

Physicochemical and Rheological properties of fortified cocoa milk containing prebiotic lactulose and stevia for individuals with Dysphagia

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Abstract

Dysphagia increases the risk of choking, aspiration, dehydration, and malnutrition, creating a need for nutritious beverages with controlled flow properties that remain stable from serving to oral temperature. To develop a fortified cocoa milk containing lactulose and stevia with rheological, physicochemical, sensory, and microbiological properties suitable for individuals with dysphagia.

Cocoa milk formulations containing 0.03–0.25% carrageenan were initially screened, and selected formulations containing 0.05–0.10% carrageenan were compared with sucrose- or maltodextrin–stevia-containing samples. Apparent viscosity, flow behaviour, and dynamic viscoelastic properties were evaluated at 10 and 35°C. Physicochemical and microbiological stability was monitored during 5 days of refrigerated storage. Sensory acceptance was assessed by 15 trained panellists. Measurements were performed in triplicate and analysed by analysis of variance followed by the least significant difference test ($p < 0.05$).

Increasing carrageenan concentration increased apparent viscosity, consistency index, storage modulus, and loss modulus. All formulations exhibited non-Newtonian shear-thinning behaviour, with power-law fits of $R^2 = 0.94–0.98$. Heating from 10 to 35°C reduced viscosity; however, maltodextrin limited this loss. At 0.10% carrageenan, the maltodextrin–stevia formulation retained an apparent viscosity of 0.066 Pa·s at 35°C, compared with 0.033 Pa·s for the sucrose formulation. The selected formulations achieved viscosity characteristics corresponding to International Dysphagia Diet Standardisation Initiative Levels 0–2. No visible sedimentation occurred in the selected formulations, and physicochemical and microbiological properties remained within acceptable limits during storage. The 0.10% carrageenan–stevia formulation received the highest taste and overall acceptability scores. Carrageenan combined with maltodextrin can provide temperature-stable cocoa milk for dysphagia management, while lactulose and stevia enable functional fortification and sugar replacement without compromising product stability or sensory acceptance.

1. Introduction

Dysphagia is a swallowing disorder commonly found in the elderly. In this condition, individuals experience difficulty swallowing both solid and liquid foods ^{1–3}. Often, dysphagia is a secondary consequence of other diseases such as Alzheimer's, Parkinson's, neuromuscular disorders, etc., and it can lead to dehydration, malnutrition, lung infection, choking during swallowing, and even death ⁴. Fortunately, by modifying the texture of solid foods and increasing the viscosity of liquids, this condition can be managed, and its risks mitigated ^{5,6}. In order to standardize food texture modification for individuals with swallowing disorders, the International Dysphagia Diet Standardisation Initiative (IDDSI) was introduced in 2016 with the aim of developing terminology and standardizing global definitions.

IDDSI categorized Individuals with swallowing disorders into three levels, where level one individuals can only tolerate diets consisting of liquids up to pureed textures, while level two and three individuals can

tolerate higher textures with more elasticity. Generally, hydrocolloids are considered one of the best texture modifiers available for standardizing foods according to the needs of dysphagia patients as defined in the IDDSI standard. The main reason for the widespread use of hydrocolloids in foods is their ability to modify the rheology of food systems, namely flow behavior (viscosity) and texture alteration ⁷. Overall, several key rheological parameters are evaluated for assessing dysphagic beverages, including viscosity, elastic modulus (G'), viscous modulus (G''), loss tangent ($\tan\delta$), stability index (k), and others ¹.

In this study, considering insufficient fluid intake as a common consequence of dysphagia and the avoidance of fluid consumption by individuals due to fear of choking during swallowing, a dairy beverage tailored to their needs has been formulated to have appropriate rheological properties ^{8,9}. According to studies conducted in 2010, it was found that over the past 40 years, per capita fluid milk consumption has continuously decreased in the United States ¹⁰. Milk is considered a complete food useful for providing balanced proportions of nutrients (fat, protein, and carbohydrates) and micronutrients (calcium, selenium, riboflavin (vitamin B2), cobalamin (vitamin B12), and pantothenic acid (vitamin B5)) (32). Cocoa milk is one of the most popular and widely consumed milk substitutes in the world. Cocoa present in cocoa milk is rich in polyphenols (12–18%), mainly flavonoids, which can act as heart protectors, anti-inflammatory, anti-obesity, anti-cancer, and neuroprotective agents ¹¹. Nowadays, the lactose intolerance suppressive effect of cocoa through its flavanols has been proven in various studies. Therefore, considering the importance of the health of the elderly, there has been an increased demand for the production of nutritious foods and beverages rich in bioactive compounds. Functional foods have been designed to improve metabolic functions and biochemical parameters in the human body and help prevent non-communicable diseases such as cardiovascular diseases (CVDs), cancer, type 2 diabetes, and osteoporosis ¹².

One of the most common methods for enhancing the functionality of food is the use of probiotics and prebiotics. The benefits of lactulose prebiotic have been recognized for over 50 years, and numerous studies have confirmed the health benefits of lactulose as a food substance, including its role as a selective stimulant for gut flora, laxative effect, and improvement in calcium absorption ¹³.

Every year, the number of people affected by diabetes, obesity, high blood pressure, and heart diseases increases. Therefore, artificial sweeteners have received much more attention nowadays. A study has shown that the consumption of beverages containing artificial sweeteners can also lead to type 2 diabetes, and therefore, they cannot be considered suitable substitutes for sucrose in these products ¹⁴. Hence, there is a growing demand for natural sweeteners, especially calorie-free sweeteners, as they are powerful, beneficial, safe, and low-calorie sugar substitutes ¹⁵. Steviol glycosides, as one of these natural and calorie-free sweeteners, have gained much attention and usage today. Steviol glycosides have a wide range of health benefits, including anti-diabetic, anti-hypertensive, anti-inflammatory, anti-caries, and anti-cancer properties (11,20). Steviol glycosides are used in various products such as seafood, soft drinks, plant waters, dairy products, bakery products, and fruit drinks ^{16–18}.

The aim of this study was to prepare a functional cocoa milk suitable for the needs of patients with swallowing disorders, and efforts were made to achieve levels one and two as defined by the IDDSI standard using different concentrations of carrageenan in the product so that ultimately the rheological properties measured in the product would comply with standards and previous studies to create safe swallowing in these patients. Considering that this disease has various causes, all rooted in aging, and taking into account major aging problems such as osteoporosis, diabetes, and multiple gastrointestinal problems including constipation, there is a need to prepare a dairy product to alleviate osteoporosis ¹¹. Therefore, prebiotics lactulose and stevia were used in cocoa milk samples to achieve these goals in this study.

2. Material and methods

In this study, non-fat dry milk powder from Pegah Company (Tehran, Iran), cocoa powder from Farmand Company (Iran), carrageenan gum from Ceamsa (Spain), lactulose from Fresenius Kabi (India), maltodextrin from Tianjin Harvest (China), stevia in extract form under the brand name Stevira from Asareh Talaye Sabz Arvand Company (Iran), and sucrose from Golpira Company (Iran) were obtained. Carrageenans were used in amounts of 0.03–0.25% to achieve levels one to three of dysphagia ¹⁹.

Lactulose content

The maximum tolerable intake of lactulose before inducing laxative effects is approximately 10 g per day ²⁰. By comparing this with the composition of the lactulose-containing syrup (77.2%), the appropriate level of addition for this study was determined. Accordingly, the formulation was adjusted to include 2.94% lactulose syrup, corresponding to the amount that would provide approximately 10 g of lactulose in a 500 mL serving of the cocoa milk beverage, an amount consistent with the recommended daily intake used.

Sample preparation

To prepare cocoa milk samples, distilled water was first heated using a thermal mixer to a temperature of $75 \pm 5^\circ\text{C}$, distributed into separate containers and then various concentrations of carrageenan (0.25%, 0.15%, 0.1%, 0.08%, 0.06%, 0.05%, 0.03%) was added and homogenized for 45 minutes at a temperature of $75 \pm 5^\circ\text{C}$ to absorb water ^{19,21}. The temperature was then reduced to 60°C , and 2.94% lactulose syrup was added, followed by adding 3% maltodextrin and 1% stevia to some samples and 7% sucros to others ²². The mixtures were homogenized for a short period using a thermal mixer, then the temperature was reduced to $40 \pm 5^\circ\text{C}$, and cocoa powder (1.3%) and non-fat dry milk powder (9.24%) were added to the solution and mixed on a heater for a few minutes ²³. Subsequently, the cocoa milk was homogenized for 5 minutes at 6500 rpm using a homogenizer and pasteurized at a temperature of 80°C for 5 minutes before cooling to 4°C . Finally, the samples were refrigerated for 24 hours ²⁴.

All stability tests on samples were conducted during 5 days of storage based on the shelf life of pasteurized products and were performed in triplicate.

Rheological tests

For rheological evaluations, a protocol similar to that described in the previous study on dysphagia-oriented beverages²⁵ was followed. Rheological properties such as viscosity, storage shear modulus (G'), loss shear modulus (G''), loss tangent ($\tan \delta$), and flow behavior were measured using an Anton Paar MCR302 rheometer in a cylinder with a diameter of 50 millimeters and a gap size of 0.206 mm equipped with a P-PTD200-SN82454135 temperature control system and a concentric cylinder probe PP40/SN16891. All tests were performed in triplicate.

The apparent viscosity of samples was measured at two constant shear rates: 50 s^{-1} (represent the shear rate by the mouth to food) and variable shear rate $1\text{-}100 \text{ s}^{-1}$ (basis for comparing thick beverages suitable for dysphagia patients) at two temperatures of 10°C (serving temperature) and 35°C (mouth temperature).

The flow behavior of samples was tested over a shear rate range of $1\text{-}100 \text{ s}^{-1}$ at two temperatures of 10°C and 35°C . The rheological analysis was done using rheoplus software.

After strain sweep test and determination of linear viscoelastic region (LVR) values, frequency tests were conducted at variable frequencies of $0.1\text{--}10 \text{ Hz}$ with a constant strain of 0.1% and an LVR range of 1.

All oscillatory tests were performed at two temperatures of 10°C and 35°C , followed by evaluation of, storage modulus, loss modulus, and loss tangent.

Acidity measurements

According to National Iranian Standard No. 2852 for acidity determination, a volume of 10 milliliters of the sample was poured into an appropriate container, and 0.5 milliliters of phenolphthalein indicator was added. Titration with 1.0 N sodium hydroxide was performed until a pale pink color appeared that remained stable for at least 5 seconds. The acidity was calculated in terms of lactic acid using the following formula:

$$\text{Acidity (\% lactic acid)} = \frac{N \times 0.009 \times 100}{V}$$

Where N = Normality of NaOH used for titration and V = titration volume.

pH measurements

pH measurement was performed using a pH meter device where calibration of the device was first carried out using buffer solutions with pH 7 and pH 4. After calibration, the electrode of the device was

rinsed twice with distilled water, dried, placed inside the sample, and after a short period of time, the pH value was read.

Color measurements

The color measurement of the samples was conducted using an image processing method adopted from the previous studies and Adobe Photoshop CS5 software, where parameters L^* , a^* , b^* , R, G, and B were obtained. L^* represents the brightness level of the sample color ranging from 0 (pure black) to 100 (pure white), a^* indicates the proximity of the sample color to green or red with a variable range from - 120 (pure green) to + 120 (pure red), and finally, b^* indicates the proximity of the sample color to blue or yellow with a variable range from - 120 (pure blue) to + 120 (pure yellow). Additionally, to ensure the accuracy of the results, three other indices R (redness level), G (green color level), and B (blue color level) in the samples were also examined. The method involved calibrating the software using a white paper, pouring a small amount of the sample into a petri dish, capturing an image of the sample against a white background with a camera (ISOELL CMOS sensorre, Samsung), and obtaining the parameters using the software ^{1,26}.

Brix measurement

The Brix measurement in cocoa milk samples was determined using a refractometer at room temperature. Initially, the prism of the device was cleaned with distilled water, and a small amount of sample (2–3 drops) was placed on the prism. The Brix value was then read through the refractometer eyepiece. ¹.

Microbial tests

The total viable microbial count was determined using Plate Count Agar (PCA) medium. Cocoa milk samples were prepared and stored in sterile Falcon tubes for five consecutive days. The culture medium and dilution solution were sterilized in an autoclave at 121.5°C for 15 minutes. The medium was then poured into sterile Petri dishes, and serial dilutions of the samples were prepared for microbial testing. After incubation at 37°C for 48 hours, colonies were counted and expressed as colony forming units (CFU/mL) ²⁷.

Sensory evaluation test

The sensory evaluation was conducted using a 5-point hedonic scale by 15 trained panelists (male and female) aged 20–60 years from Shahid Beheshti University. Parameters including color, taste, aroma, texture, and overall acceptability were evaluated. Panelists provided their ratings on a scale of 1 to 5, where 1 represented the lowest and 5 the highest level of preference (Aami, Farhoodi, Ebrahimi Pure, Razaghi Jahromi, et al., 2025). Informed consent was obtained from all participants prior to their participation in the sensory evaluation.

Statistical analysis

Statistical analyses were carried out using IBM SPSS Statistics version 26. Data were evaluated using analysis of variance (ANOVA), and mean comparisons were performed using the least significant difference (LSD) test. Differences were considered statistically significant at $p < 0.05$. (Aami, Farhoodi, Ebrahimi Pure, Razaghi Jahromi, et al., 2025)

3. Results

Viscosity

The viscosity resulting from carrageenan in a product depends on concentration, temperature, type of carrageenan, and its molecular weight. To evaluate viscosity, it was first evaluated at a constant shear rate (s^{-1}) of 50 in two temperatures of 10°C and 35°C. According to Fig. 1, it can be observed that with an increase in carrageenan concentration, the viscosity of cocoa milk samples increased, which was also investigated by Zuo et al. (2020) who studied the effect of adding four hydrocolloids kappa-carrageenan, gelatin, pectin, and curdlan in cocoa milk and found that adding hydrocolloids increases viscosity²⁸.

With an increase in temperature to 35°C, the viscosity of the samples decreased as shown in Fig. 1. This decrease in viscosity is likely due to the physicochemical changes resulting from heat, which could potentially affect the interaction between kappa-carrageenan and milk proteins^{28,29}. Subsequent studies have reported hysteresis in carrageenan gels, where the gels are stable at room temperature but can be melted again by heating to a temperature 5 to 30°C higher than the gelation temperature. Additionally, upon cooling, the gel system regains its strength without losing gel strength or changing texture under neutral conditions¹⁹. However, this does not cause any disruption during swallowing and according to Chen et al., food remains in the mouth for a very short period, typically a few seconds, so the temperature of 35°C does not have a significant effect on food texture³⁰. Furthermore, saliva in the mouth has elastic properties and increases the viscosity of non-Newtonian fluids, so the thickened liquid texture in mouth temperature undergoes minimal changes and its viscosity will not significantly decrease, reaching the safe bolus into the stomach^{30,31}. As shown in Fig. 2, 3 and Table 1, the addition of 3% maltodextrin together with carrageenan (Ca) reduced viscosity loss at elevated temperature. At 10°C, samples containing either sucrose (Su) or maltodextrin–stevia (M + St) displayed slightly higher viscosity than the control, reflecting the thickening effect of both sugar and polysaccharide-based stabilizers. However, when the temperature increased to 35°C, only the samples containing carrageenan combined with maltodextrin–stevia maintained their viscosity, whereas the sucrose-containing and control samples showed a pronounced decrease. This behavior highlights the synergistic interaction between carrageenan and maltodextrin, which enhances thermal stability and prevents viscosity breakdown³².

The viscosity profiles of all samples across the shear-rate range of 1–100 s^{-1} demonstrated shear-thinning (pseudoplastic) behavior typical of non-Newtonian fluids. This trend reflects structural rearrangements within the carrageenan–milk protein matrix, where increasing shear disrupts the

entangled network, allowing the fluid to flow more easily. The temperature effect was more pronounced at higher shear rates, indicating that the carrageenan interactions were partially disrupted under combined thermal and mechanical stress. Notably, the maltodextrin–stevia formulation exhibited the smallest difference between viscosities at 10 and 35°C, confirming its superior rheological stability under oral conditions ($\approx 35^{\circ}\text{C}$). The presence of maltodextrin likely contributes to a denser and more cohesive matrix that minimizes structural collapse, while stevia’s hygroscopic nature may further enhance water retention and molecular interactions within the network. This enhanced stability is particularly beneficial for individuals with dysphagia, as maintaining viscosity within the IDDSI Level 2 range under oral conditions ensures a consistent bolus texture and controlled flow during swallowing. These findings align with previous studies on thickened dairy and fruit-based beverages ¹, confirming that appropriate combinations of hydrocolloids and carbohydrate stabilizers can achieve desirable texture and safety properties without compromising sensory acceptance.

Table 1: Impact of Maltodextrin and stevia compared to sucrose on viscosity changes at different carrageenan concentration at 10 and 35 °C at a constant shear rate of 50 s⁻¹

Carrageenan Concentration (%)	Viscosity (Pa.s)					
	10 °C			35 °C		
	Blank	Maltodextrin + stevia	Sucrose	Blank	Maltodextrin + stevia	Sucrose
0.05	0.0456	0.0555	0.0478	0.0111	0.0285	0.0102
0.08	0.0918	0.0991	0.0928	0.0209	0.0389	0.0173
0.1	0.122	0.133	0.125	0.0303	0.0659	0.0329

Investigation of Flow Behavior of Solutions

The flow behavior of cocoa milk samples containing different carrageenan concentrations (0.05–0.10%) was evaluated to better understand the effect of formulation and temperature on rheological stability (Figs. 3 and 4). All samples exhibited non-Newtonian, shear-thinning behavior, indicating that viscosity decreased as the shear rate increased. This behavior is typical of hydrocolloid-thickened systems, where structural breakdown occurs under shear due to partial disentanglement of polymer chains ^{33–35}.

The flow curves demonstrated that increasing carrageenan concentration resulted in higher shear stress at any given shear rate, confirming that viscosity and flow resistance increased proportionally with hydrocolloid content. At 10°C, the samples displayed greater shear stress values compared with 35°C, suggesting stronger molecular associations and more extensive gel-like network formation at lower temperature. The reduction of shear stress at 35°C reflects partial disruption of carrageenan–protein and carrageenan–carrageenan interactions caused by thermal weakening of hydrogen bonding.

The relationship between shear stress (σ) and shear rate ($\dot{\gamma}$) was modeled using the power-law equation:

$$\sigma = k (\dot{\gamma})^n$$

where $\dot{\gamma}$ is Shear rate, k is the consistency index (a measure of apparent viscosity or structural strength) and n is the flow behavior index (a measure of deviation from Newtonian flow). The model provided an excellent fit for all experimental data, with coefficients of determination ($R^2 = 0.93\text{--}0.98$) across the full temperature and concentration range (Table 2).

The R^2 value in this model was higher than any other model, and with an increase in concentration, the numerical value of R^2 increased. Following the power law model, the best fit was observed with the Herschel-Bulkley model, which has been reported to have the highest conformity in studies related to dysphagia beverages. In another study by Zhu et al.²⁸ focusing on cocoa milk texture, the highest agreement with the power law model was reported, aligning with the results obtained in this study .

As carrageenan concentration increased, the flow behavior index (n) decreased (from 0.72 to 0.63 at 10°C), confirming stronger shear-thinning characteristics and a more structured gel network at higher polymer content. A lower n value reflects a greater extent of structure breakdown under shear, typical of systems dominated by entangled or cross-linked biopolymer chains. In contrast, the consistency index (k) increased markedly (from 0.18 to 0.58 Pa·s ^{n}), showing that higher carrageenan levels generated more viscous and stable matrices.

Increasing temperature from 10°C to 35°C slightly increased n values (indicating a transition toward less pronounced shear-thinning) while decreasing k values, consistent with lower overall viscosity. These findings imply that the structural integrity of carrageenan gel complexes weakens upon heating, resulting in easier flow but reduced resistance to deformation. Nevertheless, even at oral temperature (35°C), all samples maintained shear-thinning behavior suitable for controlled swallowing in dysphagia-targeted beverages¹.

Overall, the observed concentration- and temperature-dependent variations in n and k are in agreement with previous reports on carrageenan-stabilized dairy systems^{32,35} and confirm that rheological modeling can reliably describe the flow characteristics of such functional formulations.

Table 2
Power law values for different carrageenan concentrations at 10 and 35°C

Carrageenan Concentration (%)	parameter	10°C	35°C
0.05	n	0.72069	0.77077
	k	0.18201	0.034
	R2	0.95	0.94
0.08	n	0.65671	0.75621
	k	0.40841	0.061
	R2	0.96	0.94
0.1	n	0.63055	0.73899
	k	0.58379	0.1.36
	R2	0.98	0.95

Measurement of Dynamic Rheological Properties

The dynamic rheological behavior of cocoa milk samples containing different carrageenan concentrations (0.05–0.10%) was investigated through oscillatory frequency sweeps at 10°C and 35°C (Fig. 5). Both the storage modulus (G') and loss modulus (G'') increased with carrageenan concentration, demonstrating that higher polymer content enhanced the structural rigidity and energy storage capacity of the system. The increase in G' was consistently greater than that in G'' , indicating a predominantly elastic or weak gel structure rather than a purely viscous one. This viscoelastic nature is typical of polysaccharide-protein systems where physical junction zones and entanglements contribute to partial gel formation.

Increasing the oscillation frequency resulted in higher values of both G' and G'' , confirming their frequency dependence. This effect was more pronounced at 10°C than at 35°C, suggesting that the structural network was more stable and resistant to deformation at lower temperature. The observed trends are consistent with previous studies on hydrocolloid-thickened beverages, where an increase in frequency and gum concentration both led to higher moduli^{1,36}. At elevated temperature (35°C), the overall moduli decreased significantly due to the partial disruption of carrageenan–protein interactions and thermal weakening of the gel network.

As shown in Fig. 5, all samples displayed $G' > G''$ across the entire frequency range, confirming their predominantly elastic character. This dominance of elastic over viscous response became more evident with increasing carrageenan concentration. At 10°C, where the samples exhibited stronger gel-like behavior, both moduli showed smaller changes with frequency, indicating reduced frequency dependency and a more cohesive network. In contrast, at 35°C, partial melting of the carrageenan

network led to a stronger frequency dependence, as the weakened matrix responded more readily to deformation.

The loss tangent ($\tan \delta = G''/G'$) further supports these findings. As carrageenan concentration increased, $\tan \delta$ values decreased, confirming an enhancement in the elastic component of the system. All samples remained within the safe viscoelastic range ($0.1 < \tan \delta < 1.0$), as defined for dysphagia-oriented fluids, suggesting that the samples maintained sufficient cohesiveness while remaining swallowable. Lower $\tan \delta$ values at higher carrageenan concentrations indicate a more solid-like character, which can contribute to controlled flow and improved bolus integrity during swallowing. These results align with previous reports on gum-thickened milk systems^{1,37} and confirm that the viscoelastic structure of the cocoa milk formulations supports their functional suitability for dysphagia management.

Acidity

The acidity values of the cocoa milk samples during five days of refrigerated storage are presented in Table 3. Samples containing sucrose exhibited significantly higher acidity than those formulated with stevia at equivalent carrageenan concentrations. This difference is attributed to the greater availability of fermentable carbohydrates in sucrose-containing formulations, which can be utilized by residual microorganisms, leading to increased acid production and consequently higher titratable acidity. In contrast, stevia is a non-fermentable sweetener and therefore did not promote microbial acidogenesis. Consequently, the observed differences in acidity are primarily attributed to the type of sweetener rather than the gum concentration.³⁸

During storage, a gradual increase in acidity was observed up to day 4, likely due to metabolic activity of lactic acid bacteria surviving pasteurization. Interestingly, a slight reduction in acidity occurred on day 5, which may be related to interactions between milk proteins and carrageenan or to buffering effects that limit further accumulation of free organic acids.

The control sample (without any stabilizers or sweeteners) consistently showed the highest acidity among all treatments. This behavior reflects the absence of carrageenan, which otherwise contributes to pH stabilization by forming electrostatic complexes with casein micelles and reducing microbial accessibility to lactose.

Overall, the combined presence of carrageenan and stevia resulted in the lowest acidity values and the most stable profile over the 5-day storage period, suggesting a synergistic effect in maintaining product quality and reducing fermentation-related changes

Table 3
acidity changes during 5-day storage.

Day	Control	0.05% C + M-St	0.05% C + S	0.08% C + M-St	0.08% C + S	0.1% C + M-St	0.1% C + S
0	0.06 ± .0 ^{aD*}	0.03 ± 0.0 ^{Aba}	0.04 ± 0.0 ^{Cb}	0.031 ± 0.0 ^{Ad}	0.04 ± 0.0 ^{BCb}	0.03 ± 0.0 ^{Aa}	0.041 ± .0 ^{Cb}
1	0.063 ± .0 ^{Fab}	0.03 ± 0.0 ^{Aa}	0.043 ± 0.0 ^{CDEa}	0.037 ± 0.0 ^{Ba}	0.042 ± 0.0 ^{CDc}	0.031 ± 0.0 ^{Aba}	0.045 ± .0 ^{Dea}
2	0.064 ± .0 ^{Db}	0.034 ± 0.0 ^{Ca}	0.045 ± 0.0 ^{Ba}	0.04 ± 0.0 ^{Aabc}	0.044 ± 0.0 ^{Ba}	0.037 ± 0.0 ^{Aab}	0.046 ± .0 ^{Ba}
3	0.07 ± .0 ^{Fc}	0.036 ± 0.0 ^{Ea}	0.048 ± 0.0 ^{Ca}	0.042 ± 0.0 ^{Ac}	0.046 ± 0.0 ^{ABCDa}	0.038 ± 0.0 ^{Dab}	0.047 ± .0 ^{ABCa}
5	0.075 ± .0 ^{Dd}	0.034 ± 0.0 ^{Aba}	0.033 ± 0.0 ^{Abc}	0.039 ± 0.0 ^{Cab}	0.033 ± 0.0 ^{Ad}	0.035 ± 0.0 ^{Aba}	0.037 ± .0 ^{ABCc}
*Values with same letters (upper letters for rows and lower letters for columns) have no significant difference at 5% significance level							

pH

The pH values of cocoa milk samples during five days of refrigerated storage are shown in Table 4. In all formulations containing carrageenan, a slight but consistent increase in pH was observed over time, whereas the control sample exhibited a gradual decrease in pH. The observed increase in pH, particularly after the first 24 hours of storage, may be attributed to the hydration and gel formation of carrageenan, which can entrap free hydrogen ions and thereby reduce their measurable concentration in the solution. This trend is in line with the findings of Ozturk et al. ³⁹, who reported that the incorporation of hydrocolloid stabilizers in cocoa milk led to an overall rise in pH values.

The carrageenan used in this study had an intrinsic pH between 8 and 11 (as reported in the product specification sheet), which likely contributed to the observed pH increase, especially at concentrations above 0.03%. In contrast, the control sample (without carrageenan) showed a steady decline in pH during storage. This reduction is consistent with previous reports by Shoaie et al. ⁴⁰, indicating that post-pasteurization microbial activity and lactic acid formation lead to increased acidity and, consequently, lower pH in non-stabilized milk systems.

No statistically significant differences ($p > 0.05$) were observed in pH between samples sweetened with sucrose and those containing stevia at carrageenan concentrations below 0.1%. Although Shoaie et al. ⁴⁰ reported a slight pH reduction upon the addition of stevia in cocoa milk, other studies, such as Alizadeh et al. (2014) on stevia substitution in fruit juices, found no significant influence of sweetener type on pH. These discrepancies suggest that the effect of sweeteners on pH may depend strongly on the food matrix composition and its buffering capacity.

Overall, the formulations containing both carrageenan and stevia maintained relatively stable pH values throughout the 5-day storage period, supporting the earlier findings on acidity (Section 3.4). The combined stabilizing effect of carrageenan and the non-fermentable nature of stevia likely contributed to the enhanced chemical and microbial stability of the fortified cocoa milk.

Table 4
pH changes during 5-day storage

Day	Control	0.05% C + M-St	0.05% C + S	0.08% C + M-St	0.08% C + S	0.1% C + M-St	0.1% C + S
0	6.69 ± 0.0 ^{Cc*}	6.66 ± 0.0 ^{Aba}	6.64 ± 0.0 ^{Abb}	6.68 ± 0.0 ^{Abc}	6.65 ± 0.0 ^{Abb}	6.64 ± 0.0 ^{Aba}	6.64 ± 0.0 ^{Ba}
1	6.68 ± 0.0 ^{Cc}	6.77 ± 0.0 ^{Abb}	6.81 ± 0.0 ^{Aa}	6.81 ± 0.0 ^{Aab}	6.73 ± 0.0 ^{Bc}	6.78 ± 0.0 ^{Abb}	6.75 ± 0.0 ^{Bb}
2	6.64 ± 0.0 ^{Cbc}	6.80 ± 0.0 ^{Abbc}	6.83 ± 0.0 ^{Bab}	6.83 ± 0.0 ^{Bab}	6.80 ± 0.0 ^{Aba}	6.77 ± 0.0 ^{Ab}	6.85 ± 0.0 ^{Bc}
3	6.61 ± 0.0 ^{Dab}	6.82 ± 0.0 ^{ABCbc}	6.84 ± 0.0 ^{Ca}	6.84 ± 0.0 ^{Ca}	6.83 ± 0.0 ^{Bca}	6.81 ± 0.0 ^{ABCbc}	6.83 ± 0.0 ^{BCc}
5	6.59 ± 0.0 ^{Ca}	6.83 ± 0.0 ^{Abc}	6.80 ± 0.0 ^{Ab}	6.80 ± 0.0 ^{Ab}	6.84 ± 0.0 ^{Aba}	6.85 ± 0.0 ^{Bc}	6.85 ± 0.0 ^{Abc}

*Values with same letters (upper letters for rows and lower letters for columns) have no significant difference at 5% significance level.

Brix

The Brix values of cocoa milk samples increased with rising carrageenan concentration, primarily due to the higher proportion of total soluble solids in the formulations. Similarly, samples containing sucrose exhibited higher Brix values than those prepared with stevia, reflecting the greater molecular weight and solubility of sucrose compared to steviol glycosides. In contrast, formulations sweetened with stevia showed significantly lower Brix values, consistent with previous reports describing similar trends in sucrose substitution systems. Alizadeh et al. (2014) reported the same behavior in fruit-based milkshakes.

Storage at refrigeration temperature for five days did not cause a statistically significant change in Brix values ($p > 0.05$), indicating that no major compositional or phase-separation effects occurred during short-term storage. The stable Brix readings confirm that the formulated cocoa milk maintained its soluble solids content and overall formulation integrity throughout the storage period.

Color

As shown in Table 5, the L* index or brightness in cocoa milk samples containing sugar was slightly lower than in samples containing maltodextrin-stevia, but this slight difference was not statistically significant at a probability level of 5% ($p > 0.05$). On the other hand, with an increase in carrageenan concentration, this index decreased, suggesting that this decrease in brightness may be due to the interactions of carrageenan with cocoa milk compounds. During storage, none of the samples showed a significant difference in color ($p > 0.05$), and all the samples-maintained stability in all color indexes over a 5-day storage period in the refrigerator. This result was also confirmed by examining the R index, which more accurately reflects the redness of the samples.

According to Table 5, the a* index in samples containing maltodextrin-stevia at higher concentrations is greater than in samples containing sugar, and as the carrageenan concentration increases, the difference in this index becomes more pronounced. However, these differences did not create a statistically significant effect ($p > 0.05$), which was also observed in the studies by Abedini et al. ⁴¹.

The b* index in samples containing maltodextrin-stevia at concentrations of 0.05% and 0.08% carrageenan is higher than in samples containing sugar; the lower value of this index indicates the proximity of sample colors to blue. Additionally, the yellow color intensity in control samples and samples containing sugar increased slightly during storage, while in samples containing stevia, there was a decreasing trend. It can be said that none of these samples, except for the control sample, showed a statistically significant difference with each other.

Overall, significant differences were only observed between the control sample and different treatments of cocoa milk, which could be attributed to cocoa sediment in the control sample due to the absence of carrageenan.

Table 5
Coca milk samples color index (L*, a* and b*) changes during 5-day storage.

Sample		Day 1	Day 2	Day 3	Day 5
Control	L*	35.33 ± 3.0 ^{aC*}	33.67 ± 1.5 ^{aB}	35.00 ± 4.0 ^{aB}	32.67 ± 5.5 ^{aB}
	a*	7.6 ± 0.5 ^{aA}	7.3 ± 0.5 ^{aA}	8.0 ± 2.0 ^{aA}	8.6 ± 1.0 ^{aA}
	b*	8.67 ± 1.5 ^{aA}	9.00 ± 1.0 ^{aA}	9.00 ± 1.0 ^{aA}	8.67 ± 0.5 ^{aA}
0.05% C + M-St	L*	30.33 ± 0.5 ^{aBC}	29.00 ± 2.0 ^{aAB}	29.67 ± 0.5 ^{aAB}	29.67 ± 1.5 ^{aAB}
	a*	11.6 ± 0.5 ^{aC}	11.6 ± 0.5 ^{aC}	11.3 ± 0.5 ^{aB}	12.0 ± 0.0 ^{aC}
	b*	13.0 ± 1.0 ^{aB}	12.33 ± 0.5 ^{aB}	11.67 ± 0.5 ^{aB}	12.0 ± 1.0 ^{aB}
0.05% C + S	L*	30.33 ± 1.5 ^{aBC}	28.33 ± 2.3 ^{aAB}	30.0 ± 1.0 ^{aB}	27.67 ± 2.0 ^{aAB}
	a*	11.0 ± 0.0 ^{aBC}	11.0 ± 0.0 ^{aBC}	11.0 ± 0.0 ^{aBC}	10.6 ± 0.5 ^{aABC}
	b*	8.67 ± 1.5 ^{aA}	9.00 ± 1.0 ^{aA}	9.00 ± 1.0 ^{aA}	8.67 ± 0.5 ^{aA}
0.08% C + M-St	L*	29.3 ± 1.6 ^{aBC}	29.0 ± 2.0 ^{aAB}	29.0 ± 1.0 ^{aAB}	28.3 ± 1.5 ^{aAB}
	a*	11.0 ± 0.0 ^{aBC}	11.0 ± 0.0 ^{aBC}	11.33 ± 0.5 ^{aB}	11.0 ± 0.0 ^{aABC}
	b*	12.0 ± 0.0 ^{aB}	11.67 ± 0.5 ^{aB}	12.0 ± 0.0 ^{aB}	11.6 ± 0.5 ^{aB}
0.08% C + S	L*	27.0 ± 2.6 ^{aAB}	26.3 ± 3.5 ^{aA}	27.67 ± 3.5 ^{aA}	25.6 ± 3.0 ^{aAB}
	a*	8.67 ± 1.5 ^{aAB}	10.67 ± 1.5 ^{aBC}	9.67 ± 1.1 ^{aAB}	8.67 ± 1.5 ^{aAB}
	b*	11.67 ± 0.5 ^{aB}	12.33 ± 0.5 ^{aB}	11.33 ± 0.5 ^{aAB}	11.33 ± 0.5 ^{aB}
0.1% C + M-St	L*	27.3 ± 0.5 ^{aAB}	28.3 ± 0.5 ^{aAB}	28.0 ± 1.0 ^{aA}	27.6 ± 1.1 ^{aAB}
	a*	11.0 ± 1.0 ^{aBC}	11.0 ± 1.0 ^{aBC}	10.0 ± 1.0 ^{aAB}	10.6 ± 1.5 ^{aABC}
	b*	11.6 ± 0.5 ^{aB}	12.0 ± 0.0 ^{aB}	11.6 ± 0.5 ^{aB}	12.0 ± 0.0 ^{aB}
0.1% C + S	L*	24.6 ± 1.5 ^{aA}	27.3 ± 0.5 ^{aAB}	28.0 ± 1.0 ^{aA}	22.6 ± 2.0 ^{aA}
	a*	9.6 ± 1.5 ^{aABC}	11.3 ± 0.5 ^{aC}	10.3 ± 1.1 ^{aAB}	8.6 ± 0.5 ^{aA}
	b*	11.67 ± 0.5 ^{aB}	12.33 ± 0.5 ^{aB}	11.33 ± 0.5 ^{aB}	11.33 ± 0.5 ^{aB}

*Values with same letters (upper letters for rows and lower letters for columns) have no significant difference at 5% significance level.

Sediment Level

No visible sediment formation was observed in any of the cocoa milk samples during the 5-day storage period, except for the control sample. The absence of sediment in the treated formulations can be attributed to the stabilizing effect of carrageenan, which effectively maintained the suspension of cocoa particles and prevented gravitational settling through the formation of a weak gel network. This structural integrity ensured homogeneous dispersion of solids and contributed to the visual stability of the product throughout storage. However, in the formulation containing 1.0% carrageenan, signs of phase separation were detected from the first day. This phenomenon is likely related to excessive gel rigidity and localized syneresis, which can expel water from the gel matrix and create visible serum separation. Such over-gelation effects highlight the importance of optimizing carrageenan concentration to balance particle suspension and prevent destabilization due to gel contraction.⁴²

Sensory Properties Evaluation

The results of the sensory evaluation are summarized in Table 6. Among the samples, the highest aroma score was obtained for the cocoa milk containing 0.05% carrageenan with maltodextrin-stevia, whereas the lowest aroma acceptance was recorded for the samples containing 0.05% carrageenan + sucrose and 0.1% carrageenan + maltodextrin-stevia. In terms of texture, the highest panel rating corresponded to the formulation with 0.1% carrageenan + maltodextrin-stevia, while the lowest was observed in 0.05% carrageenan + sucrose.

Overall, cocoa milk samples with higher carrageenan concentrations received greater texture and mouthfeel scores, which aligns with the rheological results indicating higher viscosity and improved structural integrity at elevated carrageenan levels. The increase in viscosity likely enhanced the sensory perception of creaminess and swallowing safety, producing a smoother and more pleasant mouthfeel desirable for dysphagia-oriented products.

The taste scores followed a similar pattern, with the formulation containing 0.1% carrageenan + stevia receiving the highest rating, and the sample with 0.08% carrageenan + sucrose receiving the lowest. Notably, panelists reported that the use of stevia at appropriate levels did not impart a bitter aftertaste and produced a sweetness profile comparable to sucrose, supporting its sensory feasibility as a natural sugar substitute.

The overall acceptability ratings confirmed that the cocoa milk containing 0.1% carrageenan + stevia achieved the highest consumer preference, while the 0.05% carrageenan + sucrose formulation had the lowest acceptance. These findings demonstrate that optimizing carrageenan concentration in combination with natural sweeteners such as stevia can improve both sensory quality and functional suitability, balancing viscosity, taste, and consumer acceptability in fortified cocoa milk beverages.

Table 6
sensory evaluation

	Control	0.05% Ca		0.08% Ca		0.10% Ca	
		stevia	sucrose	stevia	sucrose	stevia	sucrose
Color	3.80 ± 0.83 ^A	3.40 ± 1.10 ^A	3.00 ± 0.70 ^A	4.60 ± 0.50 ^A	4.00 ± 1.00 ^A	4.00 ± 0.70 ^A	4.00 ± 0.70 ^A
Odor	4.60 ± 0.54 ^A	4.80 ± 0.44 ^A	4.00 ± 1.40 ^A	4.20 ± 0.80 ^A	4.20 ± 0.80 ^A	4.00 ± 0.73 ^A	4.40 ± 0.89 ^A
Taste	3.80 ± 0.83 ^A	3.80 ± 1.09 ^A	3.40 ± 0.89 ^A	4.20 ± 0.44 ^A	2.60 ± 0.54 ^A	4.40 ± 0.89 ^A	3.00 ± 0.70 ^A
Texture	3.80 ± 1.09 ^{AB}	3.80 ± 0.44 ^{AB}	3.60 ± 0.54 ^{AB}	4.00 ± 1.22 ^{AB}	4.00 ± 1.00 ^A	4.40 ± 1.34 ^B	4.00 ± 1.73 ^{AB}
Overall Acceptance	4.00 ± 0.70 ^{AB}	3.80 ± 0.44 ^{AB}	3.20 ± 0.44 ^{AB}	4.00 ± 0.70 ^{AB}	3.00 ± 0.70 ^A	4.40 ± 0.89 ^B	3.60 ± 0.54 ^{AB}

Values with same letters have no significant difference at 5% significance level

Microbiological evaluation

The total bacterial count of cocoa milk samples during refrigerated storage is shown in Fig. 6. In all formulations, a gradual increase in microbial load was observed over the 5-day storage period. This increase can be attributed to the activity of heat-resistant enzymes and bacteria that either survived pasteurization or entered through minor post-processing contamination^{43,44}. The growth pattern exhibited a short lag phase followed by a progressive increase in bacterial count after the third day, consistent with previous findings for pasteurized milk systems¹⁰.

The control sample (blank cocoa milk) showed a higher microbial load than regular milk, confirming previous studies reporting faster bacterial growth in cocoa-based dairy products compared to plain milk⁴⁵. The presence of cocoa solids and carbohydrates likely provides additional nutrients that favor microbial proliferation. The incorporation of carrageenan in cocoa milk slightly promoted microbial growth, particularly after the third day of storage. However, no significant differences ($p > 0.05$) were detected among samples containing different carrageenan concentrations, which agrees with earlier studies suggesting that carrageenan does not exert a direct antimicrobial effect but may influence nutrient dispersion and water-binding capacity⁴⁶.

In contrast, samples sweetened with sucrose exhibited a higher microbial load than those containing stevia, reflecting microorganisms' greater affinity for fermentable sugars. The slightly lower bacterial counts in stevia-containing samples may be linked to the mild antimicrobial activity of steviol glycosides reported in previous studies¹⁶.

Overall, despite a moderate increase in microbial counts over storage, none of the samples exceeded the acceptable microbial limits established by the Iranian National Standard Organization for pasteurized milk products. These results indicate that the fortified cocoa milk formulations remained microbiologically safe and stable during the 5-day refrigerated storage period.

4. Conclusion

The results of this study demonstrated that developing a fortified cocoa milk suitable for individuals with dysphagia is feasible, achieving IDDSI Levels 0–2 at both serving (10°C) and oral cavity (35°C) temperatures. Rheological analyses confirmed that increasing the concentration of carrageenan up to an optimal threshold of 1.0% enhanced viscosity, storage modulus (G'), and loss modulus (G''), thereby improving flow resistance and textural stability.

Incorporation of maltodextrin effectively mitigated viscosity loss at 35°C, stabilizing the formulation under oral conditions.

comprehensive evaluation of rheological, stability, sensory, and microbiological parameters revealed that replacing sucrose with stevia did not compromise product quality; on the contrary, stevia-containing samples exhibited higher sensory acceptance and comparable physicochemical stability. The absence of sedimentation during storage and the lack of microbial spoilage confirmed the physical and microbiological stability of the formulations.

Overall, the two main objectives of this study achieving appropriate viscosity for safe swallowing and fortifying cocoa milk with lactulose and stevia for functional health benefits, were successfully met. Although lactulose and stevia did not significantly affect viscosity or viscoelastic behavior, their inclusion improved the nutritional and sensory profile of the product. These findings demonstrate that cocoa milk enriched with carrageenan, maltodextrin, lactulose, and stevia can serve as a functional, dysphagia-friendly beverage with enhanced safety, palatability, and nutritional value.

Declarations

Authors have no conflict of interests.

There is no funding to declare.

Author Contribution

Z.B. investigation, experiments. N. R. writing – original draft, visualization, M.F. methodology, validation, supervision, formal analysis, project administration, conceptualization. A. E. P. formal analysis, methodology, validation, writing – review and editing, visualization, supervision, software, data curation, conceptualization, A.N. supervision, conceptualization

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request

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Figures

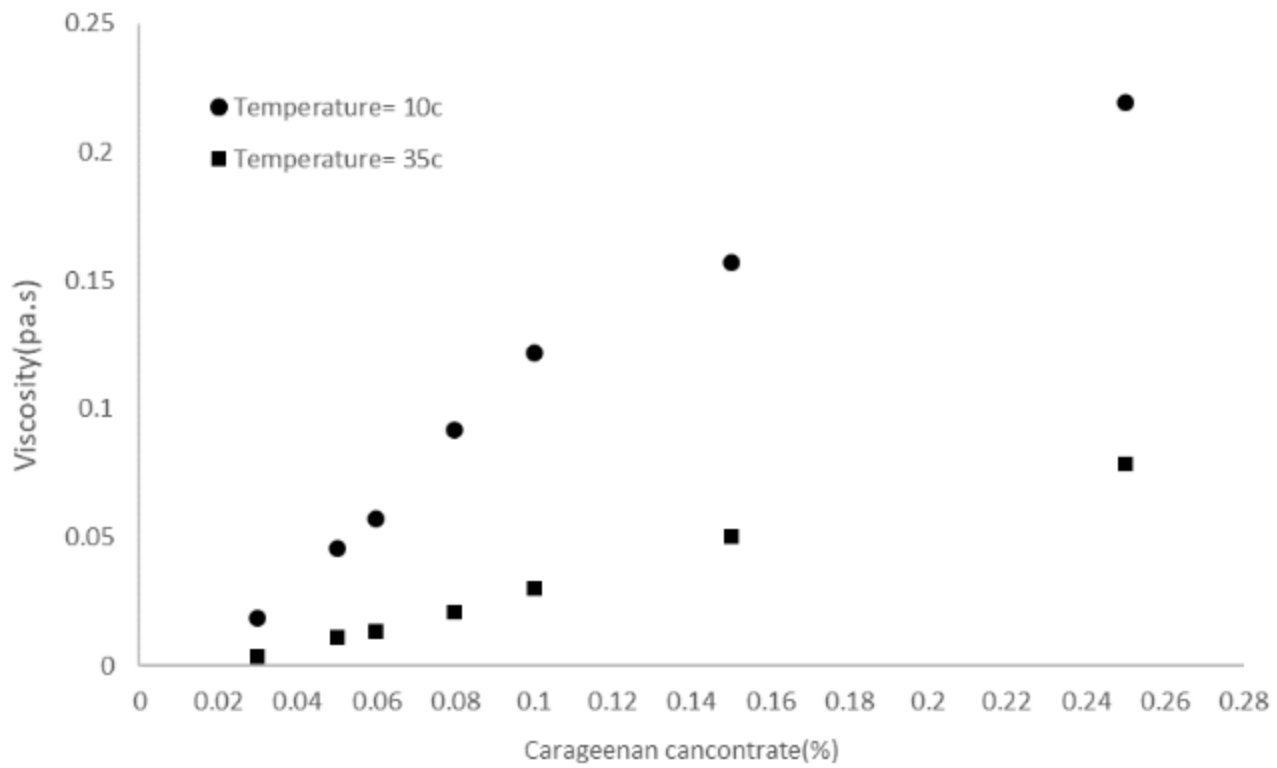


Figure 1

The impact of carrageenan concentration on viscosity of cocoa milk at a constant shear rate of 50 s^{-1} and temperature of 10 and 35°C

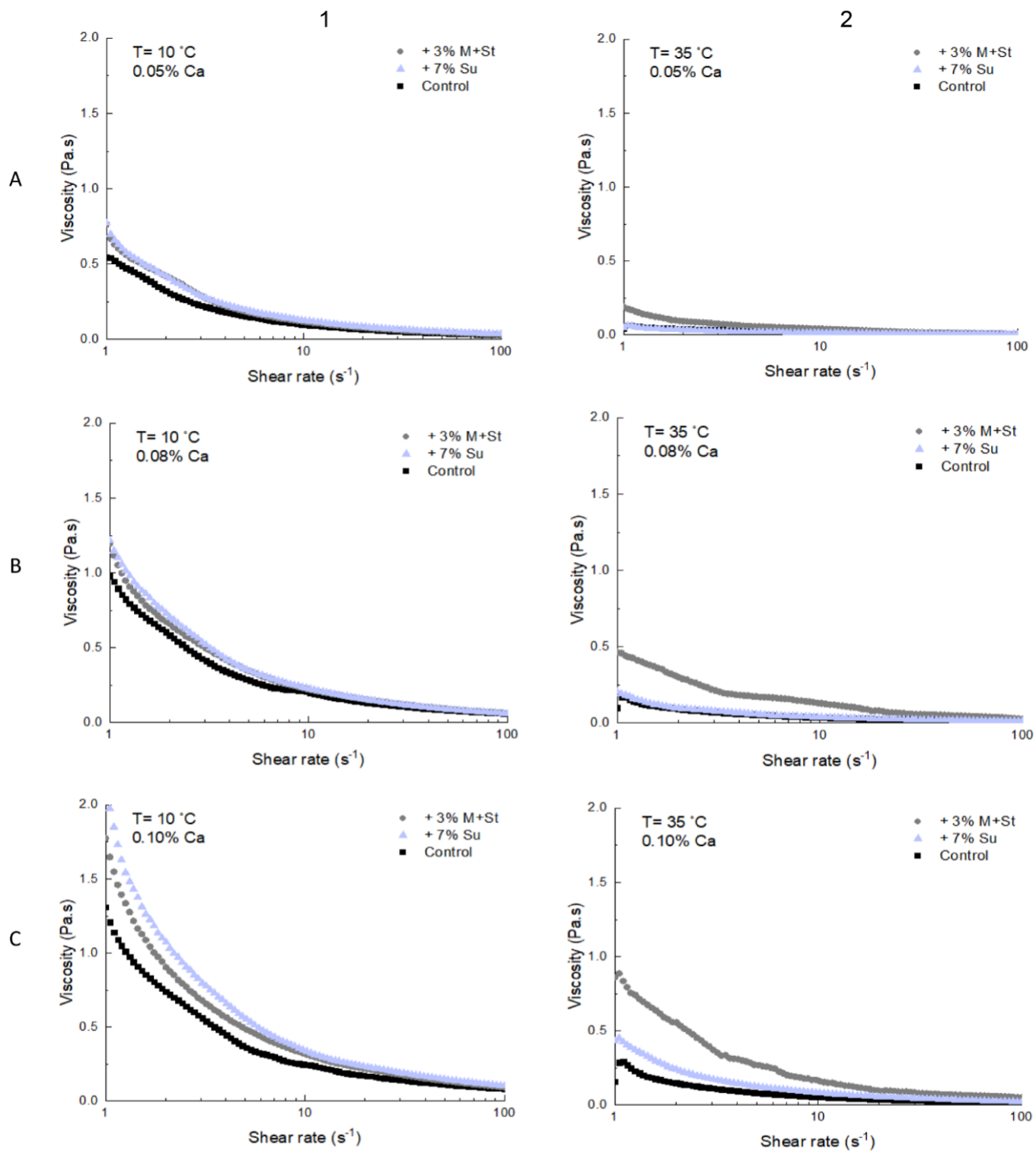


Figure 2

Impact of Maltodextrin (M) and stevia (St) against sucrose (Su) on viscosity changes at different carrageenan concentration and shear rate of 1-100 s^{-1} at 10 and 35 °C

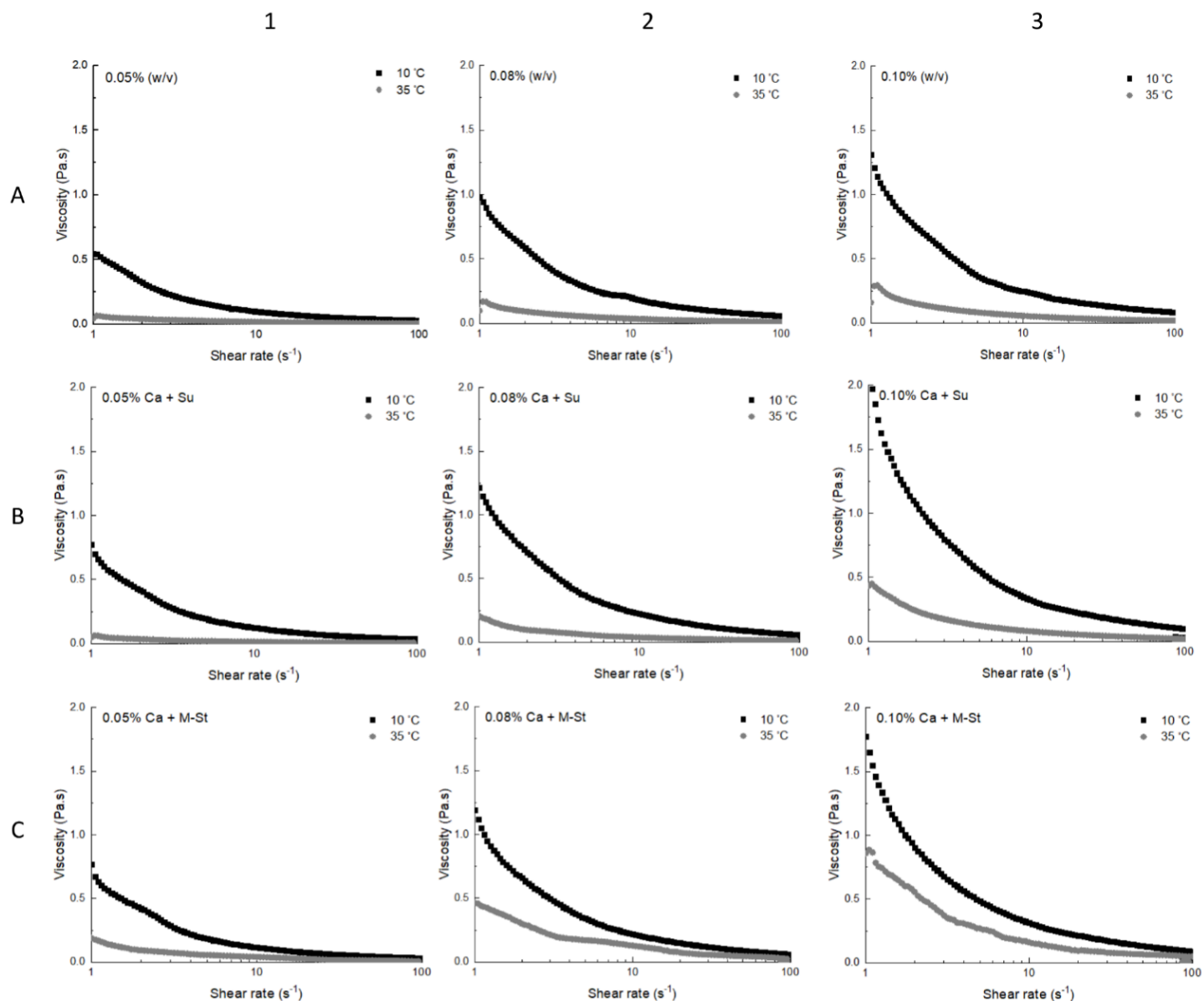


Figure 3

The impact of maltodextrin-stevia at different carrageenan concentration on viscosity loss at 10 and 35 °C

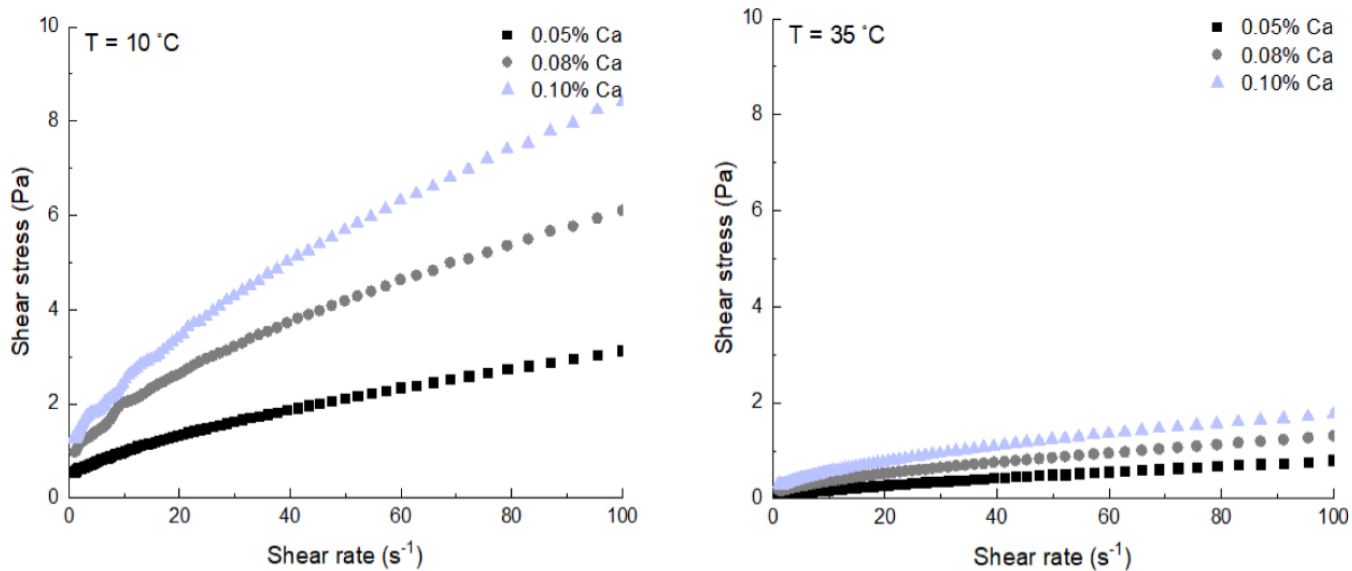


Figure 4

flow behavior characteristics of cocoa milk samples with different carrageenan concentrations at shear rate of 1-100 s^{-1} at 10 and 35 °C

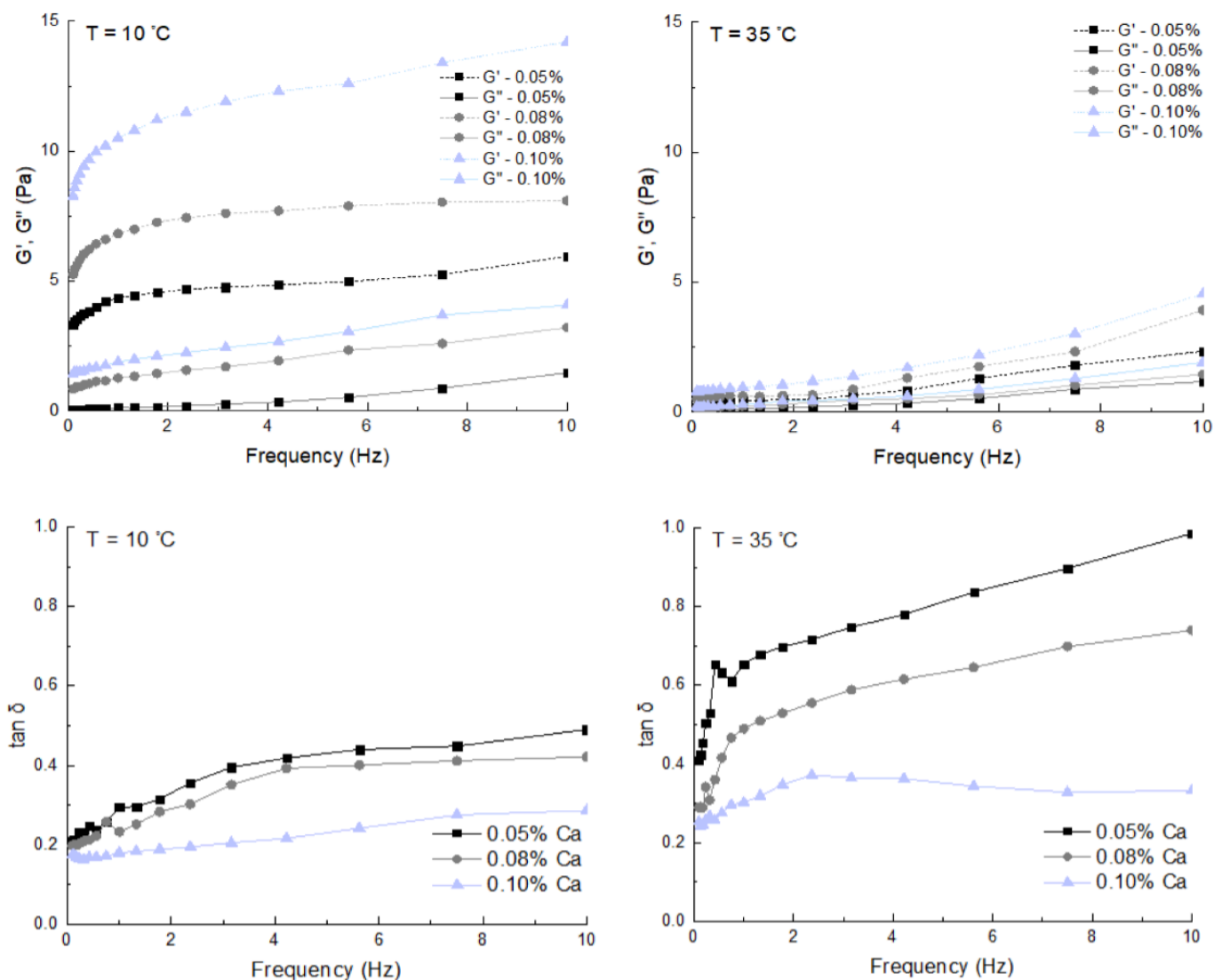


Figure 5

The impact of frequency on viscoelastic properties of cocoa milk samples at 10 and 35 °C

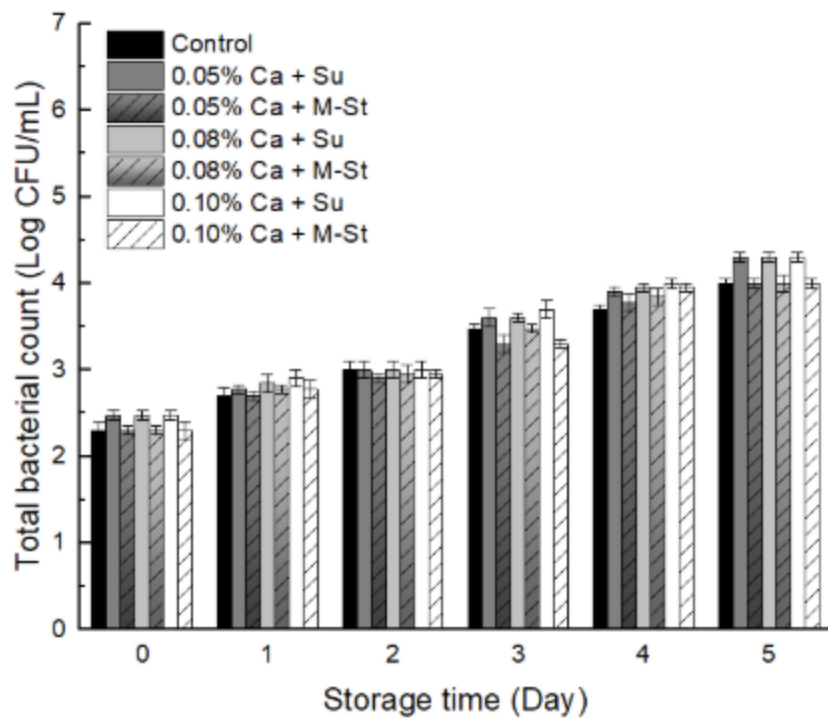


Figure 6

Total microbial count for samples from day 0 till day 5 of storage.