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The Effect Of Using Stevia And Agave Nectar as a Substitute For Sucrose On Physical, Chemical, Rheological, And Sensory Properties Of Dark Chocolate

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ABSTRACT

Despite of the fact that the use of sweeteners as sugar substitute in food products is effective in reduction of calories and related diseases, they are typically associated with change in the texture and sensory properties of the product. In the present study, the effect of partial and overall replacement of sucrose with agave nectar and stevia on chemical, physical, rheological, and sensory properties of dark chocolate was evaluated. The results indicated that the amount of water, water activity, fat, and protein of chocolate dropped with an increase in the amount of agave nectar and a decrease in stevia. There was no significant difference between fat and protein of the control sample and the one containing agave nectar while there was a significant difference between other samples. The hardness of the control sample was more than that of other ones, and replacing sugar with agave nectar and stevia led to a decrease in the chocolate hardness. L, hue, and Chroma mean of the sample with stevia was higher and that of the sample with agave nectar was lower than all other samples, which indicated the favorable quality of the sample prepared with agave nectar. Fitting the experimental data with different model indicated that Windhab Model was the best model to predict the rheological properties of dark chocolate. In Windhab Model, with an increase in the amount of agave and sucrose, linear yield stress and shear stress increased in chocolate, and the control sample experienced a higher level of yield stress. Moreover, the apparent viscosity of the control sample was more than other ones, and replacing sucrose with agave nectar and especially with stevia led to a drop in apparent viscosity. Regarding the sensory properties, the sample containing agave nectar obtained a higher score than the control sample, and the quality of the produced chocolate using agave nectar was evaluated to be the best one. The results of the present study indicated that using a combination of sucrose, agave nectar and stevia and agave nectar alone is an appropriate solution for producing chocolate, and this product can fulfill the needs of individuals with diabetes mellitus, cardiovascular diseases, and Gastrointestinal cancer.*

Keywords: agave nectar, chemical and physical properties, dark chocolate, Rheological properties, sucrose substitute, stevia, sensory properties

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INTRODUCTION

Sweet taste is one of the most popular tastes among humans since birth [1]. Consuming sweet products in different forms is one of the daily nutritional habits all over the world. Over more than 3,000 years, consuming sweet products has had an increasing and different trend, varying from types of sweet fruits and plant secretions and honey to varieties of synthetic sweeteners [2]. Despite of all its advantages as a natural sweetener with its superior performance properties, sucrose is harmful especially for diabetic patients due to its relationship with some health problems such as blood pressure, heart diseases, tooth decay, obesity, increase in blood glucose and insulin levels. On the other hand, because of economic and technological issues, numerous studies have carried out in order to replace sugar with other sweeteners [1, 3,4]. The World Health Organization [WHO] has estimated that 1 billion people are overweight, 400 million suffer from obesity, and it is expected that this figure will double by 2015 [5].Another problem that the world is faced with is that millions of people are suffering from type 1 and 2 diabetes. And, the individuals should avoid consuming high-calorie carbohydrates with high absorption in order adverse complications such as increased blood sugar [6].There are substitute natural sweeteners for sugar, which are more appropriate choices than refined sugar. Stevia and agave nectar are natural sweeteners which are potential substitutes for sugar in different products. Agave nectar has low glycaemia, antioxidant capacity, and anti-bacterial properties[7] and are 1.8 times sweeter than sugar [8].Moreover, as opposed to fruits, vegetables, and honey, fructose content in agave nectar is more than that of glucose, which is attributed to the lower level of glycaemia index in agave nectar compared to sugar[9].Fructose content, glucose, and other sugars respectively account for 90, 6, and 4 percent of all sugars in agave nectar [10].Stevia plant with the scientific name of stevia rebaudiana is of asteraceae family which grows in South America, Paraguay, and Brazil. People of South America have been using this plant as a sweetener and an herbal medicine for hundreds of years [11].Stevia plant is a natural sweetener with no calorie and a sweetness level of 250-300 times more than sugar [12].As opposed to artificial sweeteners, stevia not only has health effects in the body but it also pharmacological impacts like antibacterial activity [13].Evidence indicates that stevia plays a role in treating obesity and blood pressure and leaves a slight effect on blood glucose [14]. In December 2008, FDA announced the sweetener obtained from stevia as a GRAS compound for use in foods and juices [15].Chocolate is a high-calorie food with fast metabolism and good digestion. The increasing consumption of different types of chocolate is due to its unique taste and texture and the pleasure it gives after being eaten. However, one of the problems involved with eating this food is the high amount of its sugar which varies between 35-50% depending on its type [16]. Dark chocolate bears numerous positive effects on cardiovascular health, lower blood pressure, lower cholesterol, and stimulation of endorphin production. It seems that advantageous effects of dark chocolate is due to the performance of its flavonoids, because they have antioxidant properties and can chelate free radicals [17].Excessive consumption of dark chocolate; however, can have adverse effects on health due to the high levels of its fat, sugar, and calorie. There are few studies focusing on sucrose replacement with other compounds in chocolate in order to reduce calorie and the consequences of sugar consumption in chocolate. Shourideh *et al.*[18]studied the effect of using tagatose and inulin as substitutes for sucrose on rheological, sensory, and physical properties of dark chocolate Farzanmehr and Abbasi [19] produced a type of low-calorie milk chocolate using sucralose as the sweetener and inulin, polydextrose, and maltodextrin as bulking agents. Shah *et al.*[20] studied the production of a type of sucrose-free milk chocolate with the use of stevia rebaudiana extract as the sweetener and inulin and dextrose and the bulking agents. Golob *et al.*[21] investigated the effect of using inulin and fructose on sensory properties of milk chocolate. The present study was carried out in order to replace sugar in dark chocolate with agave nectar, stevia, and a compound of them and investigate the physical, chemical, rheological, and sensory properties of the produced chocolate.

MATERIALS AND METHODS

Ingredients

Cocoa powder, cocoa butter, and stevia obtained from Salamat Mehr Company, lecithin and vanilla powder obtained from Golha Company, and agave nectar was purchased from a reputable food store in Tehran. And other chemicals were achieved from Merck Chemicals.

The method of preparing the chocolate samples

First, the sugar was quite ground with a hand mill. Afterwards, to prepare the 100 gr samples of low-calorie dark chocolate, first cocoa butter (20 gr) was melted in a 60° C stove, then cocoa powder (26.2 gr) and sugar (42 gr) with its substitutes including agave nectar and stevia solely and in combination with each other (see Table 1), sucralose (0.02 gr) and vanilla (0.5 gr) were added to the melted cocoa butter. The obtained mixture was placed in a ball mill manufactured by Sepehr Machine Company (Tehran, Iran),and the size reduction of the grains and chocolate kneading were carried out in 65° C in a paraffin bath. The remaining of the cocoa butter (10 gr) and lecithin (0.5 gr) were added in the last 30 minutes of the kneading [19,22]. The prepared samples were stored in the 60° C stove for 24 hours, and then their temperature was reduced to 55 ° C and were kept at this temperature for 30 minutes. In order to conduct the conditional temperature actions during mixing using the mixer, the temperature of the samples was reduced to 28° C during 25 minutes and they were kept at this temperature for 10 minutes. Afterwards, the temperature of the samples was enhanced to 30° C before they were transferred to plastic molds. After this period, the samples were transferred into the molds and were kept at a temperature of 15° C for 3o minutes. After the samples were removed from the molds, they were packed in aluminum sheets and kept in a refrigerator until the experiment time [19,23].The control sample was also prepared using the same method except for using sucrose instead of sugar replacements. The balance of sweetness was employed in order to measure the level stevia in sucrose replacement treatment. Sweetness of sucrose was considered as 1, that of agave nectar as about 1.8, and that of stevia as 250 times more than that of sucrose.

Table 1. Different percentages of sweeteners in dark chocolate

Sample	Percentage of different sweeteners in dark chocolate		
	Sucrose	Agave nectar	Stevia
1	0	0	100
2	0	25	75
3	0	50	50
4	0	75	25
5	0	100	0
6	33.3	33.3	33.3
7	50	25	25
8	100	0	0

Measuring some physical and chemical properties of chocolate

The level of moisture, fat, and protein of the chocolate samples was measured by AOAC method. Water activity of the chocolate samples was also determined using Novasina Labmaster Instrument (made in Switzerland). Hunter Lab-025-9000 was used to measure the hue of the samples, and in CIELAB factors of L^*, a^*, and b^*, the amounts of Chroma (saturation degree) and Hue (brightness level) were calculated using the following equations [(24).]

$$c^* = (a^{*2} + b^{*2})^{1/2}$$

$$hue^0 = \arctan(b^* / a^*)$$

Measuring the hardness

In order to measure the hardness, Hounsfield Texture Analysis device was used. Chocolate pieces of 10×20×100 were prepared, then put in an incubator with refrigerator, and kept at a temperature of 20° C for 6 hours. After some phases of examining with swabs of different sizes, swab 1.6 was selected, and with a penetration rate of 90 mm/min the depth of 6 mm was measured and the maximum measured force was reported as the hardness index [(25).]

Measuring some rheological properties

Rheometer device (Anton Paar-MCR 301) made in Austria was used to examine the rheological properties of the samples. This device was equipped with temperature regulator and water circulator. First, the chocolate samples were placed in a closed container and kept in a stove of 50° C for at least 80 minutes. Afterwards, they were put in the geometry cup, and were mixed at a temperature of 40° C at a speed of 5 S^{-1} for 10 minutes. Then, in temperature of 40° C, shear changes were measured during 180 seconds in the range of 1 to $60S^{-1}$ (Ramp up) and 60 to $1S^{-1}$ (Ramp down). Afterwards, Bingham Mathematical Model (Eq. 1), Herschel-Bulkley Model (Eq. 2), Casson Model (Eq. 3), Windhab Model (Eq. 4), and Ostwald Wall Model (Eq. 5) were obtained for experimental data. The most appropriate mathematical model was determined based on calculation of R^2 (the coefficient of determination) and SE (standard deviation), and rheological indices were measured [(26).]

$$\sigma = \eta_{pl}\ (\gamma^{\circ}) + \sigma_0 \qquad :1$$

$$\sigma = K\,\gamma^{\circ n} + \sigma_0 \qquad :2$$

$$(\sigma)^{0.5} = K_1(\gamma^{\circ})^{0.5} + (\sigma_0)^{0.5} \qquad :3$$

$$\sigma = \sigma_0 + (\sigma_1\text{-}\sigma_0).[1\text{- }\exp(-\gamma^{\circ}/\gamma_{*})] + \eta_{\infty}.\,\gamma^{\circ} \qquad 4$$

$$\sigma = k_2\ \dot{\gamma}^{\,n_2} \qquad :5$$

Where, τ_1 is linear shear stress,$\tau_1$is yield stress, η_∞ is viscosity at high shear rates, $\gamma^{o*}$is characteristic shear rate, τ is shear stress, γ^o is shear rate, viscosity η_p is plastic, n_1 is flow behavior index, k_1 is Casson viscosity, k_2 is viscosity index, and $n_2$is power law index [(22).]

Sensory evaluation

Sensory properties of chocolate samples including sweetness, texture, melting method in the mouth, hue, and total acceptance were measured using 5-point Hedonic Scale (1=very bad, 2=bad, 3=average, 4=good, and 5=very good) by 16 evaluators aging 20-40 years old. Each sample was assigned a 3-digit code. The samples were randomly distributed among the evaluators.

Statistical analysis
Chemical and physical tests, rheological test, and sensory evaluation were carried out in a completely random design by comparing Duncan mean with a confidence level of 95% using SAS 9.1 Software. Graphs were drawn using Microsoft Excel. It should be noted that all tests were conducted with three replicates.

RESULTS AND DISCUSSION

The effect of replacing sugar with stevia and agave nectar on some physical and chemical properties of dark chocolate
Table 2 indicates physical and chemical properties of the chocolates prepared with sugar replacements. As it is obvious the moisture percentage of the samples containing stevia and agave nectar was higher than that of the control ones, and with an increase in the level of sucrose in the samples, water percentage dropped, and there was a significant difference among all of the samples. There was a significant difference among the treatments in terms of water activity, and the water activity of the control sample was less than other ones. However, there was no significant difference between sample 5 (containing agave nectar only) and sample 8 (containing sucrose only). Agave nectar and sucrose contain many hydroxyl groups which connect with water molecules and lead to a decrease in the activity and mobility of water; therefore, the water activity of the samples containing these two compounds is lower than those with stevia. As a result, it can be concluded that moisture absorption and retaining of agave nectar is like that of sucrose. Comparing moisture percentage and water activity level of samples 5 and 8 showed that although sample 5 had a higher amounts of water, its water activity was lower which can be attributed to the higher power of agave nectar to reduce water activity and its stronger link with water molecules. The reason can be that sucrose is a disaccharide and has fewer hydroxyl groups than monosaccharides of agave nectar; therefore, agave nectar has more groups to absorb water and reduce water activity more. The level of fat and protein of the samples decreased with an increase in the amount of agave nectar and a decrease in stevia, and the sample that only contained stevia had the highest levels of fat and protein. There was a significant difference among sample 1 (containing stevia only), sample 5 (containing agave nectar only), and sample 8 (containing sucrose only). Furthermore, there was no significant difference between the control sample and sample 5 in terms of the levels of protein and fat. It can be stated that in sample 1, 0.2 gr stevia (as much as it sweetened the chocolate) was used instead of 43 gr sucrose, and since there was a decrease in carbohydrate percentage, the percentage of other ingredients such as water, fat, and protein had a relative increase, and with a rise in the amount of agave nectar and sucrose (increase in carbohydrates), the percentage of water, fat, and protein decreased. There was no significant difference in pH of different samples. The same results were reported in the study carried out by Bitaraf *et al.*[27].

Table 2. The results of some chemical and physical properties of dark chocolate

Sample	Moisture percentage	Water activity	pH	Fat	Protein
1	6.07a	0.411a	6.95a	51.85a	10.45b
2	5.15b	0.373b	6.95a	47.42b	9.99c
3	4.63c	0.347c	6.93a	43.93c	9.37d
4	4.09d	0.325d	7a	41.1e	8.85d
5	3.88e	0.321d	6.99a	39.52f	7.24f
6	4.03f	0.354c	6.94a	43.49d	8.56d
7	3.75g	0.325d	6.96a	41.41e	7.75e
Control	3.36h	0.328cd	6.98a	39.34f	7.33f

The means that are presented with different letters in each column are significantly different using Duncan test and at a level of 95% ($p<0.05$)

According to Table 3 on the hue indices, the mean $L*$ of the sample containing stevia was higher than the two samples containing agave nectar and sucrose, and the hue of the prepared chocolates was significantly brighter. With an increase in the amount of agave nectar and a decrease in stevia (samples 1 to 5), L index dropped. This index in sample 5 (containing agave nectar) was lower than that of the control sample, which can be attributed to the role of agave nectar in the Maillardreactions, because sucrose is a non-reducing sugar and cannot participate in Maillard reactions unless it turns into glucose and fructose that are reducing sugars as a result of heat and other factors. Because agave nectar contains excessive fructose and glucose and thus has reducing sugar groups, it participates in Maillard reactions and cause an increase in the hue and darkening of the samples compared to the control sample and the samples containing stevia. Because the sample containing stevia does not contain reducing sugar groups, its $L*$ index is higher than other values. The results of the present study are not in agreement with those of the study conducted by Shourideh *et al.* who reported that Maillard reaction has no effect on the hue. It is noteworthy that the water amount and water activity and also the temperature used in preparing the chocolates were higher in the present study while in the one carried out by Shourideh *et al.* these parameter were lower; therefore, they concluded that due to low temperature, water activity, and low water percentage, the reducing sugars were less likely to participate in Maillard reaction and they had no specific role in hue indices [28, 29, 30, 31].

Table 3. The effect of replacing sucrose with agave nectar and stevia on hue indices

Sample	*L**	*a**	*b**	*Chroma*	*hue*
1	25.35a	4.92ab	7.13a	8.66a	55.39a
2	23.97b	4.83b	6.36b	7.98c	52.78b
3	21.62e	4.33c	5.43c	6.94d	51.43c
4	20.34f	4.06d	5.01d	6.44f	50.97cd
5	19.64g	3,82e	4.33e	5.77e	48.58e
6	23.11c	4.93ab	6.45b	8.11b	52.60b
7	21.33e	4.31c	5.29cd	6.82d	50.82d
Control	22.68d	5.14a	6.41b	8.21b	51.27c

The means that are presented with different letters in each column are significantly different using Duncan test and at a level of 95% ($p<0.05$)

As indicated in Table 3 above, the two indices of saturation level and brightness degree were highest in sample 1, and the sample containing pure stevia had higher indices of Chroma and Hue than others, i.e. they were brighter than other samples. Moreover, according to Table 2, it is obvious that the rate of these two indices in samples 1 to 5 has gradually decreased, which can be attributed to the increase in the amount of agave nectar in the sample formulation. Comparison between sample 1 and control sample to some extent indicates the role of sucrose in causing the darkness and less brightness of the sample which is probably due to sucrose breakdown into reducing sugars and its marginal participation in Maillard reaction. Therefore, according to indices of Chroma and Hue, sample 5 is an appropriate one and its brightness is low which is proper and favorable for dark chocolate.

The effect of replacing sugar with stevia and agave nectar on some mechanical and rheological properties of dark chocolate

Hardness refers to physical hardness and is directly related to the sensory properties of chocolate during consumption. Moreover, hardness is effectively used to predict the melting time during consumption ([20]). According to Figure 4, the hardness level of the prepared samples containing pure stevia is lower than other samples, and it increased with a drop in stevia amount. The hardness level of the control sample was higher than other samples, and this difference was significant. According to the results, it can be stated that replacing sugar with stevia and agave nectar led to a reduction in the hardness (being crispy) of dark chocolate. In general, the hardness of the prepared samples was lower than that of the control sample, which can be attributed to their higher level of moisture and the resistance difference of the replacements with sugar. Similar results was reported by Bitaraf *et al.* who indicated that replacing sugar with inulin, polydextrose, and maltodextrin reduced the hardness of chocolate [(27).]

Table 4. Hardness level of different samples of chocolate

Sample	1	2	3	4	5	6	7	Control
Hardness	23.3d	24.5c	26.9b	27.1b	27.2b	26.7b	28a	28.3a

The means that are presented with different letters in each column are significantly different using Duncan test and at a level of 95% ($p<0.05$)

Aeschlimann and Beckett [(32)] stated that moisture (even very low amounts) can result in chocolate hardness, which was also observed in the present study; sample 1 and control sample that respectively had the highest and the lowest levels of moisture had the lowest and the highest hardness, respectively. On the other hand, the percentage of fat and protein increased in sample 1 to 5, which can have a positive effect on hardness increase. Having the lowest levels of fat and protein, the control sample had the highest level of hardness. Regarding low-fat chocolates, the higher level of hardness is due to the decrease in the total fat and liquid fat [(33).]In the present study, the fat percentage of the control sample is lower than other ones, which can cause hardness in the control samples.In the study carried out by Shah *el al.*[(20)]who investigated the production of a sucrose-free milk chocolate using stevia rebaudiana extract, inulin, and polydextrose, sample containing inulin were harder than the control ones. The difference between the present study and that of Shah *et al.* can be attributed to the method of preparing the chocolate, the ingredients, and sugar replacements.Rheological properties play an essential role not only in determining the efficiency processes such as mixing and pumping but also in using chocolate as coating, shell forming, and molding [(34).]In order to achieve the best and most appropriate model for evaluating the flow behavior of dark chocolate, the data obtained through the five models of Windhab, Casson, Bingham, Herschel-Bulkley, and Ostwald Wall were fitted, and based on the highest coefficient of determination and the lowest standard deviation, the appropriate model was selected. After the coefficients (See Table 5) were examined, it was specified that Windhab Model was the most appropriate model followed by Casson and Herschel-Bulkley models. Since 2001, the appropriateness of this model for melted chocolate (the temperature of 40° C) in the shear speed range of 1 to 100 mm/sec has been approved by International Confectionery Association (ICA), and now it is used as an international standard to measure chocolate viscosity [(35).]Windhab viscosity of different samples is presented in the table. Casson Model in the study carried out by Bitaraf *et al.*[(27)] and Windhab Model in the study of Shourideh *et al*[.(18)] were selected as the most appropriate model for dark chocolate. The difference between the present study and the one conducted by Bitaraf *et al.*can be attributed to the type of the devices used to knead the chocolate; therefore, the structure formed in the prepared chocolate is different.

Table 5. The coefficient of determination and standard deviation in 5 common rheological models in the dark chocolate samples

Sample	Model	Coefficient of determination	SD	Sample	Model	Coefficient of determination	SD
1	Windhab	0.98259	1.22	5	Windhab	0.99951	1.87
	Casson	0.98112	1.62		Casson	0.99911	2.1
	Herschel-Bulkley	0.98013	2.23		Herschel-Bulkley	0.98980	3.29
	Ostwald Wall	0.97925	3.41		Ostwald Wall	0.98650	3.98
	Bingham	0.96525	3.82		Bingham	0.97990	5.15
2	Windhab	0.98881	1.75	6	Windhab	0.99989	1.13
	Casson	0.98732	1.98		Casson	0.99981	1.65
	Herschel-Bulkley	0.98389	2.28		Herschel-Bulkley	0.99673	2.84
	Ostwald Wall	0.98213	2.88		Ostwald Wall	0.99182	3.98
	Bingham	0.98003	4.52		Bingham	0.97900	5.86
3	Windhab	0.99332	0.68	7	Windhab	0.99996	0.93
	Casson	0.99046	1.16		Casson	0.99990	1.23
	Herschel-Bulkley	0.98937	1.43		Herschel-Bulkley	0.99890	1.88
	Ostwald Wall	0.98842	3.33		Ostwald Wall	0.99579	2.36
	Bingham	0.99821	2.84		Bingham	0.99015	4.16
4	Windhab	0.99911	1.28	Control	Windhab	0.99999	1.46
	Casson	0.99896	2.22		Casson	0.99968	1.95
	Herschel-Bulkley	0.99679	2.98		Herschel-Bulkley	0.99932	2.61
	Ostwald Wall	0.99187	3.63		Ostwald Wall	0.98994	3.68
	Bingham	0.98888	4.04		Bingham	0.98899	6.12

Yield stress is one of the properties of materials, which indicates the minimum shear stress needed for the material to flow [36]. As indicated in Table 6 below, in Windhab Model the values rose with an increase in the amounts of yield stress and linear shear stress in the prepared chocolates, and a significant difference was observed among the samples. In general, the maximum yield stress and linear shear stress were observed in the samples containing sucrose, which indicates the effect of sucrose on the increase in these parameters. Moreover, agave nectar had a lower capacity to enhance yield stress and linear shear stress than sucrose. Difference in the structure of stevia, agave nectar, and sucrose can be one of the influential factors in the interaction among the particles, and this their resistance against flowing.

Table 6. Rheological results of dark chocolate by Windhab Model

Sample	Yield stress	Linear shear stress	Viscosity in high shear rates	Apparent viscosity in 40 per second
1	29.35f	58.36g	2.1e	3.13d
2	34.32e	63.58f	2.36d	3.25c
3	38.28d	71.7e	2.79c	3.62bc
4	42.43c	78.83d	3.21b	3.88b
5	44.87b	89.83c	3.23b	3.93b
6	44.45b	93.96b	3.16b	3.42c
7	46.36b	95.14a	4.42a	5.44a
Control	48.5a	94.91a	4.45a	5.51a

The means that are presented with different letters in each column are significantly different using Duncan test and at a level of 95% (p<0.05)

According to the results, it can be stated that mechanisms that cause the formation of a new structure in high shear rates and a more decrease in viscosity in samples with high levels of stevia. In regard to energy consumption and production cost, this index is remarkable in conditions that there is a need for mixing chocolate at high shear speeds (like new generation conches), and its value in all samples is lower than sample 7 and the control sample. Apparent viscosity level also increased with a rise in the amount of agave nectar; however, the rate of this parameter in all samples was in general lower than the control sample. Comparing sample 5 and the control one indicated that sucrose's role in enhancing rheological parameter is more than agave nectar, and all parameters were evaluated higher for the control sample.

Sensory evaluation

The results of the sensory evaluation of the produced dark chocolates are presented in table 7. As indicated, there was no significant difference among the samples in terms of sweetness, which can certainly be attributed to using each sweetener according to its sweetening capacity. Regarding the texture, all samples except for those containing stevia were evaluated to be appropriate, and with a rise in the amount of agave nectar, the texture score increased, which can be attributed to the fact that stevia did not have combining capacity with other compounds like fact and protein, and its amount was so low; therefore, the texture of this chocolate was not evaluated to be appropriate. In regard to melting in the mouth, the samples containing stevia obtained lower scores.

Table 7. The effect of replacing sucrose with agave nectar and stevia on sensory indices

Sample	Sweetness	Texture	Melting in the mouth	Hue	Scent and smell	Flavor and taste	Total acceptability
1	4.21a	3.24c	3.32b	3.03d	2.53e	3.13c	3.1e
2	4.23a	3.62b	3.24b	3.56c	3.42d	3.46c	3.52d
3	4.22a	3.96ab	3.31b	3.69cd	3.78cd	3.85b	3.85c
4	4.17a	4.1ab	3.16c	3.81c	4.02c	3.91b	3.91c
5	4.15a	4.28a	3.11c	4.15a	4.75a	4.09a	4.40a
6	4.2a	4.32a	3.78a	3.95b	4.25b	3.99ab	4.45a
7	4.19a	4.4a	3.82a	3.98b	4.24b	4a	4.42a
Control	4.17a	4.51a	3.65a	3.92b	4.14bc	4.03a	4.13b

The means that are presented with different letters in each column are significantly different using Duncan test and at a level of 95% ($p<0.05$)

Regarding hue, the sample containing agave nectar obtained the highest score and had a significant difference with other samples. This results are in agreement with those of hue measurements in which this sample obtained the minimum score on Chroma and Hue, which shows its higher darkness compared to the other samples which is more appropriate and favorable for dark chocolate. These results, as was referred to before, can be attributed to the presence of reducing sugars in agave nectar compared to other samples. Similar results on hue and scent and smell were also observed, and sample 5 obtained the highest score which can also be attributed to the participation of the reducing sugars in Maillard reactions. The sample containing pure stevia obtained the lowest score in terms of hue and scent and smell, and it was evaluated unfavorable regarding panelists. In regard to flavor and taste, with a rise in the amount of agave nectar, the flavor and taste of the samples increased, and the flavor and taste of the control sample had no significant difference with samples 5, 6, and 7. The three samples of 5, 6, and 7 obtained the highest rate of acceptability, and there was no significant difference among these samples. The total acceptability of the control sample was placed after these three sample. Samples containing stevia also gained the minimum score and had a low rate of acceptability. The total result in regard to sensory properties indicated that the three samples of 5, 6, and 7 had acceptable sensory properties and they can be total or partial replacements for sucrose without any decrease in the quality, and even the sensory quality of the chocolates increased. The better sensory properties of these samples can probably be attributed to the synergistic effect of between sucrose and agave nectar and also the presence of reducing sugars in agave nectar compared to the control sample. However, stevia has a high potential to improve sensory properties, and samples containing stevia were evaluated to be more favorable.

CONCLUSION

According to the results of the present study, stevia and agave nectar had different effect on physical and chemical, mechanical, rheological, and sensory properties of the dark chocolate samples. The results of the effects of agave nectar and stevia as total or partial replacements for sucrose on the properties of dark chocolate indicated that hardness level increases with a rise in the amount of the replacements in the chocolate. Hardness level of the control sample was higher than that of the other samples. The amount of protein and fat of chocolate decreased with a rise in the amount of agave nectar and stevia. Moreover, the percentage of protein and fat of the control sample was lower than other samples. Regarding the indices of hue measurement, mean L^* of the sample containing stevia was higher than the two samples containing agave nectar and sucrose, and the color of the chocolates was significantly brighter. The level of saturation and the brightness degree of the sample containing pure stevia were higher than other samples, and the one of the sample containing agave nectar was lower than the other ones. Among the evaluated mathematical models to predict the rheological properties of dark chocolate, Windhab Model was known as the most appropriate model. With a rise in the amount of agave nectar and sucrose, yield stress and linear shear stress in Windhab Model increased in the produced chocolate. The maximum yield stress and linear shear stress were observed in the samples containing sucrose. Apparent viscosity also increased with a rise in the amount of agave nectar and sucrose in the samples, and the viscosity of the control sample was higher than other ones. Using stevia did not indicate a high potential in regard to quality and sensory properties, and samples containing stevia were not evaluated to be favorable, and using a combination of stevia and other sweeteners seemed to be favorable. The results of the present study indicated that using a combination of sucrose, agave nectar and stevia and agave nectar alone is an appropriate solution for producing chocolate, and this product can fulfill the needs of individuals with diabetes mellitus, cardiovascular diseases, and Gastrointestinal cancer**.**

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