemical and sensory analyses performed on the milk beverage samples containing the above-mentioned extracts indicated that the developed formulation satisfactorily met the expected characteristics of the final product (Table 1). The measured pH, titratable acidity, sucrose content, and sensory indices were comparable to relevant national and international standards, reflecting appropriate stability and overall quality.

At the same time, the sugar level in the initial sample was slightly higher than the desired target, which may influence perceived sweetness and consumer acceptance. The most pronounced negative effect was observed as a marked reduction in taste and overall acceptance scores, such that the formulation containing 25% sucrose achieved the lowest consumer satisfaction. In contrast, samples containing 75% and 100% sugar received higher scores. Conversely, attributes related to color, aroma, and texture were not substantially affected, and no statistically significant differences were observed for these parameters. These findings highlight the importance of maintaining an

appropriate sugar level to preserve desirable sensory properties; although sugar reduction is advantageous from a health perspective, low-sugar formulations require further optimization to improve flavor. This issue should be considered in subsequent formulation optimization phases.

In addition, the extract components (cinnamon, ginger, cardamom, and anise/star anise) successfully imparted the characteristic taste and aroma profile, as confirmed by sensory evaluation. This observation emphasizes that careful selection of natural additives can substantially contribute to final product quality.

In Figure 1, changes in the fat content of the milk-based beverage in four different treatments over 30 days of storage are presented. Statistical analysis indicated that there were no significant differences in fat content either among the treatments or throughout the storage period ($P \geq 0.05$).

Table 1. Compositional details and key parameters of the formulations examined in both natural and hydroalcoholic extracts (per 208 g product)

Treatment	Sweetener composition		Cinnamon extract content	Ginger extract content	Cardamom extract content	Anise extract content	Black tea extract content
	Sugar (g & %)	Stevia (g & %)					
SUC.100	10.6 (5.1&100%)	0 (0%)	1	1	1	1	0.8
SUC.75	7.95 (3.8&5%)	0.4 (0.192&25%)	1	1	1	1	0.8
SUC.50	5.3 (2.54&50%)	0.8 (0.384&50%)	1	1	1	1	0.8
SUC.25	2.65 (1.27&25%)	1.2 (0.576&75%)	1	1	1	1	0.8

* SUC100: 100% sucrose and 0% stevia; SUC75: 75% sucrose + 25% stevia; SUC50: 50% sucrose + 50% stevia; SUC25: 25% sucrose + 75% stevia.

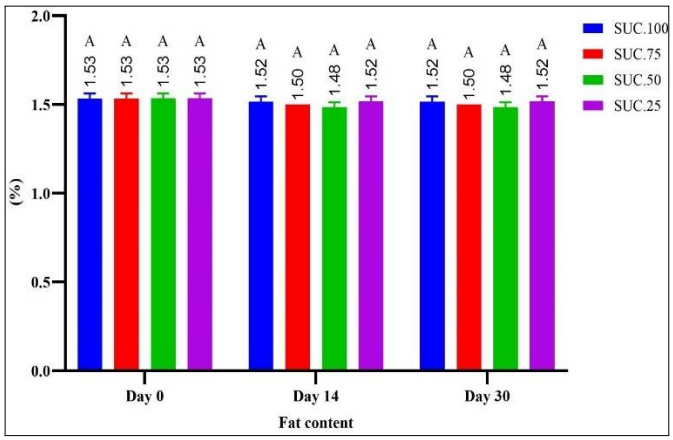


Figure 1. Fat content analysis of the milk-based beverage containing herbal extracts

This finding indicates that the replacement of sucrose with stevia had no impact on the fat content of the product, and the lipid composition of the beverage remained stable throughout the storage period. From a scientific perspective, this result is expected, as stevia is a non-lipid sweetener, and its substitution for sucrose does not alter the fat content of the formulation. Furthermore, under the storage conditions of the present study, potential lipid degradation processes, such as oxidation or hydrolysis, were not significant enough to cause a substantial change in fat content. The stability of the fat content during storage is consistent with the findings of Basharat et al. (2020) in their study on soy milk, in which they stated that lipid compounds in formulated plant-based beverages do not undergo major changes when the emulsion remains stable. Additionally, Kasapidou et al. (2023) reported that the fat content in plant-based milk alternatives is primarily a function of the raw materials (the plant base), and flavoring additives or sweeteners do not induce perceptible changes, which aligns with the results of this study regarding the lack of effect of stevia.

The acidity profile (Figure 2) illustrates changes in this parameter across the different milk beverage treatments during the 30-day storage period. The results showed that at each sampling time, no significant differences were observed among the treatments in terms of titratable acidity ($P \geq 0.05$), indicating that replacing sucrose with stevia did not have a noticeable effect on product acidity. However, evaluation of changes over storage time demonstrated a significant increasing trend in acidity in all treatments throughout the storage period ($P < 0.05$). Such an increase in acidity during the shelf life of dairy products is a common phenomenon and is mainly attributed to microbial activity and the conversion of lactose into lactic acid.

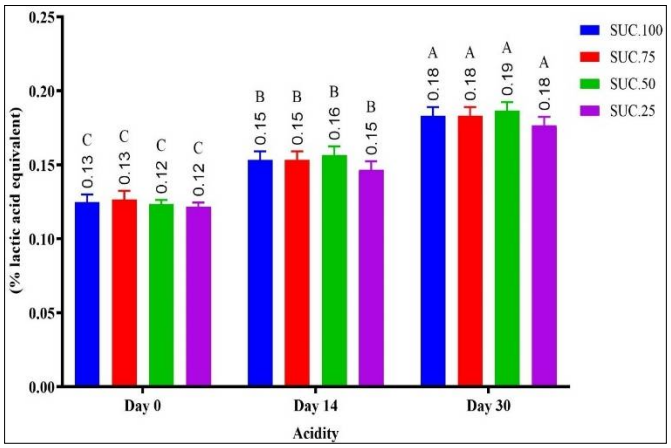


Figure 2. Titratable acidity analysis of the milk-based beverage containing herbal extracts

The pH profile (Figure 3) depicts changes in this parameter across the different milk beverage treatments during the storage period. The results showed that at each sampling time, no significant differences were observed among the treatments ($P \geq 0.05$), indicating that replacing sucrose with stevia did not have a significant effect on the beverage pH. However, assessment of changes over time demonstrated that pH decreased significantly from day 0 to days 14 and 30 ($P < 0.05$), whereas no significant difference was detected between days 14 and 30 ($P \geq 0.05$). This decline in pH during storage is a common phenomenon in dairy products and is mainly attributed to microbial metabolic activity and the gradual production of organic acids over time. This trend is also consistent with the observed increase in titratable

acidity and microbial load on days 14 and 30, suggesting that as storage time progresses and microbial activity increases, the beverage environment becomes more acidic and pH decreases. Therefore, the findings of this study indicate that although the type of sweetener was altered, this change did not significantly affect the natural trend of increasing acidity during storage.

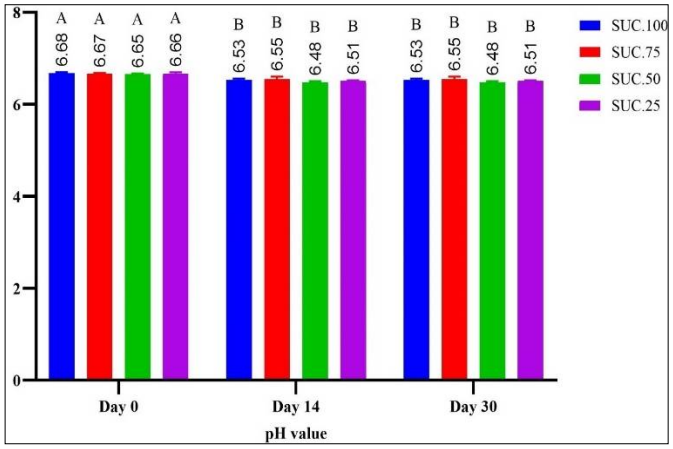


Figure 3. pH analysis of the milk-based beverage containing herbal extracts

The progressive increase in acidity and corresponding decrease in pH during storage are consistent with the findings reported by Vallath et al. (2021) for a chickpea-based beverage and by Jokar and Azizi (2022) for a milk–persimmon beverage. These changes can be attributed primarily to the growth and metabolic activity of psychrotrophic microorganisms during refrigerated storage. The utilization of available carbohydrates by these microorganisms can result in the formation and accumulation of organic acids, which subsequently increase titratable acidity and lower the pH of the beverage. Therefore, the observed changes in acidity and pH during storage may reflect ongoing microbial metabolic activity and the progressive production of organic acids. Moreover, Najman et al. (2024) noted that in commercial plant-based beverages, pH changes are less dependent on the type of sweetener than on enzymatic and microbial activities throughout shelf life, which supports the lack of significant differences among the stevia treatments in the present study.

The sucrose content profile (Figure 4) illustrates a significant reduction in sugar levels across the various treatments, showing that as the percentage of sucrose substitution with stevia increases, the sucrose content in the formulations decreases significantly ($P < 0.05$). This trend confirms that the treatment containing 100% sucrose exhibited the highest sugar concentration, while higher levels of stevia substitution led to a gradual reduction in sucrose content, which is entirely consistent with the formulations designed for this study.

On the other hand, the investigation of changes during storage revealed that sucrose content in each treatment did not change significantly over the 30-day storage period ($P \geq 0.05$), which is consistent with the non-reducing nature of sucrose and its relative stability under the mild storage conditions applied in this study. This stability indicates that the sucrose present in the beverage matrix did not undergo significant chemical degradation or fermentation throughout the shelf life, and its concentration remained nearly constant over time. Consequently, it can be stated that the storage conditions and the formulation composition used in this study successfully maintained the stability of sucrose during the storage period. These findings are in agreement with the report by Jeske et al. (2017), who demonstrated that the removal or reduction of added sugars in plant-based beverages directly leads to a decrease in the final product's sucrose content. Furthermore, Kasapidou et al. (2023) stated that modifications in sweetener levels can significantly influence the carbohydrate composition of such beverages.

The non-fat solids (SNF) profile (Figure 5) shows a significant decrease in this parameter as the level of sucrose replacement with stevia increases in the beverage formulation ($P < 0.05$). At all sampling times, the control treatment containing 100% sucrose exhibited the highest SNF value, and as the sucrose concentration in the formulation decreased, the SNF content in the other treatments declined significantly. This pattern indicates that reducing sucrose in the formulation directly lowers the non-fat solids content of the product. Scientifically, this outcome is expected because sucrose constitutes a substantial portion of the soluble solids in the beverage. In contrast, stevia (due to its very high sweetening power) is used at very low levels and contributes negligibly to the total solids; therefore, replacing sucrose with stevia reduces the overall non-fat solids.

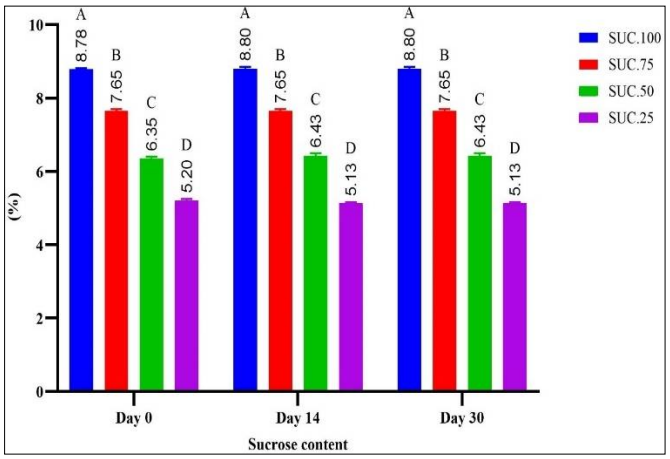


Figure 4. Sucrose content analysis of the milk-based beverage containing herbal extracts

In addition, evaluation of changes during storage demonstrated that SNF did not change significantly within any treatment over the 30-day storage period ($P \geq 0.05$). This stability suggests that the formulation components remained stable and that no substantial changes such as precipitation, degradation, or loss of solids occurred throughout the product's shelf life. Similar results were

reported by Najman et al. (2024) for rice-based beverages, where soluble solids composition was directly influenced by formulation ingredients. Moreover, Azra et al. (2025) reported that altering the initial composition of plant-based beverages can affect the overall non-fat solids content, whereas this parameter typically remains stable during storage.

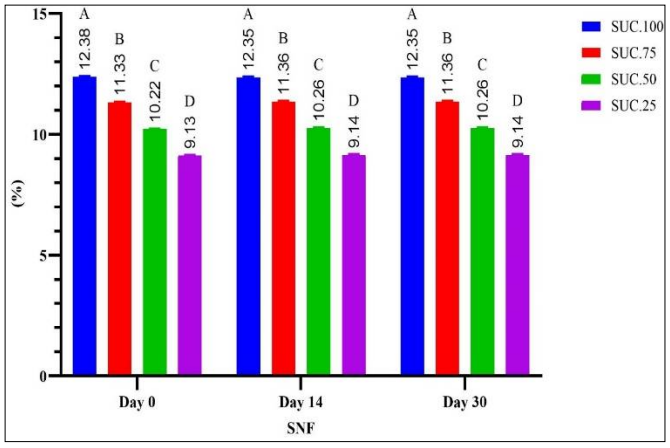


Figure 5. Non-fat solids (SNF) content analysis of the milk-based beverage containing herbal extracts

3.2 Microbiological results

The total microbial load profile (Figure 6) illustrates changes in the microorganism population in the different treatments over the 30-day storage period. The results showed that the total microbial count increased significantly from day 0 to days 14 and 30 ($P < 0.05$), indicating the gradual growth of microorganisms during storage. However, no significant difference was observed between days 14 and 30 ($P \geq 0.05$), suggesting that the microbial population reached a relatively stable phase over this interval.

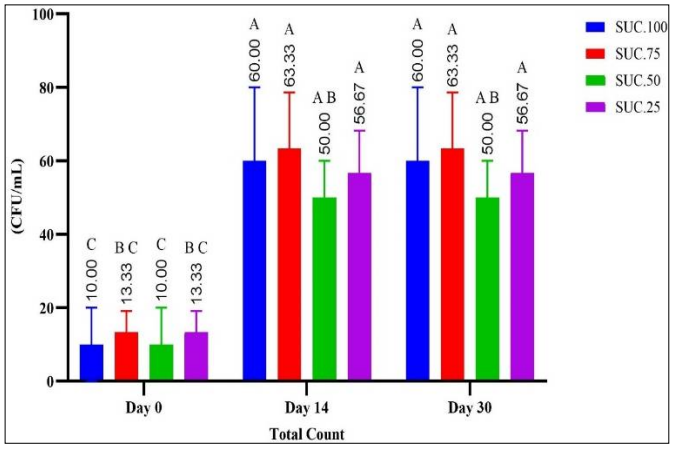


Figure 6. Total microbial count analysis of the milk-based beverage containing herbal extracts

When treatments were compared, at most sampling times no significant differences were detected among the different formulations (i.e., sucrose replacement with stevia) ($P \geq 0.05$). This finding indicates that using stevia instead of sucrose did not adversely affect the microbial stability of the product, and the stevia-containing treatments exhibited microbial behavior comparable to the control sample (100% sucrose). An increase in microbial load over storage time is considered a natural phenomenon in dairy products, mainly because the matrix provides suitable nutrients for microbial growth throughout shelf life. Overall, the results of the present study indicate that changing the type of sweetener did not significantly affect the general trend of microbial growth.

A time-dependent increase in microbial load is a common pattern in plant-based beverages and was also reported by Enayatian et al. (2025) in plant-based desserts. The lack of a significant effect of stevia on microbial growth is consistent with the findings of Hidalgo-Fuentes et al. (2024), who stated that non-nutritive sweeteners such as stevia, unlike sucrose, do not provide an ideal substrate for rapid microbial fermentation; however, at low concentrations they also do not exert strong antimicrobial effects that would create a marked difference compared with sugar-containing treatments.

In assessments for the presence of coliform-group bacteria, no growth was observed, and the result of this test was reported as negative for all samples. This outcome indicates compliance with GMP principles and appropriate hygienic conditions during extraction and processing.

3.3 Sensory results

The sensory evaluation profile (Figure 7-A) demonstrates that as the level of sucrose replacement with stevia increases, scores for certain sensory attributes, particularly taste, overall acceptability, and to some extent, texture decline significantly ($P < 0.05$). The control treatment (containing 100% sucrose) consistently received the highest scores across most sensory attributes, including taste, color, and overall acceptability, and in many instances, outperformed treatments with higher levels of stevia substitution.

As sucrose levels were reduced to 75% and 50%, a relatively moderate decrease in certain sensory characteristics, such as taste and texture, was observed; however, these samples still maintained an acceptable level of consumer preference. In contrast, the treatment with the highest level of stevia substitution (SUC.25) exhibited a substantial decline in taste and overall acceptability scores, indicating that excessive replacement of sucrose negatively impacts the sensory quality of the product. Among the evaluated attributes, color was the least affected by the formulation changes, remaining at a relatively favorable level across most treatments. Overall, the results of the sensory evaluation indicate that stevia can be utilized at moderate substitution levels without causing a severe decline in sensory quality; conversely, complete or near-complete replacement of sucrose with stevia may lead to a perceptible reduction in sensory desirability, particularly regarding the taste and overall acceptability of the product.

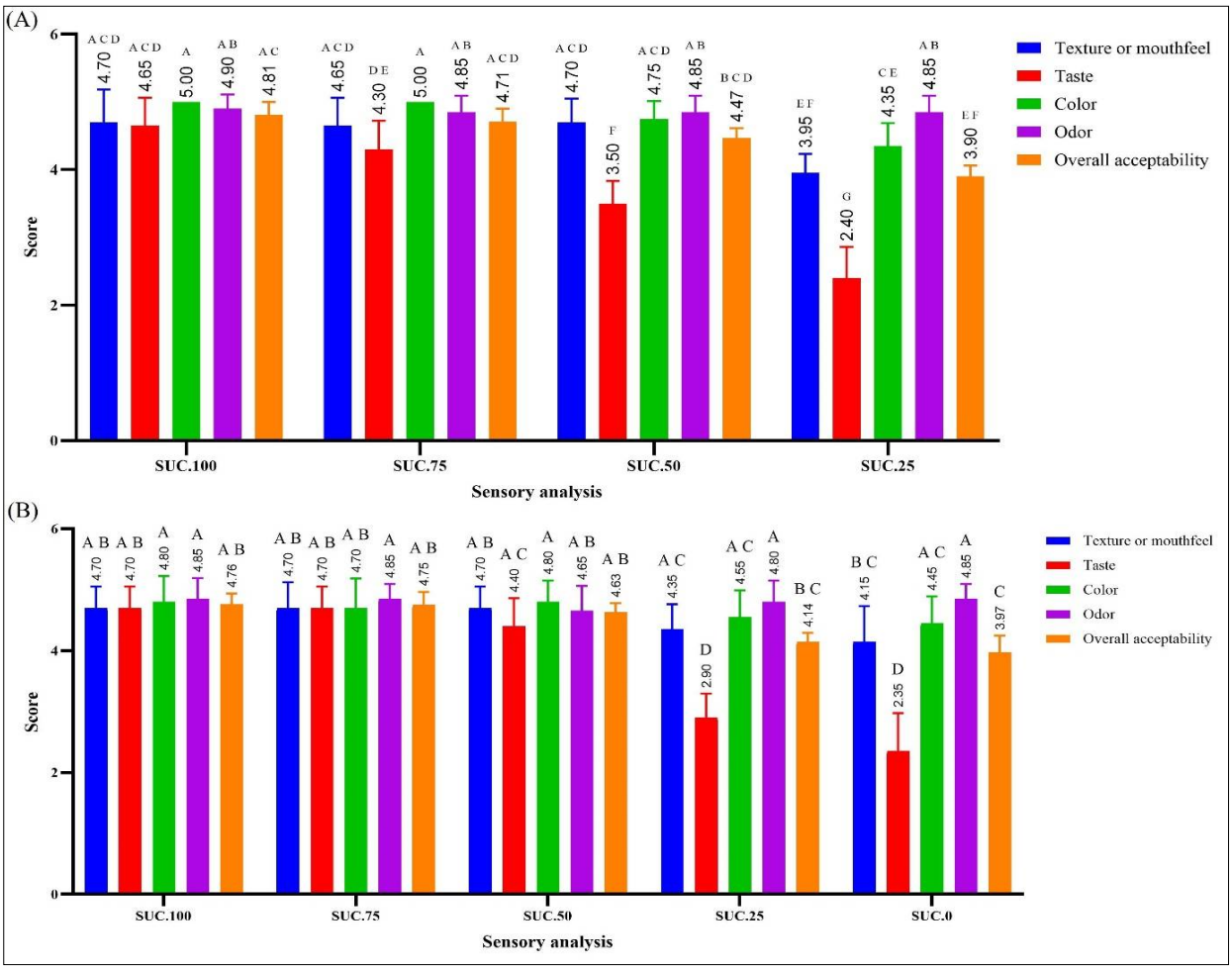


Figure 7. Sensory evaluation analysis of the milk-based beverage formulated with the same herbal components prepared as hydroalcoholic extracts (A) or natural extracts (B)

The sensory evaluation of the natural extracts (Figure 7-B) indicates that as the level of sucrose replacement with stevia increases, certain sensory attributes are affected, and a significant decline is observed in some traits. The results showed that color and odor did not differ significantly among the treatments ($P \geq 0.05$), suggesting that these attributes remained stable in the presence of natural stevia extract and were not noticeably influenced by formulation changes. In contrast, attributes such as taste, texture/mouthfeel, and overall acceptability exhibited a significant downward trend as sucrose levels decreased ($P < 0.05$). In particular, treatments with the lowest sucrose content showed a more pronounced reduction in taste and overall acceptability, indicating the negative impact of extensive sucrose replacement with natural stevia extract on the product's sensory acceptance.

On the other hand, the treatments containing 100%, 75%, and 50% sucrose showed no significant differences from one another for most sensory attributes, indicating that moderate sucrose replacement with natural stevia extract, especially up to approximately 50%, can be implemented without a noticeable loss in sensory quality. Overall, these findings suggest that using natural stevia extract at moderate levels may be a suitable strategy for reducing sucrose, whereas high substitution levels may lead to diminished sensory desirability, particularly in terms of taste and overall acceptability.

The decline in taste and overall acceptability scores with increasing stevia concentration is consistent with the findings of Frühauf et al. (2023) who noted the "bitter aftertaste" of plant-based sweeteners in commercial beverages. In addition, Lee et al. (2025) reported in nut-based beverages that complete sucrose replacement can affect mouthfeel and texture due to reduced viscosity resulting from sugar removal. The stability of color in the present study also aligns with Kolniak-Ostek et al. (2025) who showed that the use of colorless sweeteners does not affect the optical properties of plant-based beverages.

A comparison of sensory results between the two extract types indicated that natural extracts performed more consistently in maintaining appearance- and aroma-related attributes, including color and odor, and retained high scores across all treatments. In contrast, samples prepared using hydroalcoholic extracts showed greater variability, and with

increasing sucrose replacement, a gradual decrease was observed in the scores for these attributes. As the sucrose replacement level increased, particularly in the treatments with the highest substitution, taste and overall acceptability scores decreased significantly for both extract types ($P < 0.05$). However, for the natural extract at the 50% replacement level, sensory characteristics were more similar to the control, and the product maintained relatively favorable acceptability. Conversely, in the hydroalcoholic extract, reductions in taste and overall acceptability were more evident at moderate and high substitution levels, which may be attributed to the development of potential aftertaste and bitterness associated with the extraction process. Overall, the results suggest that the natural extract, due to better preservation of aromatic compounds and a lower tendency to generate strong off-flavors, may be a more suitable option for producing a milk-based beverage containing herbal extracts while maintaining sensory properties closer to the traditional product.

3.4 Bioactive compound characterization

To identify and characterize the herbal extracts of cinnamon (*Cinnamomum verum*), ginger (*Zingiber officinale*), cardamom (*Elettaria cardamomum*), star anise (*Illicium verum*), black tea (*Camellia sinensis*), and stevia (*Stevia rebaudiana*), comprehensive evaluations of physicochemical parameters and key bioactive constituents were conducted.

In addition to evaluating the physicochemical properties of the formulated beverage, the bioactive constituents of the herbal extracts incorporated into the formulation were characterized to assess their potential functional benefits. The analysis revealed considerable variation in the type and concentration of the major bioactive compounds among the extracts. Cinnamon extract exhibited a total phenolic content of 0.92 mg/mL, determined by UV spectrophotometry, while ginger extract contained 0.43% 6-gingerol, quantified using high-performance liquid chromatography (HPLC). Cardamom and Illicium verum extracts showed similar phytochemical profiles, each containing 0.6% total polyphenols as measured by UV analysis. Black tea extract was characterized by a total catechin content of 0.2%; Stevia extract, which served as the principal sugar-replacement ingredient in the formulation, contained 50.11% total steviol glycosides and 98.04% rebaudioside A, as determined by HPLC; these values are reported to characterize this key formulation component rather than to imply a direct comparison with the crude herbal extracts. These compounds are widely recognized for their antioxidant, antimicrobial, and health-promoting properties and may contribute significantly to the functional quality of food products. The presence of these bioactive constituents at appreciable levels indicates the satisfactory quality of the extracts used in the formulation and highlights their potential role in enhancing the nutritional and functional value of the beverage. Therefore, beyond providing desirable sensory attributes, the selected herbal extracts may improve the overall health-promoting characteristics of the final product through their diverse phytochemical composition.

4. Conclusion

The findings of this study demonstrate that partial replacement of sucrose with the natural sweetener stevia in a milk-based beverage containing herbal extracts maintains the product's physicochemical stability (including pH, titratable acidity, and fat content) during 30 days of storage. Stevia substitution did not adversely affect the microbiological quality of the beverage, as no statistically significant differences in microbial counts were observed among the formulations, although counts expectedly increased over time. The primary challenge of high stevia replacement levels was a significant decline in sensory acceptability, particularly due to its bitter aftertaste. While the formulation with 100% sucrose received the highest sensory scores, the SUC50 formulation (50% sucrose replacement) represents a balanced approach to formulating a reduced-sugar beverage with acceptable physicochemical stability and sensory quality.

Authors' Contributions

Nesa Azimzadeh: Conceptualization; Investigation; Writing-original draft; Formal analysis; Data curation; Resources; Methodology; Funding acquisition. **Adel Mirza Alizadeh:** Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Writing-review and editing. **Majid Aminzare:** Conceptualization; Formal analysis; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Writing-review and editing. **Hassan Hassanzadazar:** Conceptualization; Investigation; Methodology; Validation. **Fataneh Hashempour-Baltork:** Conceptualization; Investigation; Methodology; Validation. **Majid Shirmohammadi:** Conceptualization; Investigation; Methodology; Validation.

Funding

This project was financially supported by Zanjan University of Medical Sciences (project code: 1490).

Conflicts of Interest

The authors declare that there is no conflict of interest.

Acknowledgments

This paper was extracted from Nesa Azimzadeh's M.Sc. thesis and supported by Zanjan University of Medical Sciences, Zanjan, Iran.

Ethical Considerations

Informed consent was obtained from all participants in the sensory evaluation, and the study was approved by the Ethics

Committee of Zanjan University of Medical Sciences (Ethical Code: IR.ZUMS.BLC.1404.084).

Using Artificial Intelligence

AI tools were used solely for language editing and improving the manuscript's clarity.

References

Abdolmaleki, A., Akram, M., Muddasar Saeed, M., Asadi, A., & Kajkolah, M. (2020). Herbal medicine as neuroprotective potential agent in human and animal models: A historical overview. *Journal of Pharmaceutical Care*, *8*(2), 75-82.

Alias, A. H. D., & Shafie, M. H. (2024). Star anise (*Illicium verum* Hook. F.) polysaccharides: Potential therapeutic management for obesity, hypertension, and diabetes. *Food Chemistry*, *460*, 140533.

Alizadeh, M., Azizi-Lalabadi, M., Hojat-Ansari, H., & Kheirouri, S. (2014). Effect of *Stevia* as a substitute for sugar on physicochemical and sensory properties of fruit based milk shake. *Journal of Scientific Research and Reports*, *3*(11), 1421-1429.

Azra, J. M., Nurdiani, R., Nasution, Z., Aries, M., & Sutiari, N. K. (2025). Physicochemical and sensory properties of plant-based milk alternative produced from pigeon pea and soybean. *Journal of Food Technology & Industry/Jurnal Teknologi & Industri Pangan*, *36*(2), 167.

Baghy Nia, N., Oryan, Sh., Fani, A., & Maleky Rad, A. (2008). The effect of Cardamom-tea watery extract on oxidative stress. *Journal of Arak University of Medical Sciences*, *11*(4), 1-7.

Basharat, S., Ijaz, A., Tufail, T., Ain, H. B. U., Abid, F., Azhar, S., . . . & Sikander, S. (2020). Nutritional and physicochemical characterization of soymilk. *International Journal of Biosciences*, *16*(5), 256-264.

Dyduch-Siemińska, M., Najda, A., Gawroński, J., Balant, S., Świca, K., & Żaba, A. (2020). *Stevia rebaudiana* Bertoni, a source of high-potency natural sweetener-biochemical and genetic characterization. *Molecules*, *25*(4), 767.

Enayatian, A., Anvar, S. A. A., & Nowruzi, B. (2025). Evaluation of semi prepared probiotic dessert containing phycoerythrin and *Lactobacillus plantarum* for period of up to two months within the refrigerator. *Scientific Reports*, *15*(1), 26357.

Firmino, D. F., Cavalcante, T. T. A., Gomes, G. A., Firmino, N. C. S., Rosa, L. D., de Carvalho, M. G., & Catunda Jr, F. E. A. (2018). Antibacterial and antibiofilm activities of *Cinnamomum* sp. essential oil and cinnamaldehyde: Antimicrobial activities. *The Scientific World Journal*, *2018*(1), 7405736.

Frühauf, V., Egea, M., Hernandes, T., & Takeuchi, K. (2023). Relationship between physicochemical and sensory characteristics of commercial plant-based beverages. *Journal of Culinary Science & Technology*, *21*(6), 886-902.

Granato, D., Branco, G. F., Cruz, A. G., Faria, J. d. A. F., & Shah, N. P. (2010). Probiotic dairy products as functional foods. *Comprehensive Reviews in Food Science and Food Safety*, *9*(5), 455-470.

Gürakan, G., Cebeci, A., & Özer, B. (2009). Probiotic dairy beverages: Microbiology and technology. In F. Yildiz, *Development and manufacture of yogurt and other functional dairy products* (pp. 165-197). CRC Press.

Hidalgo-Fuentes, B., de Jesús-José, E., Cabrera-Hidalgo, A. D. J., Sandoval-Castilla, O., Espinosa-Solares, T., González-Reza, R. M., . . . & Aguilar-Toalá, J. E. (2024). Plant-based fermented beverages: Nutritional composition, sensory properties, and health benefits. *Foods*, *13*(6), 844.

Hinneburg, I., Dorman, H. D., & Hiltunen, R. (2006). Antioxidant activities of extracts from selected culinary herbs and spices. *Food Chemistry*, *97*(1), 122-129.

INSO. (2014). Flavored milk-specifications & test methods, NO. 1527, 2nd. Revision, ICS:67.100.10. *Iranian National Standardization Organization.* standard.inso.gov.ir

INSO. (2016). Pasteurized milk specifications & test method, NO. 93, 5th. Revision, ICS: 67.100.10. *Iranian National Standardization Organization.* standard.inso.gov.ir

Jelen, P. (2009). Whey-based functional beverages. In P. Paquin, *Functional and speciality beverage technology* (pp. 259-280). Elsevier.

Jeske, S., Zannini, E., & Arendt, E. K. (2017). Evaluation of physicochemical and glycaemic properties of commercial plant-based milk substitutes. *Plant Foods for Human Nutrition*, *72*(1), 26-33.

Jokar, A., & Azizi, M. H. (2022). Formulation and production of persimmon milk drink and evaluation of its physicochemical, rheological, and sensorial properties. *Food Science & Nutrition*, *10*(4), 1126-1134.

Kasapidou, E., Basdagianni, Z., Papatzimos, G., Papadopoulos, V., Tsiftsi, E., Neki, I., . . . & Mitlianga, P. (2023). Chemical composition, antioxidant profile and physicochemical properties of commercial non-cocoa-and cocoa-flavoured plant-based milk alternatives. *European Food Research and Technology*, *249*(12), 3011-3026.

Khajebishak, Y., & Payahoo, L. (2015). Pomegranate: Its health effects from the Holy Quran and nutrition science view. *Journal of Islamic and Iranian Traditional Medicine*, *5*(4), 310-323.

Kinghorn, A. D. (2001). *Stevia: The genus Stevia*. CRC Press.

Kolniak-Ostek, J., Kita, A., Giacalone, D., Vázquez-Araújo, L., Noguera-Artiaga, L., Brzezowska, J., & Michalska-Ciechanowska, A. (2025). Physicochemical and instrumental flavor analysis of plant-based drinks with plant powder additions. *Foods*, *14*(15), 2593.

Lee, W., Ahn, H., Yim, J., Kim, Y., & Lee, K. G. (2025). Physicochemical properties and sensory attributes of nut-based milk coffee. *Scientific Reports*, *15*(1), 24238.

Mahdian, E., Mazaheri Tehrani, M., & Shahidi, F. (2011). Evaluation of the effect of soy flour on rheological properties of ice cream. *Journal of Food Science and Technology*, *8*(31), 107-114.

Mashhadi, N. S., Ghiasvand, R., Askari, G., Hariri, M., Darvishi, L., & Mofid, M. R. (2013). Anti-oxidative and anti-inflammatory effects of ginger in health and physical activity: Review of current evidence. *International Journal of Preventive Medicine*, *4*(Suppl 1), S36-S42.

Mohamadi Sani, A., Rahbar, M., & Sheikhzadeh, M. (2019). Traditional beverages in different countries: Milk-based beverages. In A. M. Grumezescu, & A. M. Holban, *Milk-based beverages* (pp. 239-272). Elsevier.

Najman, K., Ponikowska, P., Sadowska, A., Hallmann, E., Wasiak-Zys, G., Świderski, F., & Buczak, K. (2024). Physicochemical properties and nutritional relevance of rice beverages available on the market. *Applied Sciences*, *14*(19), 9150.

Ozdemir, T., & Ozcan, T. (2020). Effect of steviol glycosides as sugar substitute on the probiotic fermentation in milk gels enriched with red beetroot (*Beta vulgaris* L.) bioactive compounds. *LWT*, *134*, 109851.

Özer, B. H., & Kirmaci, H. A. (2010). Functional milks and dairy beverages. *International Journal of Dairy Technology*, *63*(1), 1-15.

Prasad, S., & Tyagi, A. K. (2015). Ginger and its constituents: Role in prevention and treatment of gastrointestinal cancer. *Gastroenterology Research and Practice*, *2015*, 142979.

Robinson, E. E., Maxwell, S. R., & Thorpe, G. H. (1997). An investigation of the antioxidant activity of black tea using enhanced chemiluminescence. *Free Radical Research*, *26*(3), 291-302.

Sakr, E. A., & Massoud, M. I. (2021). Impact of prebiotic potential of stevia sweeteners-sugar used as synbiotic preparation on antimicrobial, antibiofilm, and antioxidant activities. *LWT*, *144*, 111260.

Shahbazi, Y., Ahmadi, F., & Fakhari, F. (2016). Voltammetric determination of Pb, Cd, Zn, Cu and Se in milk and dairy products collected from Iran: An emphasis on permissible limits and risk assessment of exposure to heavy metals. *Food Chemistry*, *192*, 1060-1067.

Sukhikh, S. A., Astakhova, L. A., Golubcova, Y. V., Lukin, A. A., Prosekova, E. A., Milenteva, I. S., . . . & Rasshchepkin, A. N. (2019). Functional dairy products enriched with plant ingredients. *Foods and Raw Materials*, *7*(2), 428-438.

Tewari, Sh., Dubey, K. K., & Singhal, R. S. (2018). Evaluation and application of prebiotic and probiotic ingredients for development of ready to drink tea beverage. *Journal of Food Science and Technology*, *55*(4), 1525-1534.

Vallath, A., Shanmugam, A., & Rawson, A. (2021). Evaluation of physicochemical and organoleptic properties of plant based beverage developed from chickpea. *The Pharma Innovation International Journal*, *10*(10), 1871-1875.