

Original Article



Effects of Sugar Substitution with Stevia on Physicochemical and Sensory Properties of Chocolate Milk

Arezoo Asghari¹ , Afshin Javadi², Zeinab Nikniaz³, Fatollah Pourali⁴, Leila Nikniaz^{5*}

¹Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran

²Department of Food Hygiene, Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran

³Liver and Gastrointestinal Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

⁴Nutrition Improvement Group, Tabriz University of Medical Sciences, Tabriz, Iran

⁵Tabriz Health Services Management Research Center, Health Management and Safety Promotion Research Institute, Tabriz University of Medical Sciences, Tabriz, Iran

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*Corresponding Author:

Leila Nikniaz,

Email: nikniazleila@gmail.com

Abstract

Background: In this study, the effects of sugar substitution with stevia on physicochemical and sensory properties of chocolate milk were investigated.

Methods: Chocolate milk samples were formulated using sugar, stevia, and inulin at different concentrations. A total of 19 different samples were obtained, and their physicochemical and sensory properties were evaluated.

Results: The replacement of sugar with stevia led to a decrease in pH value and an increase in acidity level. The viscosity of samples containing 50% stevia was not significantly different from that of the control sample ($P < 0.05$). Formulations 2 [50% sucrose (3.5 g/100 g), 50% stevia (0.012g/100g), and inulin (0.03 g/100 g)] and formulation 12 [20.27% sucrose (1.4 g/100 g), 79.73% stevia (0.018 g/100 g), and inulin (0.01 g/100 g)] had the lowest energy level and highest acceptability. They were not significantly different from the control sample in terms of acidity and viscosity.

Conclusion: Stevia can partially be used as a substitute for sucrose without a significant effect on physicochemical characteristics and sensory properties of chocolate milk.

Keywords: Chocolate milk, Stevia, Sweetener, Inulin, Response surface methodology

Introduction

In recent decades, it has been widely accepted that food plays a key role in improving the physical condition and health of humans.¹ However, it has been demonstrated that the consumption of foods containing large amounts of sucrose is associated with adverse health effects such as hypertension, cardiovascular diseases, tooth decay, obesity, and diabetes.^{2,3} Therefore, concerns about the nutritional and caloric value of food are increasing in recent years and consumers are interested in using healthier and safer products.³

There is a growing interest in the use of low-calorie alternatives to sucrose in the food industry.⁴ For this purpose, sweeteners have been used as substitutes for sugar in different foods and beverages.^{2,3,5} Therefore, the consumption of foods containing non-nutritive sweeteners has increased among people of all ages in recent years.¹

Stevia, a natural sweetener, is a glycoside extracted from the leaves of *S. rebaudiana* (Bertoni). This plant is a shrub-like plant from the *Asteraceae* family, originating

from South America.^{1,6} Because of consumer demand for natural foods and beverages, especially low-calorie ones, stevia has become increasingly popular in recent years⁶ and the attention of many scholars has been attracted to extracts of *Stevia rebaudiana* for its sweetening qualities.⁴ Glycosides such as rebaudioside A and stevioside, which are mainly found in the leaves of stevia, are responsible for the sweet taste of stevia.^{4,6} Stevioside and rebaudioside are 200-300 times sweeter than sucrose.⁷

Unlike artificial sweeteners, no adverse effects have been reported for stevia so far and it has been approved by the Joint Food and Agriculture Organization/World Organization Expert Committee on Food Additives. Stevia has also been reported to have beneficial effects on human health.^{2,8,9} It has been shown that stevia exhibits a dose-dependent hypoglycemic activity and reduces insulin resistance in humans. Additionally, it prevents the formation of dental plaque with bactericidal activity. Furthermore, this zero-calorie sweetener is suitable for use in the dietary plan of patients with diabetes and obesity.^{1,6,7,10} It also has some functional properties such



as high stability in acidic and alkaline media, good solubility, and stability to heat (up to 200°C) and light.^{8,9} Stevia has recently been used as a no-calorie sweetener in baked goods, soft drinks, soy sauce, chewing gum, dairy products, and many other foods.^{1,8,9}

Recently, several studies have been carried out on the effect of the substitution of sugar with stevia on the properties of varied products such as low-fat and whole milk set yoghurt,⁴ sucrose-free chocolate,¹¹ fruit-based milk shake,¹² cola and lemon-lime beverages,⁶ strawberry-flavored yoghurt,² and carbonated drinks.¹³ Their results showed that stevia is a good alternative to sucrose in low-calorie products. However, there was little information about the use of stevia in chocolate milk.

The purposes of this research were (1) to optimize the levels of stevia to replace sucrose in producing reduced-calorie chocolate milk and (2) to compare the physicochemical characteristics and sensory properties of optimized formulation with the control sample.

Methods

Production of Chocolate Milk Samples

The UHT milk with 1.5% fat was purchased from Pegah Company (Tabriz, Iran). Stevia was purchased from Techfa Company (Tehran, Iran). Prior to the addition of stevia powder, it was diluted according to the manufacturer's instructions. Chocolate milk samples were formulated using sugar, stevia, and inulin at different concentrations listed in Table 1. In this study, 19 different samples were obtained. After the addition of chocolate (7%) to the

samples, they were homogenized by an agitator. Then, the samples were pasteurized at 78°C for 15 seconds and immediately cooled to <4°C.

Physicochemical Characteristics

For this purpose, the pH of samples was measured using a pH meter (Model 430, Corning, USA) which was calibrated with pH 7 buffer. Acidity was evaluated by titration method using 0.1 N NaOH and reported in Dornic degrees (°D). The dry matter content of the samples was quantified by a moisture analyzer (Sartorius MA35, Germany) (AOAC, 2005). Viscosity was measured using a rheometer after 24 hours of production (Anton Paar, Austria) by spindle of R3 type with 200 RPM for 30 seconds.

Density was evaluated by a thermo-lactodensimeter (Anton Paar, Graz, Austria) which was calibrated with water and dry air (Valdez et al, 2001). The Gerber method was used to measure the fat content by a special calibrated butyrometer while protein was measured using the Kjeldahl method.¹⁴ Total sugar of the samples was evaluated by Fehling's solution with methylene blue indicator.¹⁵ Energy was measured by the factors 4.0, 4.0, and 9.0 kcal/g for protein, carbohydrate, and fat, respectively. All tests were performed in triplicate for each treatment.

Evaluation of Sensory Properties

Eight trained panelists were selected for the evaluation of sensory properties. In order to assess the acceptance

Table 1. Coded and Actual Independent Variables (Sucrose, Stevia, and Inulin Concentrations) Used in the Response Surface Design

Formulation No.	Coded Independent Variables			Sucrose (%)	Actual Independent Variables			
	X ₁ *	X ₂ *	X ₃ *		Sucrose (g/100 g)	Stevia (%)	Stevia (g/100 g)	Inulin (g/100 g)
1	-1	-1	+1	20.27	1.4	20.27	0.005	0.05
2	0	0	0	50	3.5	50	0.012	0.03
3	-1	-1	-1	20.27	1.4	20.27	0.005	0.01
4	+1.68	0	0	100	7	50	0.012	0.03
5	-1.68	0	0	0	0	50	0.012	0.03
6	0	0	0	50	3.5	50	0.012	0.03
7	+1	+1	-1	79.73	5.6	79.73	0.018	0.01
8	0	0	0	50	3.5	50	0.012	0.03
9	+1	+1	+1	79.73	5.6	79.73	0.018	0.05
10	0	0	+1.68	50	3.5	50	0.012	0.06
11	+1	-1	+1	79.73	5.6	20.27	0.005	0.05
12	-1	+1	-1	20.27	1.4	79.73	0.018	0.01
13	0	0	-1.68	50	3.5	50	0.012	0
14	0	-1.68	0	50	3.5	0	0	0.03
15	0	+1.68	0	50	3.5	100	0.023	0.03
16	-1	+1	+1	20.27	1.4	79.73	0.018	0.05
17	0	0	0	50	3.5	50	0.012	0.03
18	+1	-1	-1	79.73	5.6	20.27	0.005	0.01
19	+1.68	-1.68	-1.68	100	7	0	0	0

*Sucrose (X₁) (0, 20.27, 50, 79.73, and 100%), stevia (X₂) (0, 20.27, 50, 79.73, and 100%), and inulin (X₃) (0, 0.01, 0.03, 0.05, and 0.06)

level of different formulations, a 5-point Likert scale (5=extremely like, 4=like, 3=neither like nor dislike, 2=dislike, and 1=extremely dislike) was used. Taste, texture, and overall acceptability were compared between the samples as well as between the optimum formulations and the control sample (formulation No. 19) using Design Expert software version 6.0.2.

Statistical Analysis

To optimize physicochemical and sensory properties of chocolate milk, response surface methodology (RSM) was used. In this study, central composite design (RSMCC0318) with 18 tests (including four tests at the center point) was used. Independent variables were designed at five levels (-1.682, -1, 0, +1, +1.682), which included sucrose (X1) (0, 20.27, 50, 79.73, and 100%), stevia (X2) (0, 20.27, 50, 79.73, and 100%), and inulin (X3) (0, 0.01, 0.03, 0.05, and 0.06) (Table 1).

The experimental data matched with second degree polynomial equation, and the correlation coefficient was calculated. The quadratic polynomial equation used in the analysis was as follows:

$$Y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^k \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=2}^k \beta_{ij} X_i X_j$$

In this equation, Y is the dependent variable or predicted response of the model. β_0 , β_i , β_{ii} , and β_{ij} are regression coefficients for constant (intercept), linear, second-order, and quadratic effects, respectively. X_i and

X_j are independent variables.

Dependent variables including pH, acidity, dry matter, viscosity, density, fat, protein, total sugar, and energy of samples were analyzed using one-way ANOVA and factorial analysis in a completely randomized design. Data were expressed as mean $\pm$ standard deviation. Data analysis was performed using SPSS version 16.0. *P* values lower than 0.05 were considered statistically significant. Duncan's multiple-range test was used to compare the groups.

Results

The experimental results about the effect of sucrose, stevia, and inulin combination on the physicochemical properties of chocolate milk are shown in Table 2. Generally, these properties were affected by the level of processing factors. Results showed that the pH of samples ranged between 6.58 and 6.70. The lowest pH (6.58) and the highest acidity (16.5°D) belonged to treatment 5 containing 0% sucrose, 50% stevia, and 0.03% inulin. This formulation also had the minimum dry matter content (11.63%) and density (1.050). The highest dry matter content (18.53%) and viscosity (24 cp) were observed in treatment 4 [100% sucrose (7 g/100 g), 50% stevia (0.012 g/100 g), and inulin (0.03 g/100 g)] (Table 2).

Only minor variations were observed in the fat (between 1.50% and 1.55%) and protein levels (between 3.05% and 3.10%) (Table 2). There were significant differences between the samples in terms of total sugar ($P < 0.05$). In addition, there were significant differences between the formulations in terms of the energy level ($P < 0.05$). The

Table 2. Effects of Different Formulations on Physicochemical Characteristics of Chocolate Milk

Treatment No.	Energy (kcal/g)	Total sugar (%)	Protein (%)	Fat (%)	Viscosity (cp)	Density (g/cm ³)	Dry matter (%)	Acidity (°D)	pH
1	68.94 $\pm$ 0.17	10.78 $\pm$ 0.01	3.08 $\pm$ 0.010	1.50 $\pm$ 0.001	20.9 $\pm$ 2.0	1.051 $\pm$ 0.001	13.01 $\pm$ 0.00	15 $\pm$ 0.020	6.61 $\pm$ 0.001
2	60.02 $\pm$ 0.18	8.52 $\pm$ 0.04	3.10 $\pm$ 0.008	1.50 $\pm$ 0.008	20.0 $\pm$ 1.1	1.055 $\pm$ 0.008	15.23 $\pm$ 0.11	15 $\pm$ 0.002	6.61 $\pm$ 0.003
3	69.83 $\pm$ 0.06	10.9 $\pm$ 0.02	3.07 $\pm$ 0.003	1.50 $\pm$ 0.002	20.0 $\pm$ 2.7	1.052 $\pm$ 0.002	13.03 $\pm$ 0.31	15.5 $\pm$ 0.001	6.62 $\pm$ 0.001
4	76.50 $\pm$ 0.09	12.7 $\pm$ 0.01	3.05 $\pm$ 0.004	1.50 $\pm$ 0.004	24.0 $\pm$ 0.3	1.058 $\pm$ 0.004	18.53 $\pm$ 0.03	15 $\pm$ 0.008	6.65 $\pm$ 0.002
5	53.98 $\pm$ 0.00	7.02 $\pm$ 0.03	3.10 $\pm$ 0.001	1.50 $\pm$ 0.001	21.0 $\pm$ 0.2	1.050 $\pm$ 0.001	11.63 $\pm$ 0.00	16.5 $\pm$ 0.003	6.58 $\pm$ 0.003
6	6138 $\pm$ 0.33	8.89 $\pm$ 0.05	3.08 $\pm$ 0.003	1.50 $\pm$ 0.003	22.2 $\pm$ 0.3	1.056 $\pm$ 0.003	15.30 $\pm$ 0.00	15 $\pm$ 0.005	6.65 $\pm$ 0.002
7	76.63 $\pm$ 0.09	12.6 $\pm$ 0.01	3.07 $\pm$ 0.004	1.55 $\pm$ 0.001	21.7 $\pm$ 0.8	1.053 $\pm$ 0.001	17.03 $\pm$ 0.01	15 $\pm$ 0.002	6.70 $\pm$ 0.003
8	61.90 $\pm$ 0.006	9.00 $\pm$ 0.04	3.10 $\pm$ 0.030	1.50 $\pm$ 0.004	22.0 $\pm$ 0.1	1.059 $\pm$ 0.004	15.39 $\pm$ 0.33	15.5 $\pm$ 0.002	6.65 $\pm$ 0.005
9	76.67 $\pm$ 0.17	12.6 $\pm$ 0.02	3.08 $\pm$ 0.040	1.55 $\pm$ 0.005	21.2 $\pm$ 0.2	1.052 $\pm$ 0.005	17.17 $\pm$ 0.01	15 $\pm$ 0.004	6.68 $\pm$ 0.004
10	73.46 $\pm$ 0.008	11.90 $\pm$ 0.01	3.09 $\pm$ 0.003	1.50 $\pm$ 0.003	20.3 $\pm$ 0.0	1.053 $\pm$ 0.003	15.39 $\pm$ 0.01	15 $\pm$ 0.001	6.69 $\pm$ 0.004
11	73.50 $\pm$ 0.003	11.90 $\pm$ 0.03	3.10 $\pm$ 0.001	1.50 $\pm$ 0.001	20.9 $\pm$ 0.0	1.060 $\pm$ 0.001	16.98 $\pm$ 0.23	15.5 $\pm$ 0.003	6.63 $\pm$ 0.007
12	66.22 $\pm$ 0.007	10.08 $\pm$ 0.04	3.10 $\pm$ 0.005	1.50 $\pm$ 0.002	22.1 $\pm$ 0.8	1.058 $\pm$ 0.002	13.12 $\pm$ 0.01	15 $\pm$ 0.008	6.67 $\pm$ 0.001
13	70.63 $\pm$ 0.033	11.08 $\pm$ 0.01	3.09 $\pm$ 0.007	1.55 $\pm$ 0.001	21.0 $\pm$ 0.1	1.055 $\pm$ 0.001	15.19 $\pm$ 0.00	15 $\pm$ 0.011	6.68 $\pm$ 0.003
14	70.02 $\pm$ 0.004	11.05 $\pm$ 0.02	3.08 $\pm$ 0.003	1.50 $\pm$ 0.003	22.8 $\pm$ 0.5	1.050 $\pm$ 0.003	15.39 $\pm$ 0.01	15 $\pm$ 0.004	6.63 $\pm$ 0.002
15	73.46 $\pm$ 0.001	11.90 $\pm$ 0.01	3.09 $\pm$ 0.002	1.50 $\pm$ 0.005	21.0 $\pm$ 0.0	1.052 $\pm$ 0.001	15.29 $\pm$ 0.00	15.5 $\pm$ 0.010	6.63 $\pm$ 0.003
16	70.18 $\pm$ 0.003	11.07 $\pm$ 0.04	3.10 $\pm$ 0.008	1.50 $\pm$ 0.002	18.7 $\pm$ 0.9	1.055 $\pm$ 0.002	13.2 $\pm$ 0.25	15.5 $\pm$ 0.002	6.64 $\pm$ 0.001
17	61.74 $\pm$ 0.004	8.96 $\pm$ 0.03	3.10 $\pm$ 0.001	1.50 $\pm$ 0.001	20.9 $\pm$ 0.0	1.056 $\pm$ 0.001	15.35 $\pm$ 0.00	15.5 $\pm$ 0.330	6.65 $\pm$ 0.002
18	73.58 $\pm$ 0.003	11.92 $\pm$ 0.01	3.10 $\pm$ 0.004	1.50 $\pm$ 0.004	21.3 $\pm$ 0.0	1.052 $\pm$ 0.004	17.11 $\pm$ 0.00	15.5 $\pm$ 0.033	6.67 $\pm$ 0.003
19	74.30 $\pm$ 0.002	12.10 $\pm$ 0.02	3.10 $\pm$ 0.002	1.50 $\pm$ 0.002	22.8 $\pm$ 0.0	1.052 $\pm$ 0.002	18.52 $\pm$ 0.14	15.5 $\pm$ 0.010	6.60 $\pm$ 0.003

Data are presented as mean $\pm$ SD.

results showed that treatment 5 had the lowest total sugar (7.02%) and energy level (53.98%).

The sensory scores for taste, texture, and overall acceptability of the samples are presented in Table 3. Formulation 2 [50% sucrose (3.5 g/100 g), 50% stevia (0.012 g/100 g), and inulin (0.03 g/100 g)] and formulation 13 [50% sucrose (3.5 g/100 g), 50% stevia (0.012 g/100 g), and inulin (0 g/100 g)] had the highest scores for taste and overall acceptability, while the lowest sensory scores belonged to the formulation containing no sucrose (treatment 5). The results obtained from ANOVA are presented in Table 4.

The effect of sucrose (X1) and stevia (X2) on the sensory quality of chocolate milk is represented in Figures 1 and 2 using response surface plots. Figures clearly show that increasing the concentration of sucrose and stevia affected the quality of the chocolate milk, and the highest scores were obtained for taste (Figure 1) and texture (Figure 2).

To determine the optimum formulation, sensory scores were analyzed using Design Expert software version 6.0.2. Results showed that formulation 12 [20.27% sucrose (1.4 g/100 g), 79.73% stevia (0.018 g/100 g), and inulin (0.01 g/100 g)] and formulation 16 [20.27% sucrose (1.4 g/100 g), 79.73% stevia (0.018 g/100 g), and inulin (0.02 g/100 g)] had the highest acceptability among the examined formulations. The most optimized formulation was obtained using 20.27% sucrose (1.4 g/100 g), 79.73% stevia (0.018 g/100 g), and inulin (0.02 g/100 g).

Discussion

Based on the results of this study, the most optimized formulation was obtained using 20.27% sucrose (1.4

g/100 g), 79.73% (0.018 g/100 g) stevia, and inulin (0.02 g/100 g). The results of this study showed a significant difference in the mean pH of different treatments ($P < 0.05$). It was found that the pH of samples decreased with the reduction of sucrose content. Moreover, by the complete removal of sucrose from the formulation, the acidity level of samples was increased. Hence, treatment 5 [50% stevia (0.012 g/100 g), 0% sucrose, and inulin (0.03 g/100 g)] had the highest titratable acidity (16.5 °D). In a study performed by Alizadeh et al¹² on the substitution of sucrose with stevia, no remarkable effect on the pH value and acidity of fruit-based milkshake was found. However, in accordance with the result of the present study, it has been reported that the pH of saffron syrup decreased with the increase of the concentration of stevia.¹⁶

Saniah and Samsiah¹³ reported that sucrose is the major contributor to total soluble solid content of carbonated drinks, whereas stevia has a negative effect on this parameter. Furthermore, the increased concentration of sucrose led to an increase in the viscosity of the syrup as well as the carbonated drink. It was also demonstrated that the total solid content decreased in proportion to the reduction in the sucrose content of the milk shake.¹² Similar findings were obtained in the present study. Treatment 4 [100% sucrose (7 g/100 g), 50% Stevia (0.012 g/100 g), and inulin (0.03 g/100 g)] and control sample [100% sucrose (7 g/100g), 0% stevia, and inulin (0 g/100 g)] had the highest dry matter content (18.53% and 18.52%, respectively), while treatment 5 [0% sucrose, 50% stevia (0.012 g/100 g), and inulin (0.03 g/100 g)] had the lowest dry matter content (11.63%). Besides, the viscosity increased in samples with high sucrose content. The amount of sucrose added may affect the acceptability of the product, particularly for viscosity.¹³ It has been shown that sugar in beverages not only contributes to the sweetness but also adds body and mouthfeel.¹³

The combination of sucrose and stevia in the formulation of chocolate milk did not have a considerable effect on the fat and protein levels, while total sugar content reduced from 12.7% in the control sample to 7% (treatment 5). Stevia and inulin concentrations did not have a significant effect on total sugar content. Additionally, the substitution of sucrose with stevia decreased the energy level from 73.4 Kcal/g (control) to 53 Kcal/g (treatment 5), and the energy of samples was reduced by decreasing the concentration of sucrose. In a similar study, Saniah and Samsiah found that decreasing the sucrose concentration and increasing the stevia concentration decreased the total sugar content of the carbonated drinks.¹³ Furthermore, sucrose-stevia based samples provided significantly lower calorie (32 kcal/100 mL) than the control sample (56 kcal/100 mL).

The evaluation of sensory properties of samples by panelists indicated that formulation 2 [50% sucrose (3.5 g/100 g), 50% stevia (0.012 g/100 g), and inulin (0.03 g/100 g)], formulation 9 [79.73% sucrose (5.6 g/100 g), 79.73% stevia (0.018 g/100 g), and inulin (0.05 g/100 g)] and formulation 13 [50% sucrose (3.5 g/100 g), 50% stevia

Table 3. Effect of Various Concentrations of Sucrose, Stevia, and Inulin on the Score of Taste, Texture and Overall Acceptability

Treatment No.	Overall Acceptability	Texture	Taste
1	3.25	4.125	3.5
2	4.375	4.875	4.625
3	2.875	4	2.75
4	4.125	4.375	4.125
5	2.25	4	2
6	4.5	4.75	4.75
7	4.125	4.5	4
8	4.25	4.625	4.25
9	4.375	4.75	4.25
10	3.875	4.375	3.375
11	4	4.5	4.125
12	4.5	4.625	4.375
13	4.375	4.5	4.625
14	4.25	4.875	4.25
15	3.875	4.5	3.75
16	4.5	4.5	4.375
17	3.625	4.375	3.375
18	2.75	4.125	2.75

Table 4. Analysis of Variance for the Response Surface Models in Terms of Coded Units

Response	Source	DF	Sum of square	Mean Square	F value	P value	R ² _{adj}
pH	X ₁ [*]	1	4.49	4.49	5.91	0.029	
	X ₂ [*]	1	3.45	3.45	4.54	0.051	
	X ₃ [*]	1	4.50	4.50	0.59	0.450	
	Model	3	8.40	2.80	3.68	0.038	0.321
	Lack of fit	14	0.01	7.60	-	-	
	Pure error	3	1.20	4.00	-	-	
	Total	17	0.01	-	-	-	
Acidity	X ₁	1	0.91	0.91	7.64	0.015	
	X ₂	1	0.25	0.25	2.09	0.170	
	X ₃	1	0.08	0.08	0.67	0.426	
	Model	3	1.24	0.41	3.46	0.045	0.303
	Lack of fit	14	1.67	0.12	-	-	
	Pure error	3	0.19	0.06	-	-	
	Total	17	2.90	-	-	-	
Dry matter	X ₁	1	55.55	55.55	2371.39	0.000	
	X ₂	1	3.93	3.93	0.17	0.688	
	X ₃	1	0.01	0.01	0.49	0.494	
	Model	3	55.57	18.52	790.68	0.000	0.992
	Lack of fit	14	0.33	0.023	-	-	
	Pure error	3	0.01	4.75	-	-	
	Total	17	55.90	-	-	-	
Density	X ₁	1	1.32	1.32	1.28	0.276	
	X ₂	1	3.97	3.97	0.38	0.545	
	X ₃	1	8.00	8.00	7.752	0.931	
	Model	3	1.73	5.76	0.56	0.65	0.084
	Lack of fit	14	1.445	1.032	-	-	
	Pure error	3	9.00	3.00	-	-	
	Total	17	1.61	-	-	-	
Viscosity	X ₁	1	2.60	2.60	3.28	0.145	
	X ₂	1	7.62	6.62	0.17	0.688	
	X ₃	1	4.03	4.03	3.68	0.075	
	Model	3	7.40	2.46	2.25	0.127	0.180
	Lack of fit	14	1.53	0.096	-	-	
	Pure error	3	2.75	9.16	-	-	
	Total	17	2.27	-	-	-	
Fat	X ₁	1	8.51	8.51	0.22	0.649	
	X ₂	1	1.39	1.39	0.35	0.561	
	X ₃	1	9.80	9.80	0.25	0.625	
	Model	3	3.22	1.07	0.27	0.844	0.147
	Lack of fit	14	5.52	3.94	-	-	
	Pure error	3	1.67	5.58	-	-	
	Total	17	5.84	-	-	-	

Table 4. Continued

Response	Source	DF	Sum of square	Mean Square	F value	P value	R ² _{adj}
Protein	X ₁	1	5.17	5.17	2.47	0.138	
	X ₂	1	2.07	2.07	0.09	0.758	
	X ₃	1	3.20	3.20	0.15	0.702	
	Model	3	5.70	1.90	0.91	0.463	0.017
	Lack of fit	14	2.94	2.10	-	-	
	Pure error	3	3.00	1.00	-	-	
	Total	17	3.51	-	-	-	
Total sugar	X ₁	1	18.54	18.54	9.75	0.007	
	X ₂	1	0.32	0.32	0.17	0.688	
	X ₃	1	0.29	0.29	0.15	0.701	
	Model	3	19.15	6.38	3.36	0.049	0.293
	Lack of fit	14	26.62	1.90	-	-	
	Pure error	3	0.14	0.04	-	-	
	Total	17	45.77	-	-	-	
Energy	X ₁	1	290.69	290.69	9.81	0.007	
	X ₂	1	5.69	5.69	0.19	0.667	
	X ₃	1	4.39	4.39	0.15	0.706	
	Model	3	300.77	100.26	3.38	0.048	0.269
	Lack of fit	14	414.90	29.64	-	-	
	Pure error	3	2.31	0.77	-	-	
	Total	17	715.67	-	-	-	
Taste	X ₁	1	3.61	3.61	12.26	0.003	
	X ₂	1	1.88	1.88	6.37	0.024	
	X ₃	1	0.03	0.03	0.11	0.700	
	Model	3	5.52	1.84	6.25	0.006	0.480
	Lack of fit	14	4.12	0.29	-	-	
	Pure error	3	0.70	0.23	-	-	
	Total	17	9.64	-	-	-	
Texture	X ₁	1	0.45	0.45	10.75	0.005	
	X ₂	1	0.22	0.22	5.21	0.038	
	X ₃	1	3.41	3.41	0.08	0.778	
	Model	3	0.66	0.22	5.35	0.011	0.434
	Lack of fit	14	0.58	0.04	-	-	
	Pure error	3	0.07	0.02	-	-	
	Total	17	1.24	-	-	-	
Overall acceptability	X ₁	1	2.66	2.66	9.71	0.007	
	X ₂	1	0.97	0.97	3.55	0.080	
	X ₃	1	0.25	0.25	0.90	0.359	
	Model	3	3.87	1.29	4.72	0.017	0.396
	Lack of fit	14	3.83	0.27	-	-	
	Pure error	3	0.53	0.18	-	-	
	Total	17	7.70	-	-	-	

*Sucrose (X1) (0, 20.27, 50, 79.73, and 100%), stevia (X2) (0, 20.27, 50, 79.73, and 100%), and inulin (X3) (0, 0.01, 0.03, 0.05, and 0.06)

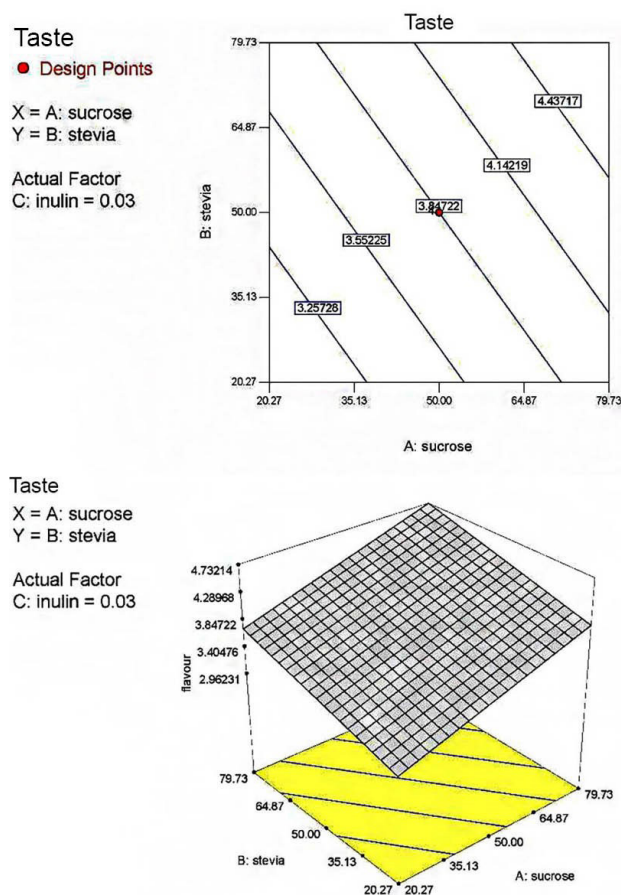


Figure 1. Response Surface Plot of the Effects of Stevia and Sucrose on the Taste of Chocolate Milk

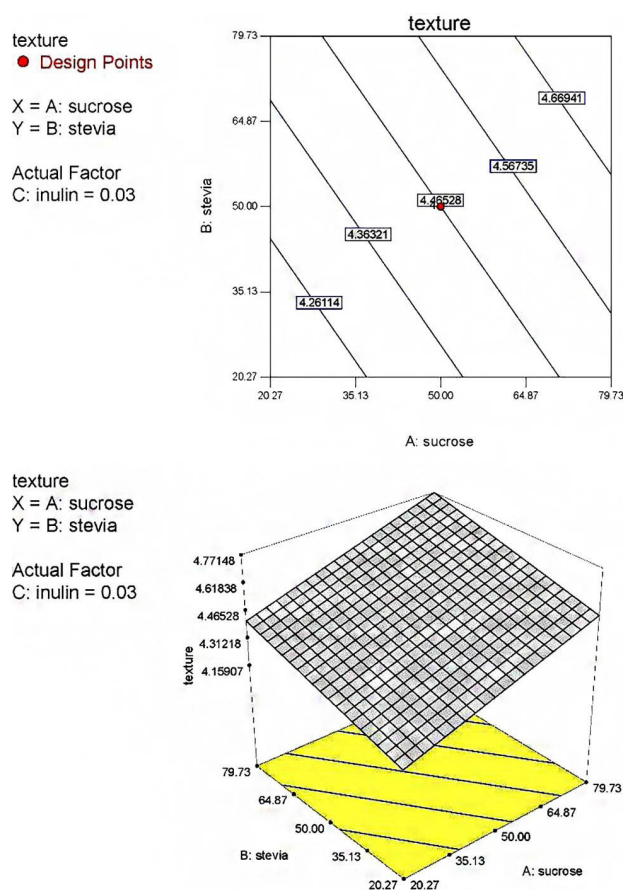


Figure 2. Response Surface Plot of the Effects of Stevia and Sucrose on the Texture of Chocolate Milk

(0.012 g/100 g), and inulin (0 g/100 g)] had the highest score for overall acceptability. These results are in line with another study which reported that the low-calorie jam can be produced using stevioside by reducing the sugar content to 50% without any changes in its sensory quality.¹⁷

In the present study, the formulation with 0% of sucrose (treatment 5) had the lowest score for taste, texture, and overall acceptability. Although stevia can be completely used for sweetness, its bitter taste should be masked in the product.¹⁸ Rebaudioside A, a component of stevia, is known to have a bitter taste when used at higher levels and it can reduce the acceptability of the product. In addition, it has been reported that increasing the concentration of rebaudioside A may lead to a decrease in the hardness and consistency of yogurt.^{4,19} Saniah and Samsiah¹³ suggested that different concentrations of sucrose can significantly affect the bitter taste of stevia and the addition of sucrose can improve the sensory quality of the product in terms of taste and texture.

In order to visualize the relationship between variables and responses, a three-dimensional response surface plot was used in this study. Using this method in the optimization process, treatment 12 [20.27% sucrose (1.4 g/100 g), 79.73% stevia (0.018 g/100g), and inulin (0.01 g/100 g)] and treatment 16 [20.27% sucrose (1.4 g/100 g), 79.73% stevia (0.018 g/100 g), and inulin (0.05 g/100 g)]

were the best formulations in terms of taste, texture, and overall acceptability. The most optimized formulation was also obtained when 20.27% sucrose (1.4 g/100 g) was used in combination with 79.73% stevia (0.018 g/100 g) and inulin (0.02 g/100 g). Alizadeh et al reported that the combination of sucrose and stevia used at the ratio of 25:75 in fruit-based milk shake had the most acceptable quality according to the panelists.¹² However, 0.25% stevia in fermented dairy products has been suggested as the most acceptable concentration.²⁰ Lisak et al² also reported that strawberry-flavored fresh yogurt sweetened with a combination of sucrose and stevia at 4.5% concentration was the most preferred formulation. It has been noted that the acceptability level of stevia depends on food products. Because overall sensory properties of beverages may be affected by some parameters such as possible interactions of added sweeteners with the sweetening components of food¹², synergistic or inhibitory effects of various sweeteners, temperature, and viscosity of food as well as the properties of dispersion media.²¹

Conclusion

Results showed that stevia can partially be used as a substitute for sucrose without a significant effect on physicochemical characteristics and sensory properties of chocolate milk. In the present study, treatment 12 [20.27 sucrose (1.4 g/100 g), 79.73% stevia (0.018 g/100 g), and

inulin (0.01 g/100 g)] and treatment 16 [20.27 sucrose (1.4 g/100 g), 79.73% stevia (0.4 g/100 g), and inulin (0.05 g/100 g)] were the optimum formulations.

However, further research in this field is needed to study the use of other concentrations of stevia in chocolate milk as well as the replacement of artificial sweeteners and sugar with stevia in other high-calorie beverages and foods.

Ethics statement

Not applicable.

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Conflict of interests declaration

Authors declare no competing interests.

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Author contributions

Conceptualization: Afshin Javadi, Leila Nikniaz.

Data curation: Arezoo Asghari.

Formal analysis: Arezoo Asghari, Fatollah Pourali.

Methodology: Afshin Javadi.

Project administration: Afshin Javadi.

Consent for publication

Not applicable.

References

- Shankar P, Ahuja S, Sriram K. Non-nutritive sweeteners: review and update. *Nutrition*. 2013;29(11-12):1293-9. doi: [10.1016/j.nut.2013.03.024](https://doi.org/10.1016/j.nut.2013.03.024).
- Lisak K, Lenc M, Jeličić I, Božanić R. Sensory evaluation of the strawberry flavored yoghurt with stevia and sucrose addition. *Croatian Journal of Food Technology, Biotechnology and Nutrition*. 2012;7:39-43.
- Vilela A, Matos S, Abraão AS, Lemos AM, Nunes FM. Sucrose replacement by sweeteners in strawberry, raspberry, and cherry Jams: effect on the textural characteristics and sensorial profile—a chemometric approach. *J Food Process*. 2015;2015:749740. doi: [10.1155/2015/749740](https://doi.org/10.1155/2015/749740).
- Guggisberg D, Piccinali P, Schreier K. Effects of sugar substitution with Stevia, Actilight™ and Stevia combinations or Palatinose™ on rheological and sensory characteristics of low-fat and whole milk set yoghurt. *Int Dairy J*. 2011;21(9):636-44. doi: [10.1016/j.idairyj.2011.03.010](https://doi.org/10.1016/j.idairyj.2011.03.010).
- Zygler A, Wasik A, Kot-Wasik A, Namieśnik J. Determination of nine high-intensity sweeteners in various foods by high-performance liquid chromatography with mass spectrometric detection. *Anal Bioanal Chem*. 2011;400(7):2159-72. doi: [10.1007/s00216-011-4937-z](https://doi.org/10.1007/s00216-011-4937-z).
- Clos JF, DuBois GE, Prakash I. Photostability of rebaudioside A and stevioside in beverages. *J Agric Food Chem*. 2008;56(18):8507-13. doi: [10.1021/jf801343e](https://doi.org/10.1021/jf801343e).
- Geuns JM. Stevioside. *Phytochemistry*. 2003;64(5):913-21. doi: [10.1016/s0031-9422\(03\)00426-6](https://doi.org/10.1016/s0031-9422(03)00426-6).
- Gardana C, Simonetti P, Canzi E, Zanchi R, Pietta P. Metabolism of stevioside and rebaudioside A from *Stevia rebaudiana* extracts by human microflora. *J Agric Food Chem*. 2003;51(22):6618-22. doi: [10.1021/jf0303619](https://doi.org/10.1021/jf0303619).
- Nunes AP, Ferreira-Machado SC, Nunes RM, Dantas FJ, De Mattos JC, Caldeira-de-Araújo A. Analysis of genotoxic potentiality of stevioside by comet assay. *Food Chem Toxicol*. 2007;45(4):662-6. doi: [10.1016/j.fct.2006.10.015](https://doi.org/10.1016/j.fct.2006.10.015).
- Carakostas MC, Curry LL, Boileau AC, Brusick DJ. Overview: the history, technical function and safety of rebaudioside A, a naturally occurring steviol glycoside, for use in food and beverages. *Food Chem Toxicol*. 2008;46 Suppl 7:S1-S10. doi: [10.1016/j.fct.2008.05.003](https://doi.org/10.1016/j.fct.2008.05.003).
- Shah AB, Jones GP, Vasiljevic T. Sucrose-free chocolate sweetened with *Stevia rebaudiana* extract and containing different bulking agents—effects on physicochemical and sensory properties. *Int J Food Sci Technol*. 2010;45(7):1426-35. doi: [10.1111/j.1365-2621.2010.02283.x](https://doi.org/10.1111/j.1365-2621.2010.02283.x).
- Alizadeh M, Azizi-lalabadi M, Hojat-Ansari H, Kheirouri S. Effect of *Stevia* as a substitute for sugar on physicochemical and sensory properties of fruit based milk shake. *J Sci Res Rep*. 2014;3(11):1421-9.
- Saniah K, Samsiah MS. The application of *Stevia* as sugar substitute in carbonated drinks using response surface methodology. *J Trop Agric Food Sci*. 2012;40(1):23-34.
- Helrich K. Official Methods of Analysis of the Association of Official Analytical Chemists. Association of Official Analytical Chemists; 1990.
- Lane JH, Eynon L. Determination of Reducing Sugars by Fehling's Solution with Methylene Blue Indicator. London: Norman Rodger; 1934.
- Hashemi N, Rabiee H, Tavakolipour H, Gazerani S. Effect of stevia (*Stevia rebaudiana*) as a substitute for sugar on physicochemical, rheological and sensory properties of dietary saffron syrup. *Saffron Agronomy and Technology*. 2015;2(4):303-10.
- Yousefi AM, Goli SA, Kadivar M. Optimization of low-calorie quince jam production with stevioside sweetener. *J Food Res*. 2012;22(2):155-64. [Persian].
- Tan SL. *Stevia: Satisfying Malaysia's Sweet Tooth*. 2009.
- Yang X, Lu Y, Hu G. Optimization of sweetener formulation in sugar-free yoghurt using response surface methodology. *CyTA J Food*. 2014;12(2):121-6. doi: [10.1080/19476337.2013.804123](https://doi.org/10.1080/19476337.2013.804123).
- Agarwal V, Kochhar A, Sachdeva R. Sensory and nutritional evaluation of sweet milk products prepared using stevia powder for diabetics. *Stud Ethno Med*. 2010;4(1):9-13. doi: [10.1080/09735070.2010.11886357](https://doi.org/10.1080/09735070.2010.11886357).
- Weber A, Hekmat S. The effect of *Stevia rebaudiana* on the growth and survival of *Lactobacillus rhamnosus* GR-1 and sensory properties of probiotic yogurt. *J Food Res*. 2013;2(2):136-43.