Journal of Agricultural Science and Technology (JAST), 28(4)
In Press, Pre-Proof Version

Assessment of the physicochemical, antioxidant, microbial, and sensory properties of camel milk fermented with *Lactobacillus plantarum* and *Lactobacillus rhamnosus*

Mohammad Bagher Kiyani Sefat[1], Marjaneh Sedaghati[1]*, and Mohammad Javad Shakouri[1]

ABSTRACT

This study was conducted aiming at evaluating the physicochemical, antioxidant, microbial and sensory properties of functional fermented camel milk (FCM) with varying β-glucan concentrations (0, 0.1, 0.2, and 0.3%), and a combination of the bacterial cultures *Lactobacillus Plantarum* and *Lactobacillus Rhamnosus.* The FCM matrix was assessed for pH/acidity, phase separation, viscosity, color, total phenolic content (TPC), antioxidant activity (AO), probiotic viability, and sensory characteristics by 12 participants. The results were analyzed using one-way ANOVA applied to data from a completely randomized design with three replicates, followed by **LSD post hoc tests** for comparison of treatment means. The pH, acidity, antioxidant activity (IC_{50}), viscosity, and probiotic viability of fortified FCM ranged from 3.46-4.4, 0.141%-0.429%, 27.01-69.67 ($mg.mL^{-1}$), 1025-2355 ($mPa.s^{-1}$), and 6.17-8.95 ($cfu.mL^{-1}$) respectively. The Results demonstrated that β-glucan fortification (0–0.3%) significantly (***P<0.05***) increased acidity, TPC, AO, viscosity, and probiotic viability in FCM, while reducing pH and phase separation**.** Increasing β-glucan concentration in the samples was associated with a significant decrease in the brightness index (L), accompanied by significant increases in the yellowness (b*)* and redness (a*) indexes (*P<0.05*). According to the sensory panelists' assessments, increasing the β-glucan concentration to 0.2% was deemed favorable. These findings suggest that fortification of fermented camel milk with 0.2% β-glucan optimally enhances its functional, physicochemical, and sensory properties, supporting its potential as a health-promoting dairy product.

Keywords: β-glucan*,* Camel milk, *Lactobacillus Plantarum*, *Lactobacillus Rhamnosus,* Probiotic.

INTRODUCTION

Currently, products that have the potential to promote physiological responses in the body or decrease the risk of illness are called functional foods. Fermentation is widely regarded as a

[1] Department of Food Science and Technology, Faculty of Biological Sciences, North Tehran Branch, Islamic Azad University, Tehran, Islamic Republic of Iran.
*Corresponding author; e-mail: marjanehsedaghati@yahoo.com

means of promoting the growth of microorganisms and enzymatic transformations in nutrient content, resulting in the production of functional foods that possess health-enhancing properties. Lactic acid bacteria are the best options due to their high fermentation potential, product safety, and compatibility during fermentation (Benkirane *et al.,* 2022). Lactic acid bacteria, which are capable of fermenting and promoting digestive health, are commonly referred to as probiotics and are consumed in the form of microbial food supplements as part of functional foods. To gain the full health benefits of probiotics, it is recommended that the product contains at least 10^7 colony-forming units (cfu) per milliliter at the time of consumption. Similarly, prebiotics are dietary components that support the growth and activity of probiotic bacteria, ultimately resulting in positive health effects (Soemarie *et al.,* 2021).

Milk from various species (goat, cow, sheep, buffalo, etc.) has been used to produce fermented milk products (Benkirane *et al.,* 2022). The appropriate choice for the production of fermented dairy products is camel milk, which has adequate nutrition and biological components. Camel milk contains high amounts of vitamin C (150 $mg.L^{-1}$) and niacin, in addition, compared to bovine milk it has a higher content of copper (Cu) and iron (Fe) (Soleymanzadeh *et al.,* 2016). Studies have also been conducted on the antioxidant and antidiabetic effects of camel milk fermented with *Lactobacillus bulgaricus* (*Lactobacillus delbrueckii* subsp. *bulgaricus*) and *Streptococcus thermophiles* (Shori and Baba, 2014). In one study, the antioxidant activity of fermented camel milk (FCM) with *Streptococcus thermophilus*, *L. acidophilus*, and *B. bifidum* was reported (Algonaiman and Alharbi, 2023). The antibacterial activity of FCM with *Lactobacillus bulgaricus* and *Streptococcus thermophilus* was also observed in a previous study (Lafta *et al.,* 2014). Furthermore, some studies have reported the functional properties of FCM (Solanki and Hati, 2018). However, there is a scarcity of relevant information in the literature regarding the camel milk properties fermented with *Lactobacillus plantarum* and *Lactobacillus rhamnosus* supplemented with yeast β-glucan.

β-glucan is a polysaccharide located in the cell walls of cereals, yeasts, marine plants, and fungi, naturally. β-glucan produced from yeast cell wall is composed of 1→ 3 β-linked glucopyranosyl residues with a few 1→ 6 β-linked branches. β-glucan is now being considered due to its prebiotic properties, immune system stimulation, limited uric acid production, reduced blood sugar, and controlled blood pressure. However, this polysaccharide is not only important in the food industry for its health benefits to consumers, but also because of its functional properties, such as its ability to form gels and thicken food products (Mykhalevych *et al.,* 2022).

Based on previous studies, *Lactobacillus plantarum*, and *Lactobacillus rhamnosus* were selected for this study because of their proper fermentative activity and potential for the production of aromatic compounds (Ma *et al.,* 2021). We propose that incorporating yeast β-glucan into fermented camel milk (FCM) with these probiotic strains will enhance its physicochemical, antioxidant, microbial, and sensory properties, thereby improving its potential as a functional food. The objective of this research was to assess the physicochemical, microbial, and sensory characteristics of FCM formulated with *Lactobacillus plantarum*, *Lactobacillus rhamnosus,* and yeast β-glucan.

MATERIALS AND METHODS

Materials

Camel milk was purchased from Asayesh Co. (Gorgan, Iran) on July 11, 2023. Yeast β-glucan from *Saccharomyces cerevisiae* (≥80% purity, yellowish fine powder) was obtained from LonierHerb Co. and stored at 4°C for 28 days. *L. plantarum* PTCC 1058 and *L. rhamnosus* PTCC 1637 were supplied by the IROST Company in Tehran. Lyophilized *L. plantarum* PTCC 1058 and *L. rhamnosus* PTCC 1637 (around 10^8 cfu.mL^{-1}) were cultured in MRS broth medium for 24 h at 37°C in a CO_2 incubator (Memmert, Munich, Germany). Analytical grades of chemicals were prepared from Merck (Germany) and used in this study.

FCM samples production

Fresh camel milk (4.9% fat, 2.7% protein, and 4.3% lactose) was pasteurized at 70°C for 20 min and afterward cooled to 37°C. The activated probiotic cultures (*L. plantarum* and *L. rhamnosus*) were centrifuged (Eppendorf 5427, Germany) at 5000×g for 15 min to harvest the bacterial biomass. β-glucan (0, 0.1, 0.2, and 0.3% w/v), and 0.2% (w/v) biomass of each probiotic culture were subsequently added. These strains have been widely referred to as probiotics based on their demonstrated functional properties in previous studies (Haghshenas *et al.,* 2016; Rezaei *et al.,* 2022). The milk was fermented at 37˚C for 6-8 hours until it reached a pH of 4.5. Thereafter, the FCM was stored in the refrigerator for 28 days at 4˚C and analyses were conducted on days 1, 7, 14, 21 and 28 (Soleymanzadeh *et al.,* 2016).

Physico-chemical analysis

Fresh camel milk was analyzed for protein, fat and lactose content. A digital pH meter (Taiwa, AZ 86502*)* was used to measure the pH of the prepared samples (El-Deeb *et al.,* 2017). The FCM samples viscosity was evaluated with a rheometer (MCR 301, Anton paar, Austria) equipped with a CC27 spindle and was sheared from 1.0 to 500 ($1s^{-1}$) at 20°C. The FCM samples were also poured into the test tubes and stored at 4°C to assess the stability of the FCM. Equation (1) was applied to assess the phase separation of FCM samples (Farahani *et al.,* 2022).

Phase separation (%)= Volume of supernatant/Volume of total sample×100 (1)

Antioxidant activity evaluation

To assess the antioxidant activity (AO), 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) was used. Three milliliters of 0.1 mM DPPH in ethanol were added to 100 µl of the FCM supernatant, which was then mixed and left to stand at room temperature for 60 minutes. Afterward, the absorption of the samples was measured using a spectrophotometer at 515 nm (Atwaa *et al.,* 2022). Inhibition values (%) were calculated using the formula: % Inhibition = [(A0 - A1) / A0] × 100, where A0 is the absorbance of the control and A1 is the absorbance of the sample being tested. The IC_{50} value for each FCM sample, the amount of FCM (mg) needed to inhibit 50% of DPPH have been calculated as a standard curve, which uses inhibition (%) values against various concentrations of FCM (Celik *et al.,* 2023).

Color measurement

To determine color parameters, i.e., L^*, a^*, and b^* indices in FCM, the Hunterlab instrument (UltraScanvis, US-Vis 1,310, USA) was used. Lightness was assessed between zero (black) to 100 (white), a^* was determined from + 127 (red) to –128 (green), and b^* was evaluated from + 127 (yellow) to –128 (blue) (Bhaskar *et al.,* 2017; Soemarie *et al.,* 2021).

Microbial analysis

To perform the microbiological analysis, a sample was mixed with 90 mL of sterile saline solution (0.95% w/v) in a sterile glass to create the initial dilution (10^{-1}). This dilution was used to prepare a series of decimal dilutions using the same diluent. To determine the count of colony-forming probiotic bacteria in liquid samples, dilutions were cultured in the bottom on MRS agar supplemented with vancomycin (10 $mg.L^{-1}$) using the Pour Plate method for enumeration (vancomycin is employed in selective media for the enumeration of *Lactobacillus plantarum* and *Lactobacillus rhamnosus* due to their intrinsic resistance to this antibiotic). The plates were incubated in a CO_2 incubator at 37°C for 72 hours, and the results were expressed as Log cfu.mL^{-1} (Sakai *et al.,* 2010).

Sensory analysis

A group of 12 trained and expert panelists, comprising six men and six women aged between 20 and 30 years, conducted a sensory evaluation using a 5-point hedonic scale ranging from 1 (extremely dislike) to 5 (extremely like). The parameters related to sensory evaluation were color, taste, flavor, texture, and overall acceptability. These characteristics were assessed on the 28th day of storage. Twenty milliliters of FCM samples were placed in labeled bottles and kept at a temperature of 4 ± 1ºC before being served to the panelists along with their meal. Following each test, panelists rinsed their mouths with water (Farahani *et al.,* 2022).

Statistical analysis

Experiments was performed in triplicate, and significant differences among means were assessed using one-way ANOVA followed by LSD post hoc tests (SPSS, version 22, 2016). The significance level was set at $P<0.05$. Nonparametric data were analyzed using the Kruskal-Wallis test (Arabshahi and Sedaghati, 2022).

RESULTS

The pH values of FCM samples during storage are presented in Figure 1a. Statistical analysis revealed that the pH of FCM samples were affected by adding yeast β-glucan significantly ($P<0.05$). By increasing the concentration of yeast β-glucan, the pH of the treated samples decreased significantly ($P<0.05$). The FCM sample with 0.3% yeast β-glucan had the lowest pH on the 28th day (3.46 ± 0.09), while the control sample (0% yeast β-glucan) had the highest pH on the first day (4.44 ± 0.06). During the storage period, the pH of samples reduced significantly ($P<0.05$).

The effects of refrigerated storage time and β-glucan concentration on the probiotic viability are shown in Figure 1b. Both factors significantly influenced the number of viable probiotic cells ($P<0.05$). Viability increased significantly after 14 days of refrigerated storage, reaching a maximum of 8.95 log (cfu.mL^{-1}) in the sample containing 0.3% β-glucan, before declining significantly by day 28 ($P<0.05$). The viscosity of FCM samples was calculated at 4 ˚C for all the tested samples (Figure 2a). The average viscosity of the FCM samples was between 1025 and 2355 mPa.s. The results showed an increase in the viscosity of camel milk samples as a result of the addition of β-glucan. The addition of β-glucan significantly increased the viscosity of camel milk samples ($P<0.05$), with the highest concentration (0.3%) producing the greatest effect. Conversely, viscosity decreased significantly during cold storage ($P<0.05$). Phase separation during refrigerated storage is presented in Figure 2b. A significant increase in phase separation was observed over time ($P<0.05$), consistent with findings by Arabshahi and Sedaghati (2022). However, the addition of β-glucan substantially reduced phase separation compared to the control ($P<0.05$). Even a low β-glucan concentration (0.1%) effectively prevented phase separation, with higher concentrations (up to 0.3%) further enhancing stability. Figure 3a presents the antioxidant activity of FCM samples measured by the IC_{50} value, which indicates the quantity of sample (in $mg.mL^{-1}$) required to scavenge 50% of DPPH radicals. Lower IC_{50} values correspond to higher antioxidant activity. On the first day, the treated sample with 0.3% β-glucan exhibited the highest antioxidant activity (69.67 ± 0.06 $mg.mL^{-1}$), while the control samples on the 28th day showed the lowest content (27.01 ± 0.02 $mg.mL^{-1}$). Statistical analysis revealed a significant increase ($P<0.05$) in antioxidant activity with increasing β-glucan concentration from 0% to 0.3%. Additionally, antioxidant activity significantly declined ($P<0.05$) in all samples over the 28-day storage period at 4°C. The color indexes L* (whiteness), a* (red–green) and b* (yellow/blueness) of FCM samples are noticed in Table 1. The L* values of FCM samples were significantly decreased in the presence of β-glucan, and a higher darkness was observed for greater amounts of β-glucan. The b* values for FCM samples were between 10.98 and 16.61. Our results indicated that the b* value or the intensity of the yellow color was slightly increased during storage. Also, the FCM samples with β-glucan had a significant increase in b* value ($P<0.05$). As the concentration of β-glucan increased, the FCM sample tended to red color. Regarding a* values, the control sample on the first day had the lowest redness, while the sample with 0.3% β-glucan exhibited the highest redness. Increasing β-glucan concentration tended to shift the color toward red.

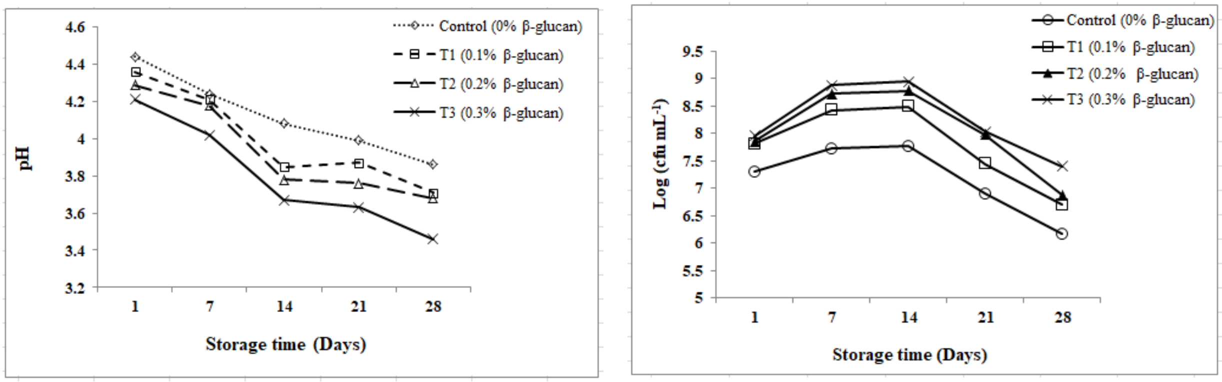

Fig. 1. The effect of β-glucan concentrations on pH (a) and probiotic viability (b) of fermented camel milk during 28 days of storage

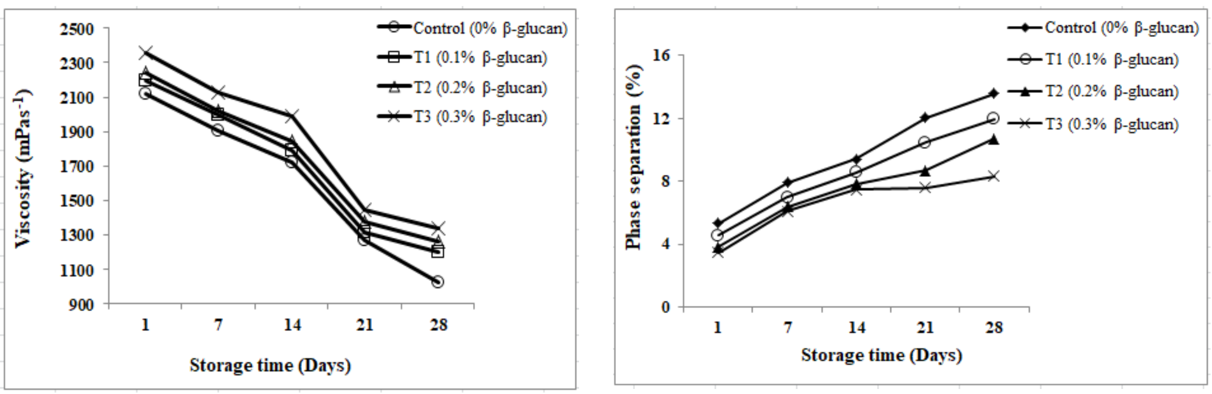

Fig. 2. The effect of β-glucan concentrations on viscosity (a) and phase separation (b) of fermented camel milk during 28 days of storage.

Table 1. The effect of β-glucan on color of fermented camel milk during 28 days of storage.

Colour properties		Storage days 1	7	14	21	28
$L*$	Control	81.86±0.36^{Aa}	79.74±0.55^{Aab}	76.99±0.67^{Ab}	75.62±0.05^{Ab}	73.58±0.16^{Ac}
	T_1	79.31±0.21^{ABa}	77.65±0.17^{ABab}	75.87±0.24^{ABb}	75.28±0.24^{Ab}	73.09±0.07^{Ac}
	T_2	77.05±0.11^{Ba}	75.61±0.55^{Bab}	74.38±0.13^{Bb}	73.79±0.13^{Bbc}	71.65±0.37^{Bc}
	T_3	74.03±0.51^{Ca}	73.61±0.36^{Cab}	72.98±0.36^{Cb}	72.58±0.39^{Cb}	70.48±0.64^{BCbc}
$b*$	Control	10.98±0.71^{Cb}	11.15±0.13^{Cb}	11.97±0.05^{Ca}	12.26±0.21^{Ca}	12.50±0.06^{Ba}
	T_1	12.70±0.14^{Bbc}	13.25±0.25^{Bb}	13.39±0.08^{Bb}	13.56±0.26^{Ba}	14.26±0.09^{ABa}
	T_2	13.37±0.18^{Bb}	13.41±0.25^{Bb}	13.67±0.03^{Bb}	13.8±0.03^{Ba}	14.45±0.02^{ABa}
	T_3	15.08±0.41^{Ab}	15.2±0.27^{Ab}	15.71±0.15^{Aa}	15.77±0.92^{Aa}	16.61±0.09^{Aa}
$a*$	Control	2.35±0.03^{ABa}	2.77±0.06^{ABab}	3.28±0.08^{ABab}	3.47±0.05^{ABab}	3.69±0.06^{Ab}
	T_1	2.81±0.06^{Aa}	3.28±0.04^{Aab}	3.48±0.02^{Ab}	3.54±0.01^{Ac}	3.88±0.04^{Ac}
	T_2	3.09±0.17^{Aa}	3.10±0.12^{Aa}	3.33±0.1^{Aab}	3.45±0.14^{Ab}	3.63±0.14^{Ac}
	T_3	3.34±0.05^{Aa}	3.34±0.02^{Aa}	3.61±0.05^{Aab}	3.72±0.02^{Aab}	3.91±0.02^{Ab}

A-D: Means within each column followed by different letters show significant differences ($P<0.05$) between treatments at the same time.
a-b: Means within each row followed by differences letters (a–b) show significant differences ($P<0.05$) at treatment during the storage period.

Figure 3b presents the results of the sensory properties of FCM on 28th day of storage. All samples showed the same acceptance of smell parameters with no significant differences (P>0.05). The highest flavor score belongs to T_2 treatment, and the presence of β-glucan significantly increased this score (P<0.05). However, only an increase of 0.2% had a positive effect on flavor acceptance, whereas a higher amount of β-glucan reduced it. The highest color score was observed in the T_2 sample, and the addition of 0.2% β-glucan significantly increased the color score (P<0.05). The addition of β-glucan only to 0.2% increased the texture score significantly (P<0.05), and a higher amount of β-glucan reduced texture acceptance. The T_2 sample had the highest texture score, whereas the control sample had the lowest. The overall acceptance scores of the FCM samples revealed a significant difference among the samples (P<0.05). The highest acceptance was observed for the T_2 treatment, and the presence of β-glucan significantly increased the acceptance score (P<0.05). However, an increase in β-glucan to only 0.2% had a positive effect on overall acceptance, while a higher amount of β-glucan reduced its score.

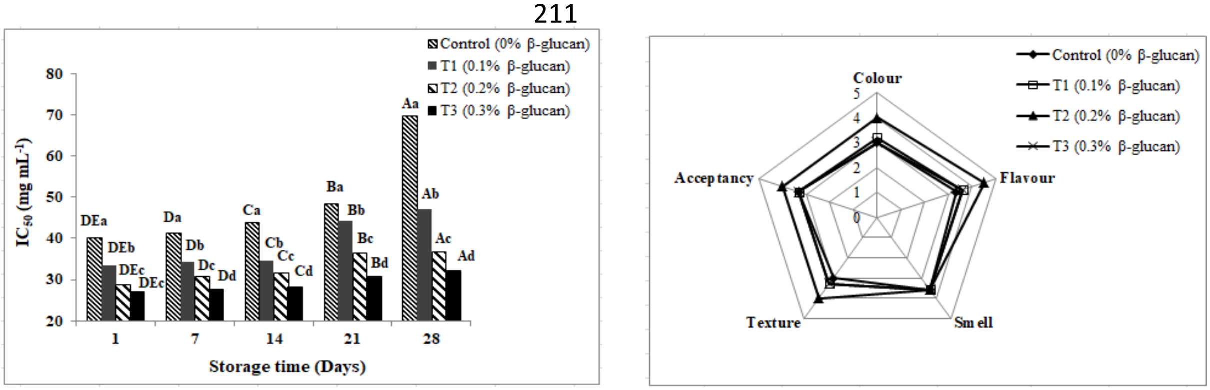

Fig. 3. The effect of β-glucan concentrations on antioxidant activity (a) and sensory properties (b) of fermented camel milk during 28 days of storage.

DISCUSSION

The decrease in pH of fermented dairy products during storage can be attributed to the creation of lactic acid and other organic acids from lactose. Biochemical activity of bacterial cultures during cold refrigeration storage results in post-fermentation acidification and subsequent acid production (Ayyash *et al.,* 2018). This is consistent with the findings of Soleymanzadeh *et al.* (2016) who reported that the pH of camel milk fermented by lactic acid bacteria decreased during storage. Similarly, Algonaiman and Alharbi (2023) observed a decrease in the pH of FCM in the presence of oats and date palms. Al-Sahlany *et al.* (2022) also reported organic acid production in bio-yogurt samples supplemented with yeast β-glucan during storage. The results for camel milk acidity decrease in the presence of β-glucan were consistent with the data on probiotic survival. It is possible that the presence of β-glucan improved the numbers and viability of probiotic bacteria, which may be related to the presence of prebiotic ingredients in β-glucan. This is consistent with the findings of Vasiljevic *et al.* (2007) who observed that yogurt probiotic survival improved in the presence of β-glucan as a non-digestible complex carbohydrate.

The incorporation of various concentrations of β-glucan significantly enhanced the population of probiotic bacteria and samples with higher percentages of β-glucan displayed a greater number of probiotic bacteria. This finding aligns with previous research demonstrating that β-glucan acts as a protective agent and prebiotic substrate, improving probiotic survival under various stress conditions. The polysaccharide's ability to form a protective matrix around probiotic cells helps shield them from environmental stresses such as acidity, bile salts, and heat, thereby enhancing their stability during storage. Moreover, β-glucan serves as a fermentable dietary fiber that can stimulate the growth and metabolic activity of probiotics, promoting their persistence in the FCM. The increase in viability observed at 0.3% concentration suggests a dose-dependent effect, where sufficient β-glucan levels provide both physical protection and a favorable substrate for probiotic metabolism (Moayednia *et al.,* 2009; Al-Sahlany *et al.* 2022). However, the number of viable cells of probiotic bacteria in FCM decreased throughout 14 to 28 days of storage due to the damages from high produced organic acids, limited nutrients and high redox potential (Anli *et al.,* 2023). Moayednia *et al.* (2009) found that the survival of *L. acidophilus* decreases during storage in the refrigerator, but the proteolytic activity of probiotic bacteria can lead to improved survival in some cases. Kurtuldu and Ozcan (2018) stated that adding β-glucan to yogurt can enhance the survival of *B. animalis* subsp*. lactis strain Bb-12* and metabolic functionality, which is attributed to the prebiotic properties of the supplement. Similarly, Sahlany *et al.* (2022) indicated that β-glucan extracted from *Saccharomyces cerevisiae* improved *Lactobacillus acidophilus* and *Bifidobacterium bifidum* viability in bio-yogurt.

The presence of β-glucan had a significant increasing effect on the viscosity of FCM samples. β-glucan's unique structure enables it to interact with water molecules and form a network that retains water within the fermented milk matrix. This network of β-glucan molecules aids in thickening the fermented dairy product. Additionally, β-glucan has been shown to possess gelling properties that can help stabilize the dairy structure and improve texture (Ahmad and Ahmed, 2016). In addition, the decrease in viscosity of FCM during the storage might be due to the production of degradative enzymes by lactic acid bacteria, which are associated with milk protein (Moradi *et al.,* 2023). Similarly, Mykhalevych *et al.* (2022) recommended the use of β-glucan for increasing viscosity in fermenting milk products. Qu *et al.* (2021) revealed that the presence of 0.3% oat β-glucan to some extent diminishes the interaction with protein particles, which reduces the fermentation process and increases the viscosity of the set-type yogurt. Salgado *et al.* (2021) reported that the apparent viscosity of donkey milk yogurt enriched with fiber reduced after 14 days of storage.

According to observations in FCM samples, increasing β-glucan concentration to 0.3% significantly reduced phase separation. This stabilization effect is attributed to two key mechanisms: (1) the gelling properties of β-glucan form a three-dimensional network that immobilizes water molecules and fat globules, preventing gravitational separation; and (2) its high water-binding capacity increases matrix viscosity, which inhibits droplet coalescence and serum release. These mechanisms collectively enhance structural integrity in colloidal systems (Zielke *et al.,* 2018; Arabshahi and Sedaghati, 2022). The non-linear concentration dependence observed in our study aligns with Bhaskar *et al*.'s findings in dahi (traditional fermented Indian yogurt), where phase separation decreased at 0.75% β-glucan but increased at 1% (Bhaskar *et al.* 2017). This suggests an optimal concentration window where β-glucan's polymer entanglement provides maximal stabilization (Algonaiman and Alharbi, 2023). Al-Sahlany *et al.* (2022) also reported a reduction in phase separation in bio-yogurts supplemented with yeast β-glucan.

Antioxidants effectively inhibit the oxidation of reactive compounds at low concentrations, thereby preserving cells from oxidative damage caused by free radicals such as singlet oxygen. Camel milk contains a high concentration of antioxidant compounds, including polyphenols, flavonoids, bioactive peptides, and vitamins (Bouhaddaoui *et al.,* 2019). The fermentation process can result in substantial improvements in the content of phenolics, primarily by activating enzymes that hydrolyze proteins and produce bioactive peptides, as well as liberating bound phenolic compounds, which enhances antioxidant activity (Benkirane *et al.,* 2022). β-glucan can improve antioxidant activity by modulating microbial enzyme production, and antioxidant peptide release (Vieira *et al.,* 2016). However, the antioxidants activity during storage may be affected by factors such as temperature, enzymes, microbial activity, and acids in the storage environment (Esparza *et al.,* 2020). Similarly, Algonaiman and Alharbi (2023) reported an increment in the antioxidant activity of fermented camel milk fortified with oat β-glucan and date palm. Also, Atwaa *et al.* (2022) reported a substantial reduction in antioxidant activity in all yogurt samples fortified with fennel extract during storage. In a related study, Soliman and Nasser (2022) discovered a significant decline in antioxidant activity in stirred yogurt samples as the storage period increased.

According the observations in FCM samples, as the concentration of β-glucan increased, L* values decreased. This decrease in L* values can be attributed to the interaction between light brown color β-glucan and milk protein, which results in a reduction in the whiteness of the products with higher concentrations (Raju and Pal, 2014). According to a study conducted by Bhaskar *et al.* (2017) the addition of β-glucan to low-fat dahi leads to a lower L* value compared to the control (Bhaskar *et al.* 2017). Similarly, Raju and Pal reported that L* value of misti dahi (traditional sweetened fermented Indian yogurt) was reduced in the presence of oat and soy fiber (Raju and Pal, 2014).

Our results indicated that the b* value of FCM samples was increased in the presence of β-glucan and during storage. This effect may be due to the light brown color of β-glucan, which causes yellowness in the fortified (Bhaskar *et al.* 2017). A positive correlation between β-glucan levels and b* increment has been reported by Gulzar *et al.* (2020) in all fortified skim milk samples containing β-glucan. Kurtuldu and Ozcan (2018) also reported a considerable increase ($p<0.05$) in the b* value of probiotic yogurt fortified with β-glucan.

Similar to the significant increase in red color intensity in samples treated with β-glucan, Bhaskar *et al.* (2017) reported a similar trend for low-fat dahi enriched with β-glucan. During storage, a* index showed an increasing trend, which was attributed to oxidation of compounds present in FCM and microbial activity that metabolized pigmented ingredients (Kurtuldu and Ozcan, 2018). Bhaskar *et al.* (2017) reported a similar increasing trend in *a** value for low-fat dahi enriched with β-glucan. Singh *et al.* (2012) found an increased redness index of set-style yogurt in the presence of 0.3% to 0.5% β-glucan.

The sensory characteristics of FCM samples such as flavor, smell, texture, color and overall acceptance are very important at the time of consumption. The proper effect of 0.2% β-glucan on FCM flavor score suggests that β-glucan can be used as a stabilizer in FCM formulations to maintain the integrity of flavor compounds and prevent flavor degradation during storage. This may prolong the FCM's flavor release and allow for a longer and more pleasant taste (Kurtuldu and Ozcan, 2018). However, Sahan *et al.* (2008) revealed that adding 0.5% β-glucan to non-fat yogurt had insignificant effect on flavor score. The Increment in the color score of FCM samples in the presence of β-glucan may due to an increase in the intensity of the red and yellow colors in treated samples. Our findings are inconsistent with Singh *et al*. (2012) who noted that the color of set-style yogurt is not affected by the presence of 0.3% β-glucan. β-glucan improved the texture score of FCM and has the ability to enhance viscosity and modify syneresis in FCM, ultimately contributing to its thickening effect. These changes can help stabilize the FCM structure, resulting in a more consistent and desirable texture. Raikos *et al.* (2018) reported an increase in the texture score of skim milk yogurt containing 0.6% β-glucan owing the increase in hardness of the yogurt samples in the presence of β-glucan. The increment in flavor, texture and color score of the treated samples only to 0.2% β-glucan was revealed improvement in the overall acceptance score and higher concentration (0.3%) reduced overall acceptance score. At 0.2% β-glucan, viscosity increases and syneresis decreases, enhancing creamy texture and mouthfeel. However, higher levels (e.g., 0.3%) may cause excessive thickness, color changes, and reduced clarity, negatively impacting visual appeal and sensory quality. Also, Raikos *et al.* (2018) reported a reduction in the overall acceptance score of skim milk yogurt by increasing β-glucan up to 0.8%, but these changes were not significant ($P>0.05$).

CONCLUSIONS

In this study, the formulation of a functional FCM incorporating β-glucan was evaluated. The results revealed that FCM fortified with β-glucan showed a considerable increase in TPC and antioxidant activity. By enhancing the total phenolic content and antioxidant activity in FCM, β-glucan fortification may help mitigate oxidative stress, a key factor implicated in aging and the pathogenesis of chronic diseases such as cardiovascular disease, diabetes, and certain cancers. The FCM sample containing 0.2% β-glucan (T_2) had an acceptable probiotic level and overall acceptability score even after 28 days of storage. The sustained viability of *L. plantarum* and *L. rhamnosus* probiotics in the β-glucan enriched FCM indicates potential for improved gut microbiota modulation. Based on the data obtained in this study, the T_2 sample was found to be the best treatment with desirable properties for creating functional FCM.

ACKNOWLEDGEMENTS

The support of the Islamic Azad University (Iran, Tehran) is gratefully acknowledged.

REFERENCES

1. Ahmad, A. and Ahmed, Z. 2016. Nutraceutical aspects of β-glucan with application in food products. *Nanotechnol. Agri-Food. Ind.,* 387-425.
2. Algonaiman, R. and Alharbi, H. F. 2023. Development of Fermented Camel Milk Incorporating Oats and Sukkari Date Palm Fruit: Nutritional, Physicochemical, Functional, and Organoleptic Attributes. *Ferment.,* **9(10):** 864.
3. Al-Sahlany, S. T. G., Al-Kaabi, W. J., Al-Manhel, A. J. A., Niamah, A. K., Altemimi, A. B., Al-Wafi, H. and Cacciola, F. 2022. Effects of β-Glucan Extracted from Saccharomyces Cerevisiae on the Quality of Bio-Yoghurts: In Vitro and in Evaluation. *J. Food. Meas. Charact.,* **16:** 3607–3617.
4. Anli, E. A., Gursel, A. and Gursoy, A. 2023. Assessment of the quality attributes of oat β-glucan fortified reduced-fat goat milk yogurt supported by microfluidization. *Foods.,* **12(18):** 3457.
5. Arabshahi, S. S. and Sedaghati, M. 2022. Production of synbiotic doogh enriched with Plantago psyllium mucilage. *J Food Sci Technol.,* 1–8.
6. Atwaa, E. S. H., Shahein, M. R., El-Sattar, E. S. A, Hijazy, H. H. A., Albrakati, A. and Elmahallawy, E. K. 2022. Bioactivity, Physicochemical, and Sensory Properties of Probiotic Yogurt Made from Whole Milk Powder Reconstituted in Aqueous Fennel Extract. *Ferment.,* **8(2):** 52.
7. Ayyash, M., Al-Nuaimi, A. K., Al-Mahadin, S. and Liu, S.-Q. 2018. In Vitro Investigation of Anticancer and ACE-Inhibiting Activity, α-Amylase and α-Glucosidase Inhibition, and Antioxidant Activity of Camel Milk Fermented with Camel Milk Probiotic: A Comparative Study with Fermented Bovine Milk. *Food. Chem*., **239:** 588–597.
8. Benkirane, G., Ananou, S., and Dumas, E. 2022. Moroccan traditional fermented dairy products: current processing practices and physicochemical and microbiological properties - a review. *J. Microbiol. Biotechnol. Food Sci.,* **12**: e 5636.

9. Bhaskar, D., Khatkar, S. K., Chawla, R., Panwar, H. and Kapoor, S. 2017. Effect of β-glucan fortification on physico-chemical, rheological, textural, colour and organoleptic characteristics of low fat dahi. *J. Food. Sci. Technol.,* **54:** 2684–2693.

10. Bouhaddaoui, S., Chabir, R. and Errachidi, F. E. 2019. Study of the biochemical diversity of camel milk. *Sci. World. J.,* **19:** 2517293.

11. Celik, O. F., Kilicaslan, M., Akcaoglu, S. and Ozturk, Y. 2023. Improving the antioxidant activity of yogurt through black and green tea supplementation. *Int. J. Food. Sci. Technol.,* **58:** 6121–6130.

12. El-Deeb, A. M., Dyab, A. S. and Elkot, W. F. 2017. Production of flavoured fermented camel milk. *Ismailia. J. Dairy. Sci. Technol.,* **5(1):** 9–20.

13. Esparza, I., Cimminelli, M. J., Moler, J. A., Jimenez-Moreno, N. and Ancin-Azpilicueta, C. 2020. Stability of Phenolic Compounds in Grape Stem Extracts. *Antioxidants.,* **9:** 720.

14. Farahani, M. V., Sedaghati, M. and Mooraki, N. 2022. Production and characterization of synbiotic *Doogh* by gum tragacanth, date seed powder, and *L. casei*. *J. Food. Process. Preserv.,* **46(11):** e16946.

15. Gulzar, S., Benjakul, S. and Hozzein, W. N. 2020. Impact of β-glucan on debittering, bioaccessibility and storage stability of skim milk fortified with shrimp oil nanoliposomes. *Int. J. Food. Sci. Technol.,* **55:** 2092–2103.

16. Haghshenas, B., Haghshenas, M., Nami, Y., Yari Khosroushahi, A., Abdullah, N., Barzegari, A., Rosli, R. and Saeed Hejazi, M. 2016. Probiotic assessment of *L. plantarum* 15HN and *Ent. mundtii* 50H isolated from traditional dairies microbiota. *Adv. Pharm. Bull.,* **6:** 37–47.

17. Kurtuldu, O. and Ozcan, T. 2018. Effect of β-glucan on the properties of probiotic set yoghurt with Bifidobacterium animalis subsp. lactis strain Bb-12. *Int. J. Dairy. Technol.,* **71:** 157–166.

18. Lafta, H., Jarallah, F. M. and Darwash, A. 2014. Antibacterial activity of fermented camel milk using two lactic acid bacteria. *JUBPAS.,* **22:** 2377-2382.

19. Ma, L., Zhao, C., Chen, J. and Zheng, J. 2021. Effects of Anaerobic Fermentation on Black Garlic by Lactobacillus: Changes in Flavor and Functional Components. *Front. Nutr*., **8:** 645416.

20. Moayednia, N., Ehsani, M. R. and Jomeh, Z. E. 2009. Effect of refrigerated storage time on the viability of probiotic bacteria in fermented probiotic milk drinks. *Int. J. Dairy. Technol.,* **62:** 204–208.

21. Moradi, H., Sedaghati, M. and Jahanbakhshian, N. 2023. Evaluation and improvement of antioxidant activity and physicochemical properties of yogurt enriched with persian gum (Amygdalus scoparia Spach) and fennel (*Foeniculum Vulgare*) extract. *Acta. Sci. Pol. Technol. Aliment.,* **22(4):** 431–440.

22. Mykhalevych, A., Polishchuk, G., Nassar, K., Osmak, T. and Buniowska-Olejnik, M. 2022. Beta-glucan as a techno-functional ingredient in dairy and milk-based products: A review. *Molecules*., **27(19):** 6313.

23. Raikos, V., Grant, S. B. and Hayes, H. 2018. Use of β-glucan from spent brewer's yeast as a thickener in skimmed yogurt: Physicochemical, textural, and structural properties related to sensory perception. *J. Dairy. Sci.,* **101:** 5821–5831.

24. Raju, P. N. and Pal, D. 2014. Effect of dietary fibers on physico-chemical, sensory and textural properties of Misti Dahi. *J. Food. Sci. Technol.,* **51(11):** 3124–3133.

25. Rezaei Z, Khanzadi S. and Salari A. 2022. A survey on biofilm formation of *Lactobacillus rhamnosus* (PTCC 1637) and *Lactobacillus plantarum* (PTCC 1745) as a survival strategy of probiotics against antibiotic in vitro and yogurt. *J Food Process Preserv.,* **46(9):** e15991.

26. Sahan, N., Yasar, K. and Hayaloglu, A. A. 2008. Physical, chemical and flavour quality of non-fat yogurt as affected by a β-glucan hydrocolloidal composite during storage. *Food Hydrocoll.,* **22:** 1291–1297.

27. Sakai, T., Oishi, K., Asahara, T., Takada, T., Yuki, N., Matsumoto, K., Nomoto, K. and Kushiro, A. 2010. M-RTLV agar, a novel selective medium to distinguish *Lactobacillus casei* and *Lactobacillus paracasei* from *Lactobacillus rhamnosus*. *Int. J. Food Microbiol.,* **139:** 154–160.

28. Salgado, M. J. G., Ramos, M. S., Assis, D. D. J., Otero, D. M., Oliveira, R. L., Ribeiro, C. V. D. M., Costa, M. P. and Oliveira, C. A. A. 2021. Impact of fiber-rich donkey milk yogurt on apparent viscosity and sensory acceptance. *LWT - Food Sci Technol*., **145:** 111494.

28. Shori, A. B. and Baba, A. S. 2014. Comparative antioxidant activity, proteolysis and in vitro α- amylase and α-glucosidase inhibition of Allium sativum-yogurts made from cow and camel milk. *J. Saudi. Chem. Soc.,* **18(5):** 456-463.

29. Singh, M., Kim, S. and Liu, S. X. 2012. Effect of purified oat b-glucan on fermentation of set-style yoghurt mix. *J. Food. Sci.,* **77:** E195–E20.

30. Soemarie, Y. B., Milanda, T. and Barliana, M. I. 2021. Fermented foods as probiotics: A review. *J Adv. Pharm. Technol. Res.,* **12(4):** 335–339.

31. Solanki, D. and Hati, S. 2018. Fermented camel milk: A Review on its bio-functional properties. *EJFA.*, **30(4):** 268-274.

32. Soleymanzadeh, N., Mirdamadi, S. and Kianirad, M. 2016. Antioxidant activity of camel and bovine milk fermented by lactic acid bacteria isolated from traditional fermented camel milk (Chal). *Dairy. Sci. Technol.,* **96:** 443–457.

33. Soliman, T. N. and Nasser, S. A. 2022. Characterization of carotenoids double-encapsulated and incorporate in functional stirred yogurt. *Front. Sustain. Food. Syst.*, **6:** 979252.

34. Qu, X., Nazarenko, Y., Yang, W., Nie, Y., Zhang, Y. and Li, B. 2021. Effect of Oat β-Glucan on the Rheological Characteristics and Microstructure of Set-Type Yogurt. *Molecules.,* **26:** 4752.

35. Vasiljevic, T., Kealy, T. and Mishra, V. K. 2007. Effects of β-glucan addition to a probiotic containing yogurt. *J. Food. Sci.,* **72:** C405–C411.

36. Vieira, E. F., Carvalho, J., Pinto, E., Cunha, S., Almeida, A. A. and Ferreira, I. M. P. L. V. O. 2016. Nutritive value, antioxidant activity and phenolic compounds profile of brewer'sspent yeast extract. *J. Food. Compos. Anal.,* **52:** 44–51.

37. Zielke, C., Lu, Y., Poinsot, R. and Nilsson, L. 2018. Interaction between cereal β-glucan and proteins in solution and at interfaces. *Colloids. Surf. B Biointerfaces.,* **162:** 256–264.

ارزیابی خواص فیزیکوشیمیایی، آنتی‌اکسیدانی، میکروبی و حسی شیر شتر تخمیر شده با لاکتوباسیلوس پلانتاروم و لاکتوباسیلوس رامنوسوس

محمد باقر کیانی صفت، مرجانه صداقتی، و محمدجواد شکوری

چکیده

این مطالعه با هدف ارزیابی خواص فیزیکوشیمیایی، آنتی‌اکسیدانی، میکروبی و حسی شیر شتر تخمیر شده فراسودمند (FCM) با غلظت‌های مختلف بتا-گلوکان (0، 0.1، 0.2 و 0.3%) و ترکیبی از کشت‌های باکتریایی *لاکتوباسیلوس پلانتاروم* و *لاکتوباسیلوس رامنوسوس* انجام شد. ماتریس FCM از نظر pH، اسیدیته، دو فاز شدن، ویسکوزیته، رنگ،

محتوای فنلی کل (TPC)، فعالیت آنتی‌اکسیدانی (AO)، زنده‌مانی پروبیوتیک و ویژگی‌های حسی توسط 12 ارزیاب بررسی شد. نتایج با استفاده از آنالیز واریانس یک‌طرفه (ANOVA) در یک طرح کاملاً تصادفی با سه تکرار تجزیه و تحلیل شد و پس از آن آزمون‌های تعقیبی LSD برای مقایسه میانگین‌های تیمارها اعمال شد. pH، اسیدیته، فعالیت آنتی‌اکسیدانی (IC50)، ویسکوزیته و قابلیت زنده‌مانی پروبیوتیک FCM غنی‌شده به ترتیب از 4.4-3.46، 0.141%-0.429%،(میلی‌گرم در میلی‌لیتر) 69.67-27.01 ، (میلی‌پاسکال ثانیه) 2355-1025 و (cfu. mL^{-1}) 8.95-6.17 متغیر بود. نتایج نشان داد که غنی‌سازی با بتا-گلوکان (0-0.3%) به طور معنی‌داری اسیدیته، TPC، AO، ویسکوزیته و قابلیت زنده‌مانی پروبیوتیک را در FCM افزایش داد، در حالی که pH و دو فاز شدن را کاهش داد (P< 0.05). افزایش غلظت بتا-گلوکان در نمونه‌ها با کاهش معنی‌دار شاخص روشنایی (L) و افزایش معنی‌دار شاخص‌های زردی (b) و قرمزی (a*) همراه بود (P< 0.05). طبق ارزیابی‌های حسی، افزایش غلظت بتا-گلوکان تا 0.2٪ مطلوب تلقی شد. این یافته‌ها نشان می‌دهد که غنی‌سازی شیر شتر تخمیر شده با 0.2٪ بتا-گلوکان، خواص عملکردی، فیزیکوشیمیایی و حسی آن را به طور مطلوب افزایش می‌دهد و از پتانسیل آن به عنوان یک محصول لبنی ارتقا دهنده سلامت پشتیبانی می‌کند.