

ORIGINAL ARTICLE

Food Engineering, Materials Science, and Nanotechnology

Determination of stabilizer concentration and process effect on the stability of milk-based cocoa beverage

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In the production of milk-based cocoa beverages, stability challenges such as sedimentation and creaming may arise due to the delicate balance of cocoa particles, proteins, and fat content, impacting the uniformity and texture of the final product. One of the most important hydrocolloids used to overcome these stabilization problems in milk-based products is κ -carrageenan. In this study, the optimization process was carried out using central composite design by adding different proportions of κ -carrageenan (0.01%–0.04%) and applying homogenization processes at different pressures (5–34 MPa) considering the norms of dairy industry. The effects of these independent variables on water-holding capacity (WHC), separation, creaming, pH, and viscosity values were investigated. Optimum sample (0.037% carrageenan and 19.38 MPa) had high WHC (97.26%), low viscosity (62.01 mPa s), and suitable separation and creaming values, indicating superior stability compared to market alternatives. The L^* , a^* , b^* color values, particle size (Z -Ave), ζ -potential value, FTIR spectra, and microscope images were measured in the optimum samples and samples obtained from market. Image processing also highlighted that optimum sample had better stability than market samples considering entropy, variance, and homogeneity values. Additionally, this research presents an innovative approach by detailing the interactions between κ -carrageenan and milk proteins, which are key to achieving the desired product stability. The fact that the levels of independent variables used in the study are appropriate for the industry will shed light on the studies to be carried out to produce products with the desired quality in the market.

KEYWORDS

carrageenan, homogenization, image processing, microstructure, milk-based cocoa beverages

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1 | INTRODUCTION

Nowadays, the consumption of flavored milk drinks is quite common, and the most popular among these products are dairy products containing cocoa. Studies on milk-based cocoa beverages generally focus on sensory and rheological properties. Due to the structural properties of cocoa, stability problems, such as sedimentation of cocoa particles, formation of large lumps, and formation of light–dark colored layers, may occur in such dairy products (Prakash et al., 2010). The addition of hydrocolloids is critical for enhancing the viscosity of cocoa-based milk, effectively combating issues such as sedimentation. The hydrocolloids maintain the stability of fat globules in each suspension phase, support the suspension of solid components, improve viscosity, reduce sedimentation rate, and maintain the quality of emulsion stability. Hydrocolloids, such as alginate, carrageenan, guar gum, and xanthan gum, exemplify this functionality. Carrageenan is the most widely used hydrocolloid because it can eliminate such stability problems in milk-based cocoa beverages and positively affect the mouthfeel of milk by providing viscosity to the products (Yanes et al., 2002). κ -Carrageenan, in particular, stands out for its high viscosity, excellent water solubility, beneficial interactions with other ingredients, and self-tolerance (Cong et al., 2022; Kumar & Verma, 2023; Sharma et al., 2022). κ -Carrageenan also has a stabilizing effect on protein–protein interactions, allowing it to maintain product stability and milk product viscosity (Pirsa & Hafezi, 2023).

In this study, we specifically focused on κ -carrageenan, as this structure plays an important role in milk-based cocoa beverages due to its capacity to provide stability and its interactions with milk proteins. Most studies devoted to the technology of milk-based cocoa beverages with the addition of κ -carrageenan focus on the influence of the main raw materials on the formation of their properties. The aim of the present research is to utilize the potential of κ -carrageenan in modeling milk-based cocoa beverages. Heating processes used during the preparation of such products may cause aggregation and precipitation of milk proteins. This problem may be attenuated through the retentive properties of hydrocolloids acting as stabilizers of protein (Lim & Baik, 2025; Nurhayati et al., 2023; Zaitoun, 2024).

Carrageenan is a sulfated polymer of galactose with an average molecular weight of 100 kDa, and its most important forms are κ -, ι -, and λ -carrageenans. It is obtained from the red seaweed species of the Rhodophyceae class. Carrageenan source seaweeds are processed to obtain the desired κ -, ι -, and λ -carrageenan ratios to meet the requirements of food technology (Langendorff et al., 2000). Although carrageenan has no nutritional value, it is used

in foods because of its thickening, gelling, stabilizing, and brightening properties. In the presence of potassium cations in κ -carrageenan and calcium cations in ι -carrageenan, these compounds exhibit gelling properties in water at concentrations above 1%, especially when subjected to heating (above 60°C) and subsequent cooling (below 35°C) process. The stability of carrageenan in foods is affected by several factors, such as pH and structural bridging between negatively and positively charged carrageenan and protein domains. There has been some controversy over the use of carrageenan as a food additive for some time. Some studies suggest that carrageenan, particularly its degraded form (poligeenan), may trigger gastrointestinal inflammation, bloating, and discomfort in sensitive individuals. There are concerns that it could contribute to conditions like irritable bowel syndrome or inflammatory bowel disease (Bixler, 2017; Tobacman, 2001; Wang et al., 2022). The Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2014) has concluded that the use of carrageenan in concentrations up to 1000 mg/L is not of concern, especially in relation to the safety assessment of its use in infant formula, based on its most recent 2014 assessment.

Carrageenan is an important hydrocolloid used to stabilize milk-based dispersions such as milk-based cocoa beverages (Zarzycki et al., 2019). Carrageenan acts as an effective stabilizer due to the interaction between negatively charged sulfate groups and positively charged components in dairy products. It is suggested that there is a specific electrostatic interaction between carrageenan sulfate groups and casein micelles so that carrageenan can contribute to stability in milk-based colloidal systems. Studies have reported that the charge density of casein micelles in milk becomes more negative as the concentration of carrageenan increases (Ji et al., 2008). The formation of these networks enhances the mouthfeel of milk-based cocoa beverages and aids in stabilizing the distribution of cocoa particles (Guo et al., 2023; Holkar et al., 2019; Prakash et al., 2010; Tran et al., 2021).

Milk-based cocoa beverages are a colloidal system containing protein, fat droplets, cocoa, carbohydrates, or dispersed particles such as carrageenan. If this mixture is stored for a long time, it becomes difficult to obtain a stable product. Solid particles such as cocoa tend to precipitate in the traditional heat-treated (UHT and pasteurized) mixtures, resulting in quality loss (Harte, 2016; Tran et al., 2021). The appropriate pressure in colloidal systems depends on the specific application, the type of colloidal dispersion, and the processing method. For most food applications, including colloidal systems, 10–100 MPa is a common range, particularly in homogenization and emulsification processes. However, the exact pressure requirement depends on the formulation, stability goals,

and equipment used (Holkar et al., 2019). Standard homogenization, used widely in dairy industry, operates with a pressure range of 20 and 60 MPa.

Despite the potential benefits of carrageenan and homogenization pressure, comprehensive studies investigating their combined effects on milk-based cocoa beverages have remained limited. Additionally, long-term shelf-life studies on cocoa-based milk have not been extensively conducted in the literature (Tran et al., 2021). Therefore, this study aims to address this gap by examining the impact of carrageenans as stabilizers and pressure treatment on the stability and structural properties of milk-based cocoa beverages. Specifically, the optimization process involves varying carrageenan concentrations and pressures to determine their optimal combination for enhancing product stability and quality. Carrageenan and pressure levels have been determined in the present study, taking into account the industrial conditions in order to be adaptable to the industry. By elucidating the synergistic effects of carrageenan and pressures on milk-based cocoa beverages, this research seeks to contribute to the development of innovative processing techniques and the improvement of product quality in the dairy industry.

2 | MATERIALS AND METHODS

2.1 | Materials

Pasteurized milk (3% fat, 3% protein) and crystal sugar were obtained from the local market. Carrageenan (Genu-lacta carrageenan type K-100) was kindly donated by CP Kelco. The type of carrageenan consists of κ -carrageenan and is sold under the trade name GENULACTA[®] Carrageenan K-100. Alkalized cocoa powder was supplied by Altinmarka, potassium phosphate was supplied by Budenheim, and potassium sorbate was supplied by Nantong. In addition, milk-based cocoa beverages containing carrageenan, coded Product-1 and Product-2, were obtained from the local market in order to compare with milk-based cocoa beverages produced at the optimum point. All chemicals used throughout the study were purchased from Sigma-Aldrich Chemie.

2.2 | Production of milk-based cocoa beverages

Sugar (6%), alkalized cocoa powder (1%), potassium phosphate (0.50%), potassium sorbate (0.05%), and carrageenan (w/w) were mixed in dry form. The dry phase was added to the pasteurized milk, and all components were mixed at 450 rpm using Thermomix (Thermomix TM6, Vorwerk). The system was heated to 75°C and immediately

homogenized with a homogenizer (Hommak C-HM1) at the pressure norms (between 5.80 and 34.10 MPa) specified in the central composite design (CCD) experimental design (Table 1). After homogenization, the sample was kept at 95°C/4 min. The system was cooled very quickly to 25°C, and the sample was then filled into glass vials with plastic caps. Milk-based cocoa beverages were produced under standard conditions, taking into account dairy industry production conditions (Anonymous, 1995).

2.3 | Water-holding capacity (%)

The water-holding capacity (WHC) of milk-based cocoa beverages was determined by using a centrifugation (Nuve, NF 400) of a 50 mL sample at $2819 \times g$ for 10 min at room temperature. The released serum was measured as a volume (mL) (Luo et al., 2019).

$$\text{WHC (\%)} = [1 - [\text{Released serum (supernatant)} / \text{original gel}] \times 100$$

2.4 | Sedimentation (%) and creaming (%) properties

Milk-based cocoa beverages were introduced into glass cylindrical tubes (length of 15 cm) and sealed with plastic caps. The samples were stored at $4 \pm 1^\circ\text{C}$ for 21 days to determine the optimum point. Sedimentation was determined as the percentage ratio of the sediment height (H_S) to the total height of the beverage (H_T) (Tran et al., 2021):

$$\text{Sedimentation (\%)} = (H_S/H_T) \times 100$$

where H_S is the height of the sediment and H_T is the total height of the milk-based cocoa beverages.

For the shelf-life studies, sedimentation values were determined monthly.

In order to observe the effect of milk on sedimentation analysis, a comparative study was carried out in which cocoa was added to water instead of milk, and all the treatments applied to milk beverages were also applied to the aqueous cocoa solution.

In milk-based cocoa beverage systems, creaming analysis is used to evaluate the stability of the emulsion by measuring the phase separation over time. When the emulsion samples separate into two layers, the separation typically occurs due to density differences and instability of dispersed fat droplets. Top layer consists primarily of fat-rich components. The bottom layer is the aqueous phase, which contains water, dissolved proteins, sugars, and some dispersed cocoa particles that have not migrated to the top.

TABLE 1 The response values obtained with the run points of the created central composite design (CCD) experimental design.

Run	X ₁ (%)	X ₂ (MPa)	WHC (%)	Sedimentation (%)	Creaming (%)	pH	Viscosity (mPa s)
1	0.010	30.0	85.3 ± 0.8	13.75 ± 1.77	4.65 ± 0.16	6.85 ± 0.02	34.1 ± 1.4
2	0.046	20.0	95.6 ± 0.6	nd	nd	6.88 ± 0.01	70.8 ± 3.1
3	0.025	20.0	94.8 ± 1.1	0.71 ± 0.18	0.69 ± 0.11	6.86 ± 0.01	44.6 ± 0.6
4	0.025	34.1	89.3 ± 0.4	2.27 ± 0.32	2.00 ± 0.11	6.87 ± 0.03	47.5 ± 0.5
5	0.040	30.0	95.4 ± 0.9	nd	nd	6.84 ± 0.02	65.9 ± 1.2
6	0.004	20.0	83.9 ± 0.5	17.00 ± 0.47	4.40 ± 0.14	6.86 ± 0.02	31.2 ± 0.3
7	0.010	10.0	87.5 ± 0.5	7.96 ± 0.53	5.72 ± 0.14	6.89 ± 0.02	38.1 ± 1.0
8	0.025	5.8	94.3 ± 0.2	3.10 ± 0.32	7.23 ± 0.44	6.88 ± 0.03	46.7 ± 0.8
9	0.025	20.0	95.4 ± 0.6	0.01 ± 0.00	nd	6.87 ± 0.01	52.3 ± 2.1
10	0.025	20.0	95.3 ± 0.7	0.88 ± 0.06	nd	6.86 ± 0.03	46.7 ± 0.9
11	0.040	10.0	97.3 ± 0.4	nd	5.27 ± 0.06	6.89 ± 0.01	68.6 ± 1.2
Model			Quadratic	Quadratic	Quadratic	Linear	Linear
<i>p</i> value			0.0002*	0.0003*	0.0001*	0.0428**	<0.0001*
R ²			0.9846	0.9804	0.9854	0.5451	0.9526
Adj.-R ²			0.9692	0.9607	0.9708	0.4314	0.9408
Pred.-R ²			0.8949	0.8660	0.9162	0.0113	0.9156

Note: X₁: carrageenan concentration, X₂: pressure.

Abbreviations: nd, not detected; WHC, water-holding capacity.

*Significant.

**Not significant.

Regarding creaming, the prepared milk-based cocoa beverages were transferred into glass cylindrical tubes (length of 15 cm), sealed with plastic caps, and stored at 4 ± 1°C for 21 days to find the optimum point. During this time, the emulsion samples were monitored for phase separation into distinct layers. These layers were distinguished by visual appearance (Janahar et al., 2020). For the shelf-life studies, these values were determined monthly. The creaming index (CI) of emulsions is calculated by the following equation:

$$CI (\%) = (H_C / H_T) \times 100$$

where H_C is the height of the cream layer (top) and H_T is the total height of the milk-based cocoa beverages. The CI is quantified by dividing the height of the top phase by the total height of the sample, providing a measure of creaming instability.

2.5 | pH, viscosity, and color values

Prior to the analysis of the samples, the pH meter was calibrated using standard buffers of pH 4.0 and 7.0 at 25°C.

pH values of the different products were measured at 25°C 1 day after production.

Viscosity measurements were carried out with a viscometer (Lamy B-One Plus) at a controlled temperature of 25°C with a chosen speed of 100 rpm. Measurements were taken 2 min after the spindle (Spindle No. R-2) was immersed in each sample to allow thermal equilibrium in the samples. Each measurement was performed in triplicate.

Color was determined with a digital colorimeter (Konica Minolta, Chroma meter CR-410). L^* indicates the lightness, a^* and $-a^*$, redness and greenness, and b^* and $-b^*$, yellowness and blueness, respectively, according to the CIE L^*, a^*, b^* color space.

2.6 | ζ-Potential value and particle size determination

The milk-based cocoa sample was diluted 100 times with phosphate buffer (5 mM). ζ-Potential of the diluted sample was measured using Malvern Mastersizer Nano ZS90 (Malvern Instrument Co. Ltd) at 25°C, and measurements were performed in triplicate. Droplet size distribution was

measured by a Mastersizer 2000 (Malvern Instrument Co. Ltd). Regarding particle size measurement, 1 mL sample was diluted 1000 times with distilled water. The refractive indices were set at 1.33 for the continuous phase and 1.46 for the dispersed phase. The mean diameter value ($d_{4,3}$) was calculated by

$$d_{4,3} = n_i d_i^4 / n_i d_i^3$$

$$\text{Specific surface area} = \frac{6}{\rho \times d_{3,2}}$$

where n_i , $d_{3,2}$, and ρ are the number of droplets of diameter d_i , surface mean diameter, and density of sample, respectively. The measurements were performed after the milk-based cocoa beverages were prepared, and results were obtained from the average of three readings.

2.7 | FTIR analysis

FTIR spectra of optimum point milk-based cocoa beverages were recorded by a FTIR spectrophotometer (Thermo Nicolet, AVATAR-370 FTIR) at 4000–400 cm^{-1} wavenumber range (Rasool et al., 2019).

2.8 | Microstructural properties and image processing

The microstructure of milk-based cocoa beverage produced with carrageenan at optimum point and commercial products were examined using a microscope (Zeiss EVO[®] LS 10) magnified 10 and 40 times under the microscope.

The microstructures of the samples were also analyzed using SEM-EDS (JEOL JIB-4601 MultiBeam FIB-SEM system, Japonya). Samples were frozen at -20°C and freeze-dried for 48 h in a vacuum freeze dryer (Liyolife, FD5CT). Scanning electron microscopy (SEM) and energy-dispersive spectroscopy (EDS) analysis were carried out to reveal the microscopic features and chemical mapping.

To explore the properties of the images, each image was analyzed using a combination of gray level co-occurrence matrix (GLCM) and a local window analysis procedure, implemented in MATLAB software. Specifically, the analysis began with the extraction of non-overlapping $W \times W$ patches from the images of the samples, where W represents the size of the local window. Entropy, variance, and homogeneity derived using GLCM were used to assess microstructural properties of the samples. Entropy was computed as a measure of randomness or disorder within each patch, capturing the complexity of the microstruc-

ture. Variance was calculated to quantify the spread of pixel intensity values around their mean, reflecting the degree of contrast within the patches. Homogeneity, derived from GLCM, measured the uniformity of intensity distribution. The method systematically varied the window W to evaluate its impact on the calculated features, ensuring a robust comparison across different samples. By leveraging MATLAB's computational capabilities to consider a range of patch sizes and extract multiple features, the approach provided a comprehensive characterization of the samples' microstructural properties.

2.9 | Storage analysis

Samples produced under optimum conditions, as well as most known products (Product-1 and Product-2) obtained from a local market, were subjected to storage analysis over a period of 9 months. During the storage period, parameters such as WHC, sedimentation, creaming, pH, and viscosity were measured on a monthly basis. WHC, sedimentation, and creaming rates were monitored to evaluate long-term stability and the preservation of product homogeneity. Viscosity measurements were performed to observe potential changes in product consistency due to interactions between components or structural changes over time. pH values were measured to determine the maintenance of the product's acidic properties. All analyses were conducted at specified time points, and the results were statistically evaluated.

2.10 | Experimental design

The experimental design matrix and all data analyses were performed using Design-Expert[®] (version 7.0, Stat-Ease Inc.). Response surface methodology CCD with the rotatable option was used to examine the effects of carrageenan concentration (X_1) and pressure levels (X_2), two independent variables, on milk-based cocoa beverage properties. The levels of the created experimental design are shown in Table 1. This experimental design consists of 11 experimental steps, and the effects of independent variables on WHC, sedimentation, creaming, pH, and viscosity values were investigated. Production was done twice for the experimental design model. The experimental data were fitted to quadratic models to obtain the regression coefficients. All responses were evaluated for statistical significance of the factors by ANOVA (Tukey) test. For each response variable, a second-order polynomial model equation was defined. Finally, validation studies were carried out under the optimum conditions obtained, and L^* , a^* , b^* color values, ζ -potential value, particle size, FTIR, and



FIGURE 1 Images of milk-based cocoa beverages produced according to the experimental design created.

microscope images were measured in the samples under these conditions.

3 | RESULTS AND DISCUSSION

This study was carried out in three parts. In the first part, response surface CCD was established to determine the effect of carrageenan concentration and pressure values on milk-based cocoa beverage. The response values observed with the experimental design in Table 1 and the milk-based cocoa beverage images produced at these experimental points are shown in Figure 1. Model parameters, such as model type established and p and R^2 values are also presented in Table 1. In the second part, optimization and verification were each conducted based on the quality properties, and the optimum sample was compared with market samples. In the third part, the quality properties of the samples were observed during the storage period.

3.1 | Quality properties of the milk-based cocoa beverages

3.1.1 | Water-holding capacity (WHC)

The effects of different parameter combinations on WHC, sedimentation, creaming, pH, and viscosity values are shown in Figure 2. According to the ANOVA test, changes in carrageenan concentration and pressure affect the WHC at a statistically significant level ($p < 0.05$). The WHC at 11 test points in the experimental design ranged from 83.93% to 97.30%. It was observed that the WHC value increased

at the test points where the carrageenan concentration was high. This increase is statistically significant. The increase in pressure caused a decrease in WHC value, and this decrease is statistically significant. In studies on milk properties of κ -carrageenan, it has been stated that carrageenan reaches its WHC through the mechanism of strengthening the gel structure by reacting with milk proteins (Karadag & Aslan, 2021; Liu et al., 2023).

3.1.2 | Sedimentation and viscosity

Carrageenan concentration had a statistically significant effect on the sedimentation value ($p < 0.05$), whereas changes in pressure were statistically insignificant ($p > 0.05$). The sedimentation value was higher in samples with lower carrageenan concentrations, and pressure did not influence sedimentation values. In the experimental design, sedimentation values at 11 test points varied between 0% and 0.017%, with values reaching zero when carrageenan concentration exceeded 0.025%.

The hypothesis that higher pressures reduce cocoa particle size, leading to slower settling, has been explored to enhance stability in milk-based cocoa beverages. To test the accuracy of this hypothesis, a comparative study was conducted where cocoa was added to water instead of milk, and all the processes applied to milk were also applied to the aqueous cocoa solution. The results showed that cocoa powder in water tended to precipitate earlier than cocoa powder in milk. This indicates that stability in dairy products containing cocoa cannot be achieved by homogenization process alone, highlighting the importance of the milk matrix in stabilizing cocoa particles (Tran et al., 2021). This confirms the finding that the sedimentation

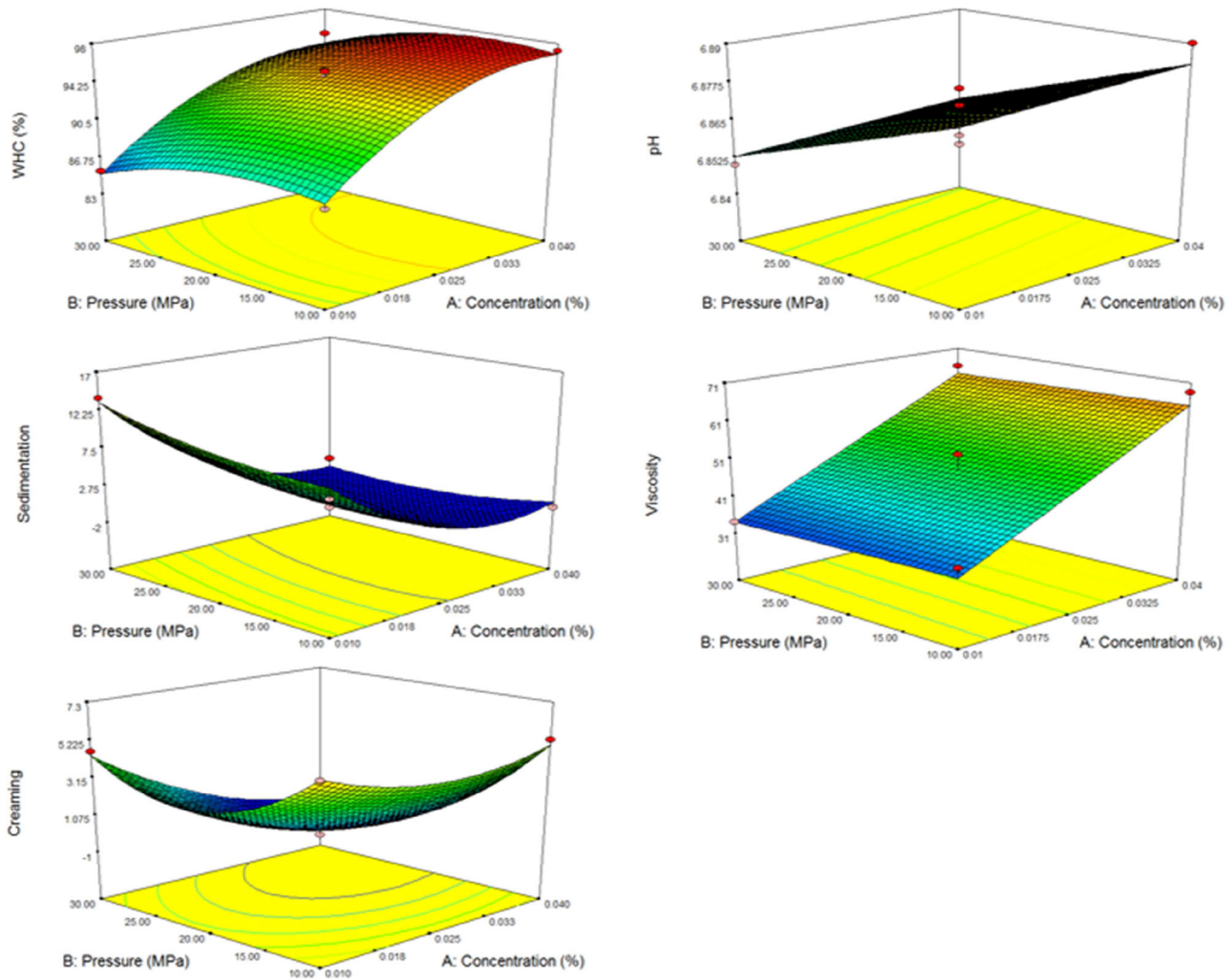


FIGURE 2 Visuals showing the interaction of parameters and responses according to the experimental design created.

parameter in the experimental design applied in this study was statistically significantly affected only by the carrageenan concentration.

The reason why the effect of the change in pressure on the sedimentation parameter was found to be statistically insignificant may be that low-level application of homogenization process conditions was sufficient. For instance, fat globules are typically homogenized at pressures around 15–20 MPa, whereas the structural modification of casein micelles generally requires pressures as high as ~35 MPa. Therefore, the pressures used in this study might not have been high enough to impact casein micelle interactions, which are essential in contributing to sedimentation stability. In addition, the composition of the milk and the amount of carrageenan used may have been sufficient to prevent sedimentation. Studies have reported that carrageenan is effective in preventing sedimentation problems (Holkar et al., 2019). Additionally, the fat-to-protein ratio, primarily influenced by casein, plays

a crucial role in sedimentation behavior in milk-based cocoa beverages. In whole milk systems, this ratio dictates the extent of milk protein adsorption onto fat globules, thereby affecting emulsion stability. To facilitate optimal protein adsorption onto fat and mitigate sediment formation during prolonged storage, the fat-to-protein ratio should be maintained below 4. Additionally, application of homogenization process ensures uniform dispersion of milk fat and proteins, effectively reducing sedimentation and enhancing the overall stability of the system (Kie, 2006).

The results also show that viscosity increases with higher carrageenan concentrations, whereas samples with lower carrageenan content exhibit lower viscosity. The viscosity of the samples ranged between 31.17 and 70.79 mPa s depending on carrageenan concentration. A study on the milk properties of κ -carrageenan confirmed that κ -carrageenan contributes to viscosity enhancement in milk samples (Liu et al., 2023; Seo & Yoo, 2021).

3.1.3 | Creaming

On the other hand, when the ANOVA test is examined, the changes in carrageenan concentration and pressure affect the creaming value statistically significantly, similar to the WHC response ($p < 0.05$). The creaming values of the samples ranged from 0% to 7.23%, depending on the carrageenan concentration. As the carrageenan concentration increased, the creaming value decreased, and this decrease was statistically significant. When the applied pressure increased, the creaming value decreased significantly. The creaming value was found to be zero if the carrageenan concentration was more than 22% and the pressure was more than 18.50 MPa. It has been reported that the addition of carrageenan in milk beverages reduces creaming, κ -carrageenan creates a stronger structure than ι -carrageenan, and creaming is observed less (Tijssen et al., 2007).

3.1.4 | pH

Carrageenan concentration and pressure did not have a statistically significant effect on pH value ($p > 0.05$). This situation shows that a reliable formulation is provided in terms of pH stability of the beverage and pH fluctuations can be kept at minimum levels throughout the shelf life of the product.

3.2 | Optimization and verification

For the optimization of carrageenan concentration and pressure values of milk-based cocoa beverages, the numerical point estimation method was applied using Design-Expert software, and it was selected based on maximizing WHC and minimizing the sedimentation and creaming values. pH values were not included in the optimization, and the viscosity value was chosen within the range of the values determined at the trial points. Looking at the results, optimum conditions were determined as 0.037% of carrageenan concentration and 19.38 MPa pressure value. According to these conditions, WHC value was found to be 97.26%, and no sedimentation and creaming were observed, and the pH and viscosity values were found to be 6.86 and 62.01 mPa s, respectively. The desirability value, which is the accuracy criterion in the optimization processes, was found to be 0.999. This value is very close to 1 and indicates a good desirability ratio.

In order to verify the theoretically determined optimum carrageenan concentration and the pressure value, milk-based cocoa drink was produced under these conditions. The theoretically obtained optimum conditions coincided with the validation data. The validation data were compared with the cocoa-containing dairy prod-

uct from the two most common brands on the market (Table 2). According to the results, the WHC value is not less than the products on the market. Sedimentation and creaming values are better than the products on the market. Based on the experimental design data, because carrageenan concentration and viscosity influence sedimentation, the improved sedimentation value can be attributed to the increased viscosity resulting from higher carrageenan concentrations. Accordingly, as both the carrageenan concentration and the pressure value affect the creaming value, these two factors may have provided superiority over the products in the market. It was found that the viscosity value was statistically significantly lower than the products on the market. Results for pH value were similar to those for viscosity. The high pH values of the products supplied from the market may be an indication of the high use of buffer salts as additives. Additives and ingredients used in products supplied from the market may cause similarities and differences for all these parameters.

When the color values are examined, it was determined that the milk-based cocoa beverages obtained from the market were statistically significantly lighter in color by looking at the L^* values. The samples on the market are generally known to slightly darken in color as chocolate powder is added. According to the L^* value given in Table 3, the color of the milk sample containing cocoa produced at the optimum point is lighter in color than the other products.

The viscosity was lower in the optimum sample compared to other products. The pH values of these two market products were also found to be considerably higher than the product produced at the optimum point, indicating that extra salt may have been added.

When particle sizes were examined, the confirmation sample had an acceptably low particle size compared to commercially available milk-based cocoa beverages (Product-1 and Product-2). Pressure, which had a statistically insignificant effect on the parameters according to the experimental design, may have been effective in the formation of low molecular sizes. The threshold value of 40 mV is often cited as a benchmark for colloidal stability (Hunter, 1981).

Negative surface charge not only ensures the homogeneous distribution of the particles but also shows that the particles have high stability in liquid solution and that the particles repel each other (Gün Gök et al., 2021). The milk-based cocoa beverage produced under optimal conditions had a ζ -potential of -37.80 mV, indicating that it is close to the ideal stable surface charge density (Table 3). When compared with other products, it was observed that the ζ -potential was statistically significantly different.

Regarding FTIR analysis, a broad band varying between 3100 and 3600 cm^{-1} , corresponding to the hydrophilic character of the seaweed, has emerged from the

TABLE 2 Optimum point obtained by central composite design (CCD), analysis results of verification sample, and two brands obtained from the market.

Products	WHC (%)	Sedimentation (%)	Creaming (%)	pH	Viscosity (mPa s)
Optimum product	97.3	nd	nd	6.86	62.01
Verification point	97.5 ± 0.3 ^b	nd	nd	6.88 ± 0.01 ^a	67.2 ± 0.4 ^a
Product-1	95.2 ± 0.6 ^b	1.05 ± 0.11 ^a	1.74 ± 0.18 ^a	7.73 ± 0.02 ^c	83.5 ± 0.7 ^c
Product-2	86.5 ± 0.8 ^a	3.72 ± 0.34 ^b	2.95 ± 0.16 ^b	7.37 ± 0.03 ^b	76.2 ± 0.5 ^b

Note: The standard deviation is represented by ± values ($n = 3$). Different letters in each column (according to the Tukey test) are statistically significantly different ($p < 0.05$).

Abbreviation: WHC, water-holding capacity.

TABLE 3 Analysis results of verification sample and two brands obtained from the market.

Products	L^*	a^*	b^*	Z-Ave (d nm)	ζ-Potential (mV)
Optimum product	52.0 ± 0.2 ^c	8.16 ± 0.04 ^a	8.80 ± 0.11 ^a	770.2 ± 29.7 ^a	-37.8 ± 1.4 ^c
Product-1	39.0 ± 0.3 ^a	9.62 ± 0.10 ^b	8.04 ± 0.25 ^a	1443.0 ± 54.0 ^b	-19.9 ± 0.7 ^a
Product-2	44.5 ± 0.2 ^b	10.11 ± 0.10 ^b	11.51 ± 0.17 ^b	707.0 ± 57.1 ^a	-27.0 ± 0.1 ^b

Note: The standard deviation is represented by ± values ($n = 3$). Different letters in each column (according to the Tukey test) are statistically significantly different ($p < 0.05$).

O—H stretching vibration from the hydroxyl group of the polysaccharides. Another peak observed at 1550–1650 cm^{-1} corresponds to the stretching of the carbonyl group. For all carrageenan structures, the 1010–1080 cm^{-1} region is attributed to the glycosidic linkage, associated with the C—O and C—C stretching vibrations of the polysaccharide molecules, which is the region indicating the presence of starch (Pourfarzad et al., 2015; Rasool et al., 2019). When the FTIR data were examined, the transmittance and wave number values of the verification sample and the cocoa-containing dairy products obtained from the market were found to be similar to each other (Figure 3).

3.3 | Microstructure and image processing

Microscopic images clearly showed that carrageenan inhibits macroscopic serum separation in milk protein/polysaccharide mixed solutions. Carrageenan forms a network by binding to milk proteins, preventing cocoa sedimentation and enhancing stability. From microscopic images, the analysis involved extracting non-overlapping $W \times W$ (dimensions of the region) patches from the images and calculating their variance and entropy. Across varying local window sizes W , verification point exhibited the lowest entropy and variance, indicating a more structured, consistent, and compact composition compared to the market samples (Figure 4). As entropy is a measure of disorder, this suggests that verification point maintains

a more ordered structure, whereas Product-2 consistently displayed the highest entropy, reflecting its irregular structure. Similarly, the variance, which measures the deviation from the mean, was highest for Product-2 and lowest for verification point, further indicating that the latter forms a tighter, more uniform network. Additionally, homogeneity structural features derived from GLCMs were evaluated for the related samples. Verification point achieved the highest values for homogeneity metric, indicating stronger relationships between pixel intensities and a more homogeneous structure, even across varying offsets. These results reinforce the conclusion that the optimum sample has a more stable and compact network with lower disorder and higher uniformity compared to commercial products (Figure 4). In addition, the interaction between casein micelles and κ -carrageenan appears to be sufficient to prevent macroscopic phase separation, which is consistent with the separation and creaming results in Table 3.

Morphological characteristics of the control sample (no hydrocolloid), optimum point cocoa milk, and products from local market were also analyzed by SEM-EDS (Figures 5 and 6). Although air gaps were observed in cocoa milk samples that contained no hydrocolloids, they were significantly reduced in the cocoa milk sample containing carrageenan at the optimum point, resulting in a more integrated structure. In products without hydrocolloids, air gaps (or voids) tend to be more prominent because there was less interaction with milk and less cohesion between cocoa or casein. These air gaps could remain trapped within the structure, contributing to a looser, less stable

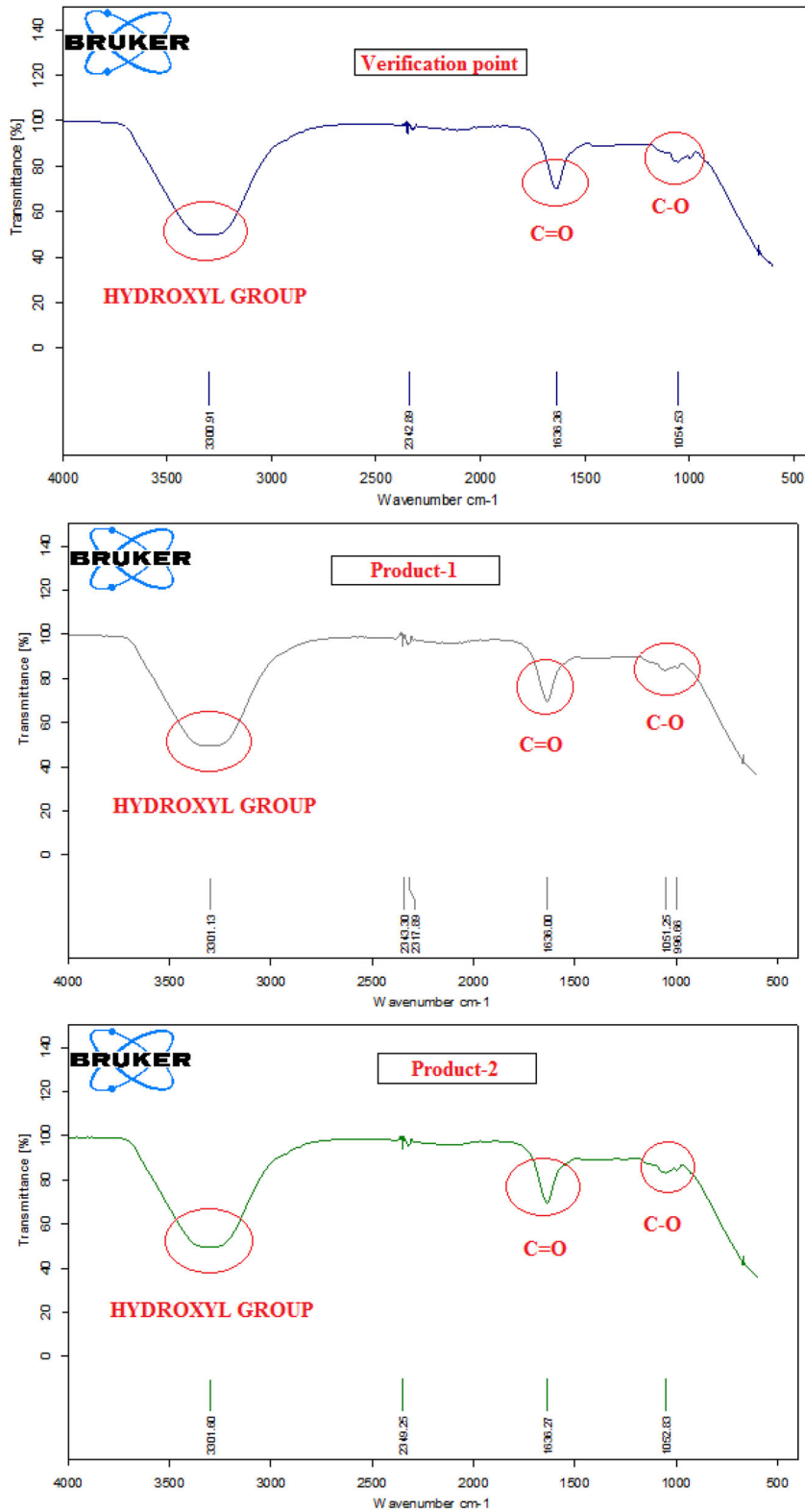


FIGURE 3 FTIR images of milk-based cocoa beverages produced at the optimum point and cocoa-containing milk procured from the market.

matrix. As a result, air gaps might persist within the matrix, leading to a more porous or loose structure that was clearly visible under SEM. When carrageenan was introduced, they absorbed water and swelled, facilitating the formation of a gel-like structure. The hydrocolloid molecules

created a network that helped close the air gaps by binding water and providing structural integrity. This results in a more uniform and compact matrix with fewer observable air gaps under SEM. The carrageenan formed a network that could effectively trap water and reduce the size of

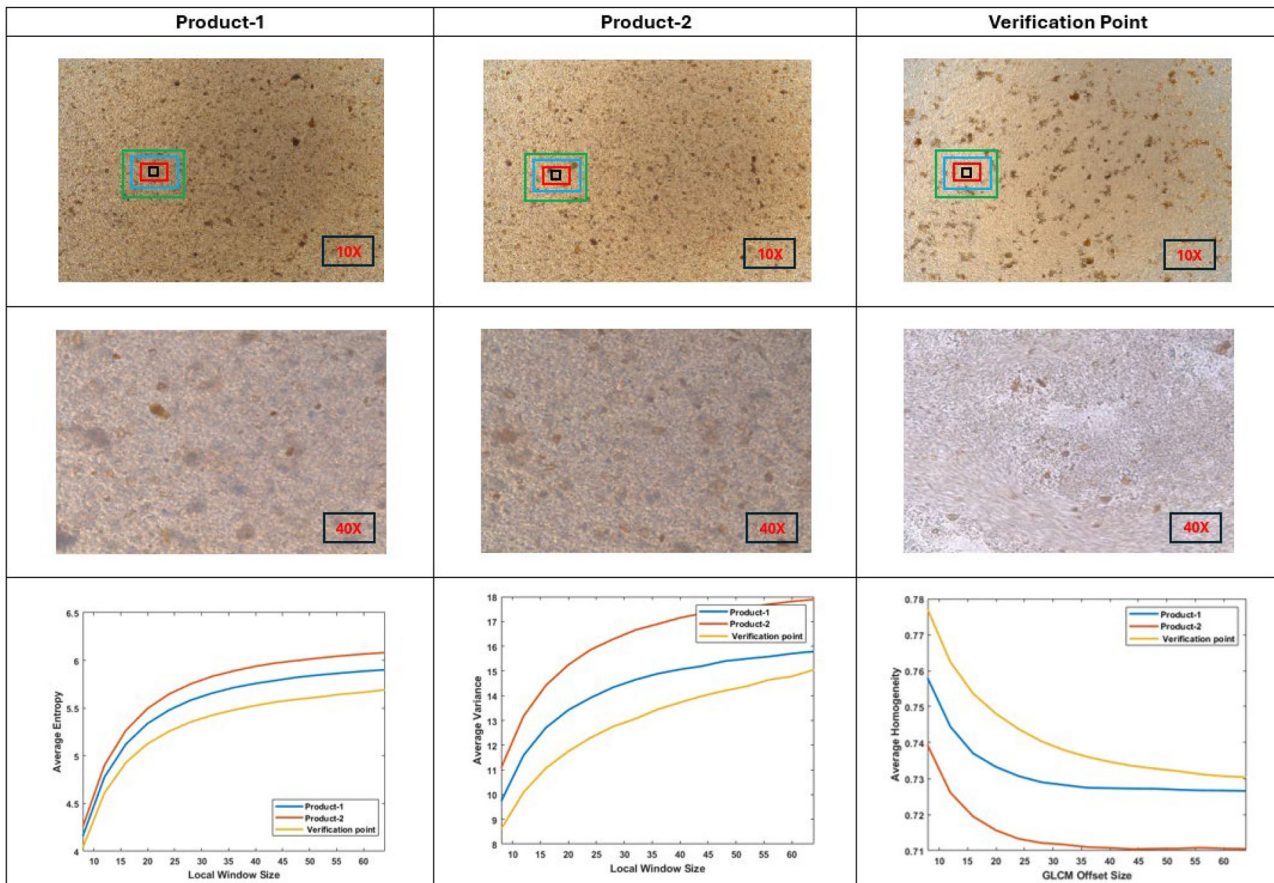


FIGURE 4 Commercially supplied Product-1, Product-2, and optimum (verification) point images of milk-based cocoa beverages under the microscope at 10- and 40-time magnifications with metrics extracted from non-overlapping patches.

voids or air gaps. This structure reduced the spaces among particles, resulting in a smoother, more compact surface when examined under SEM. Market products also had fewer air voids than cocoa milk samples without hydrocolloids and do not appear as smooth as the cocoa milk sample produced with carrageenan at the optimum point. Although the final products without hydrocolloids have a more sponge texture, the samples containing hydrocolloids are more cohesive to each other. Market product results are closer to each other. The chocolate milk sample produced at the optimum point had a smoother appearance (Wang et al., 2022).

These results consistently highlight the superior stability of optimum sample, with the lowest entropy and variance values among all samples, reflecting its highly ordered and compact network (Figure 5). In contrast, the sample without hydrocolloid and Product-1 samples showed the highest levels of irregularity, indicating a more disordered and less cohesive structure. Furthermore, the highest homogeneity was observed in the optimum sample, underscoring its tightly organized and consistent nature, likely due to the carrageenan–protein interaction that stabilizes the system and minimizes disruptions. According to the EDS results, the calcium and chloride

concentrations were found to be higher in the two most common brands (Product-1 and Product-2) compared to those without carrageenan and produced at the optimum point. Given the elevated calcium levels observed in the nutritional value table, it is hypothesized that calcium chloride may have been added (Table 4).

3.4 | Storage analysis

Table 5 presents the effects of samples produced under optimal conditions on various parameters over a 9-month storage period. The WHC exhibited a minimal decline of 3.80%, decreasing from 92.50% to 88.70% throughout the storage duration. This reduction did not reach statistical significance, suggesting that the WHC of the beverages remained substantially high even after long-term storage. Consequently, the overall stability of the formulation was preserved. Both sedimentation and creaming rates remained at zero throughout the entire storage period. This finding suggests that the particles did not separate or rise to the surface, preserving the homogeneity and stability of the product. The pH values showed a slight change from 6.86 to 6.79, which was also not statistically significant, revealing

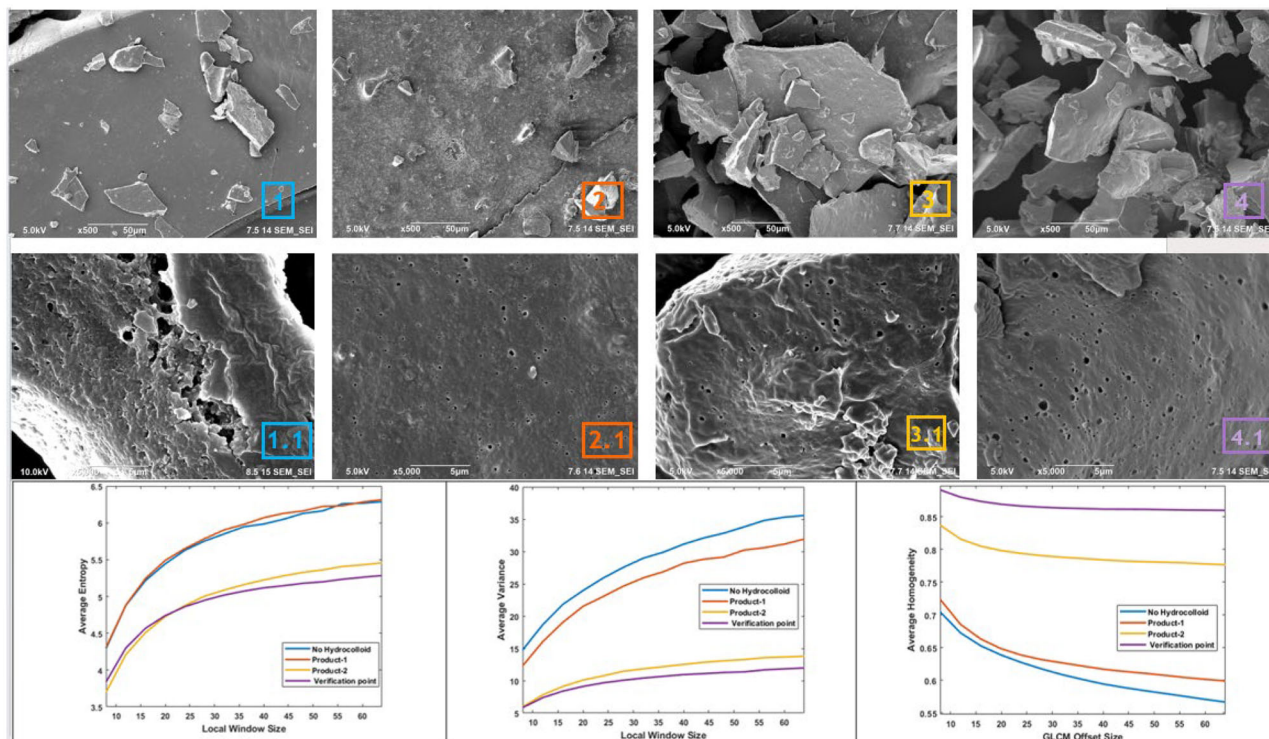


FIGURE 5 Scanning electron microscopy (SEM) images of no hydrocolloid (1), optimum point cocoa milk (2), Product-1 (3), and Product-2 (4) with 500 magnification and no hydrocolloid (1.1), optimum point cocoa milk (2.1), Product-1 (3.1) and Product-2 (4.1) with 5000 magnification with metrics extracted from non-overlapping patches.

TABLE 4 Energy-dispersive spectroscopy (EDS) results of no hydrocolloid, optimum sample, and two brands obtained from the market.

Products	C (%)	N (%)	O (%)	Na (%)	P (%)	Cl (%)	K (%)	Ca (%)
No hydrocolloid	69.19	7.25	16.50	0.60	0.00	1.30	3.72	1.45
Optimum product	68.11	5.86	17.36	0.59	0.35	1.61	4.87	1.25
Product-1	52.75	1.75	15.31	0.43	0.00	7.30	18.23	4.23
Product-2	66.08	5.55	17.59	0.67	0.00	2.26	4.76	3.08

TABLE 5 Nine-month storage results of optimum samples produced under optimum conditions.

Months	WHC (%)	Sedimentation (%)	Creaming (%)	pH	Viscosity (mPa s)	Torque (mN m)
1	92.5 ± 0.7	nd	0.012 ± 0.005	6.86 ± 0.02	59.5 ± 0.6 ^a	0.269 ± 0.007
2	92.4 ± 0.3	nd	0.012 ± 0.005	6.86 ± 0.01	61.7 ± 0.2 ^{ab}	0.275 ± 0.010
3	92.1 ± 0.1	nd	0.016 ± 0.001	6.85 ± 0.03	64.0 ± 0.2 ^{bc}	0.282 ± 0.005
4	91.5 ± 0.7	nd	0.018 ± 0.002	6.83 ± 0.02	66.6 ± 0.7 ^{cd}	0.294 ± 0.021
5	91.1 ± 0.6	nd	0.018 ± 0.002	6.83 ± 0.01	67.5 ± 0.7 ^d	0.303 ± 0.016
6	90.7 ± 0.7	nd	0.027 ± 0.005	6.80 ± 0.03	67.9 ± 0.1 ^d	0.305 ± 0.023
7	90.4 ± 0.2	nd	0.033 ± 0.008	6.80 ± 0.01	68.5 ± 0.3 ^{de}	0.308 ± 0.012
8	89.5 ± 1.1	nd	0.046 ± 0.010	6.79 ± 0.02	68.7 ± 0.5 ^{de}	0.309 ± 0.009
9	88.7 ± 1.0	nd	0.050 ± 0.010	6.79 ± 0.01	71.2 ± 0.7 ^e	0.320 ± 0.018

Note: The standard deviation is represented by ± values ($n = 3$). Different letters in each column (according to the Tukey test) are statistically significantly different ($p < 0.05$).

Abbreviation: WHC, water-holding capacity.

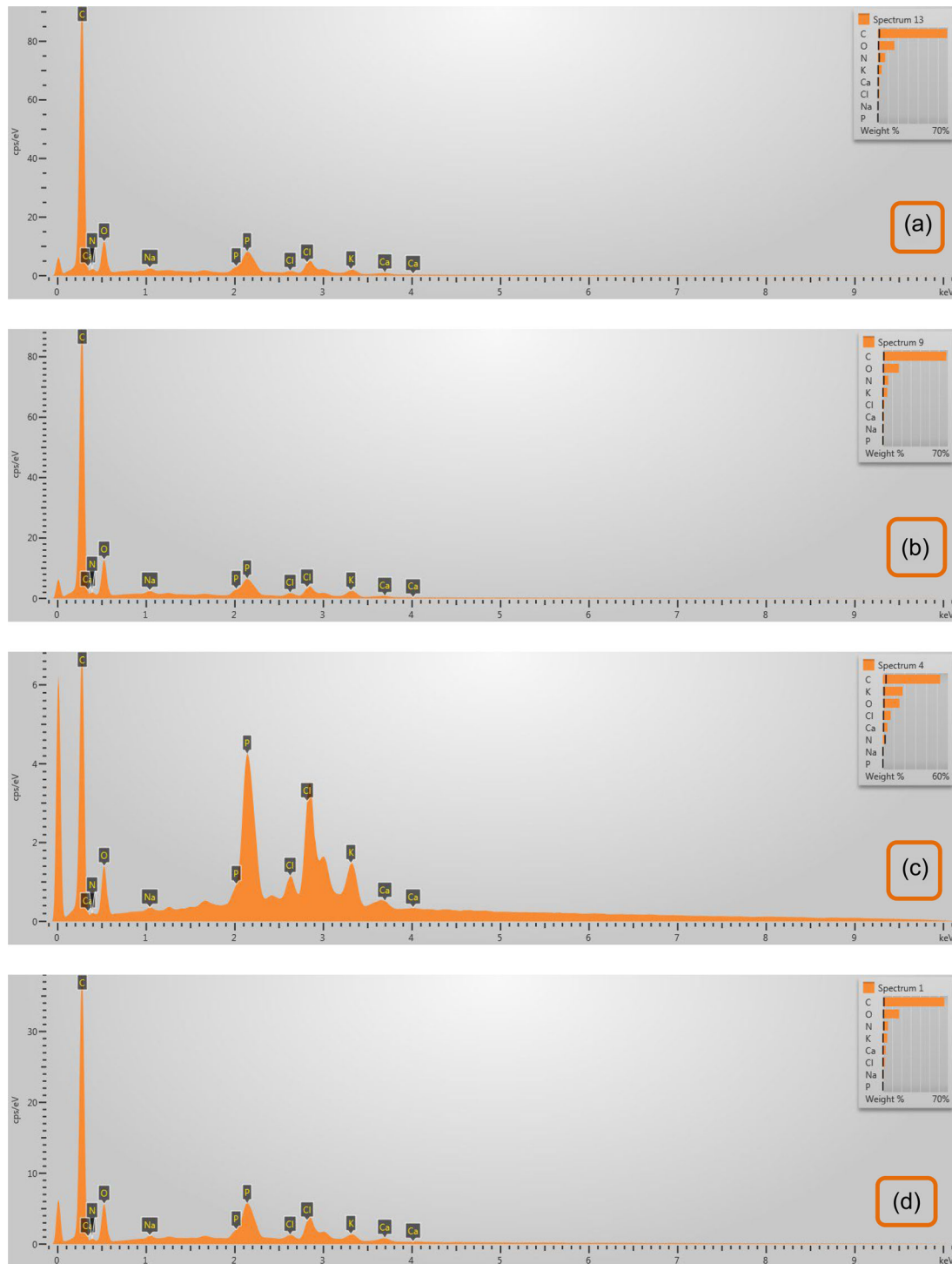


FIGURE 6 Energy-dispersive spectroscopy (EDS) images of cocoa milk samples, including no hydrocolloid (a), optimum point (b), Product-1 (c), and Product-2 (d), respectively. (Elements written on the peaks are C, Ca, N, O, Na, P, P, Cl, Cl, K, Ca, and Ca, respectively.)

that the acidic properties of the beverage were consistently maintained throughout the storage period. In contrast, viscosity values demonstrated a statistically significant increase, rising from 59.50 to 71.23 mPa s by the end of the 9 months. This increase is likely associated with interactions among the components in the formulation or structural

changes occurring over time, indicating a thickening of the product's consistency. It is important to consider that this increase in viscosity may influence consumer perception and impact the physical properties of the product.

Tables 6 and 7 present the 9-month storage results of market products (Product-1 and Product-2, respectively)

TABLE 6 Nine-month storage results of local market product (Product-1).

Months	WHC (%)	Sedimentation (%)	Creaming (%)	pH	Viscosity (mPa s)	Torque (mN m)
1	94.0 ± 1.3 ^a	1.70 ± 0.28 ^a	1.78 ± 0.04 ^a	7.72 ± 0.03 ^a	86.0 ± 1.4 ^a	0.386 ± 0.009 ^a
2	92.0 ± 1.4 ^a	2.10 ± 0.14 ^b	2.25 ± 0.35 ^b	7.71 ± 0.02 ^a	92.3 ± 3.2 ^{ab}	0.414 ± 0.015 ^a
3	90.6 ± 1.3 ^a	2.55 ± 0.21 ^{bc}	2.70 ± 0.28 ^{bc}	7.71 ± 0.04 ^a	100.0 ± 2.1 ^b	0.449 ± 0.007 ^a
4	87.1 ± 0.4 ^b	3.30 ± 0.42 ^c	3.28 ± 0.32 ^c	7.69 ± 0.02 ^a	107.4 ± 1.3 ^c	0.482 ± 0.011 ^b
5	85.0 ± 1.5 ^b	4.08 ± 0.25 ^d	3.80 ± 0.21 ^d	7.66 ± 0.01 ^a	112.0 ± 2.8 ^c	0.503 ± 0.013 ^b
6	82.2 ± 1.0 ^b	6.68 ± 0.39 ^e	4.55 ± 0.52 ^e	7.65 ± 0.03 ^a	128.5 ± 3.8 ^d	0.579 ± 0.017 ^c
7	78.8 ± 0.4 ^c	7.13 ± 0.25 ^f	4.98 ± 0.32 ^e	7.63 ± 0.02 ^a	135.1 ± 3.0 ^d	0.605 ± 0.015 ^c
8	77.7 ± 0.4 ^c	7.25 ± 0.35 ^f	5.40 ± 0.14 ^{ef}	7.61 ± 0.01 ^a	145.3 ± 1.1 ^e	0.652 ± 0.007 ^c
9	75.5 ± 0.7 ^c	7.30 ± 0.14 ^f	6.07 ± 0.40 ^f	7.60 ± 0.03 ^a	150.6 ± 1.7 ^e	0.679 ± 0.012 ^c

Note: The standard deviation is represented by \pm values ($n = 3$). Different letters in each column (according to the Tukey test) are statistically significantly different ($p < 0.05$).

Abbreviation: WHC, water-holding capacity.

TABLE 7 Nine-month storage results of local market product (Product-2).

Months	WHC (%)	Sedimentation (%)	Creaming (%)	pH	Viscosity (mPa s)	Torque (mN m)
1	85.0 ± 1.4 ^a	4.05 ± 0.14 ^a	3.20 ± 0.28 ^a	7.35 ± 0.01	79.8 ± 1.8 ^a	0.358 ± 0.008 ^a
2	83.1 ± 1.3 ^a	7.00 ± 0.28 ^b	4.80 ± 0.21 ^{ab}	7.34 ± 0.03	85.4 ± 2.6 ^{ab}	0.382 ± 0.015 ^{ab}
3	80.5 ± 0.7 ^{ab}	9.33 ± 0.11 ^c	6.00 ± 0.71 ^{bc}	7.31 ± 0.02	92.7 ± 2.3 ^{bc}	0.415 ± 0.010 ^{abc}
4	79.5 ± 0.9 ^{bc}	10.50 ± 0.42 ^{cd}	7.00 ± 0.42 ^{bcd}	7.30 ± 0.02	98.5 ± 1.6 ^{cd}	0.442 ± 0.009 ^{bcd}
5	77.6 ± 1.2 ^{bc}	11.45 ± 0.64 ^{cde}	7.65 ± 0.30 ^{cde}	7.28 ± 0.03	103.4 ± 2.8 ^{cd}	0.464 ± 0.011 ^{cde}
6	74.5 ± 0.6 ^{cd}	11.99 ± 0.09 ^{de}	8.20 ± 0.64 ^{cde}	7.27 ± 0.04	108.9 ± 1.3 ^{de}	0.489 ± 0.012 ^{de}
7	71.9 ± 0.8 ^{de}	12.38 ± 0.18 ^{de}	8.48 ± 0.25 ^{de}	7.24 ± 0.01	117.5 ± 2.3 ^{ef}	0.527 ± 0.007 ^{ef}
8	70.6 ± 1.1 ^{de}	12.74 ± 0.27 ^e	8.80 ± 0.16 ^{de}	7.23 ± 0.03	127.7 ± 1.8 ^{fg}	0.573 ± 0.005 ^{fg}
9	68.3 ± 1.2 ^e	13.00 ± 0.71 ^e	9.03 ± 0.32 ^e	7.21 ± 0.02	135.0 ± 2.8 ^g	0.606 ± 0.024 ^g

Note: The standard deviation is represented by \pm values ($n = 3$). Different letters in each column (according to Tukey test) are statistically significantly different ($p < 0.05$).

Abbreviation: WHC, water-holding capacity.

obtained from a local market. Although the WHC of Product-1 started at 93.95% and showed a consistent decline over the storage period, reaching 75.50% by Month 9 (Table 6), WHC of Product-2 started at 85.00% and reached 68.25% by Month 9 (Table 7). In both cases, the declines represent a significant decrease of about 19.70% for both products, indicating a reduced ability of local market products to retain moisture compared to the optimum samples, which maintained a high WHC. And the change in WHC value over 9 months is statistically significant.

In terms of sedimentation and creaming, both of local market products exhibited noticeable increases over time. Initially, sedimentation was observed at 1.70% for Product-1, increasing to 7.30% over time, representing a 4.3-fold increase. Similarly, sedimentation in Product-2 began at 4.05% and rose to 13.00%, indicating a 3.2-fold increase. Additionally, creaming values exhibited approximate 3.4-

and 2.8-fold increases for local market products Product-1 and Product-2, respectively. These changes suggest that, unlike the optimum samples where sedimentation and creaming remained at zero, the local market products showed instability, with the formation of layers indicative of separation and a loss of homogeneity. The pH of the local market products also showed slight variations, decreasing from 7.72 to 7.60 for Product-1 and 7.35 to 7.21 for Product-2. Although these changes were statistically insignificant, they indicate a gradual acidification of the product over time, which may affect its overall flavor profile and stability. Viscosity values for the local market products increased significantly from 86 to 150.60 mPa s for Product-1 and 79.75 to 135.00 mPa s for Product-2 during the storage period. This increase suggests that the product thickened considerably, potentially affecting the mouthfeel and consumer perception. The torque values also showed a corresponding rise, indicating greater

resistance to flow, which may influence how the product is utilized in culinary applications.

In summary, the storage results indicated that the local market products experienced a significant decline in WHC and increased sedimentation and creaming over 9 months. In contrast, the optimum samples maintained better stability and quality, highlighting the advantages of the formulation and processing conditions used in producing the latter.

4 | CONCLUSION

In milk-based cocoa drinks, stability issues such as cocoa particle precipitation, clumping, and color separation can arise due to the structural properties of cocoa. This study investigated the stabilizing effect of carrageenan, a key stabilizer enhanced by homogenization. An optimization process was conducted using a CCD with carrageenan concentrations (0.01%–0.04%) and pressures (5–34 MPa). The optimized sample was validated by comparing it with two popular and pioneering commercial products. The results indicated that increasing carrageenan concentration significantly improved WHC, sedimentation, creaming, and viscosity. The optimized sample, with 0.10% carrageenan produced at 20 MPa pressure, demonstrated a higher WHC (97.26%) and viscosity (62.01 mPa s) with lower sedimentation and creaming compared to market products. The microstructural properties and image processing supported the findings and indicated the interactions of carrageenan with milk proteins. These findings suggest that combining carrageenan and an optimum homogenization process can enhance the quality of milk-based cocoa beverages. The fact that the levels of independent variables selected in the study are suitable for the industry is very important in terms of the adaptability of the study results to the industry in the production of similar products with desired quality features. Despite the promising findings of this study, certain limitations should be acknowledged. The study primarily focused on the stabilizing effect of carrageenan in milk-based cocoa beverages, without evaluating potential alternative hydrocolloids or stabilizers that may exhibit similar or superior functionality. Future research should explore other hydrocolloids such as gellan gum, xanthan gum, pectin, or modified starches to determine their comparative efficiency in stabilizing cocoa dispersions. By addressing these limitations, future research can further enhance the formulation, processing, and stability of milk-based cocoa beverages, ultimately contributing to the development of superior-quality products for industrial applications.

AUTHOR CONTRIBUTIONS

Saniye Akyıl Öztürk: Conceptualization; investigation; writing—original draft; methodology; writing—review and editing; validation; formal analysis; data curation; supervision; resources; software; project administration; funding acquisition. **Necattin Cihat İcyer:** Conceptualization; investigation; writing—original draft; writing—review and editing; software. **Ali Can Karaca:** Methodology; validation; visualization; writing—review and editing; software. **Hatice Bekiroğlu:** Methodology; validation; formal analysis. **Osman Sagdic:** Methodology; validation; writing—review and editing. **Omer Said Toker:** Conceptualization; investigation; writing—original draft; methodology; validation; writing—review and editing; software; supervision; project administration.


ACKNOWLEDGMENTS

The authors express thanks to TUBITAK (The Scientific and Technological Research Council of Turkey) for providing open-access publication fee. SEM-EDS analysis was performed by the Sabanci University Nanotechnology Research and Application Center (SUNUM).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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How to cite this article: Öztürk, S. A., Icyer, N. C., Karaca, A. C., Bekiroglu, H., Sagdic, O., & Toker, O. S. (2025). Determination of stabilizer concentration and process effect on the stability of milk-based cocoa beverage. *Journal of Food Science*, 90, e70173. <https://doi.org/10.1111/1750-3841.70173>