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Full Research Paper

Optimization of sugar free dark chocolate product compatible for ketogenic diet and investigating its physicochemical, textural, thermal and sensory properties

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Abstract
There is a challenge in producing a portion both compatible to ketogenic diet and sufficient satiety. This study investigated the possibility of producing sugar-free chocolate product using increasing total fat and protein. The ingredients were chosen such that they do not contain any source of starch and sucrose. The cocoa powder was replaced with cocoa butter substitute (CBS) and sodium caseinate at different levels (0, 5 and 10%) along with constant amount of stevia ketogenic powder and soybean hull as sugar substitute. Results showed that cocoa powder substitution significantly ($p< 0.05$) led to an increase in moisture, water activity, fat and protein and a decrease in ash and carbohydrate amount, respectively. It was also observed that addition of sodium caseinate and CBS made the chocolate softer and to be easily melted ($p< 0.05$). Sensory analysis showed that samples with high protein and fat content got better scores in overall acceptance ($p< 0.05$). Also, principle component analysis showed that the first two components could explain about 81% of total variance. Finally, the best composition was determined by considering both TPA, DSC and sensory properties. This sample contained 5% sodium caseinate and 35% CBS. Moreover, total sugar content and calorie amount of this sample was 2.17% and 547.41 kcal, respectively. The peroxide value of optimized sample was 0.5 meq per kg immediately after production and it reached to 1.13 meq per kg after two months. Consuming 100 g of this chocolate can supply 27% of daily calorie of an adult person (assuming 2000 kcal per day for adults). Consuming this 100 g can also supply 17% and 40% of classic and atkins keto diet. These results showed that, the selected sample with 35% CBS and 5% sodium caseinate could be compatible to ketogenic diet but more clinical research should be done in future.

Keyword: Sugar free, Chocolate, Ketogenic diet, Stevia.

Introduction
Chocolate is a suspension of solid particles including sugar, cocoa and milk ingredients in a continuous fat phase usually cocoa butter (Glicerina et al., 2013). Different kinds of chocolate are assorted according to type of solid particles. Dark chocolate usually contains sugar, high cocoa amount, and less milk ingredients in comparison to milk and white ones. Dark chocolate is a rich source of

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antioxidant due to high cocoa amount in formulation (Afoakwa, 2010). Consuming 100 g of dark chocolate can supply 25% of an adult daily calorie requirement (Zugravu & Otelea, 2019). According to Codex standard, the chocolate should have cocoa butter as main fat ingredient. The chocolate produced with cocoa butter substitute (CBS) is called chocolate product. Hydrogenated palm kernel oil is a kind of cocoa butter substitute. It has sharp melting rate due to its short chain triglycerides like lauric acid (Beckett, 2009).

The ketogenic diet is a kind of eating behavior in which consumption of fat increases, protein amount moderates, and carbohydrate limits to 20 or 50 g per day. The restriction of carbohydrate intake activates the liver to break fat into ketone body and fatty acid. This state is called ketosis (Kalra et al., 2018). In high carbohydrate diet, glucose would generate adenosine triphosphate (ATP) during glycogenesis metabolic pathway but in the ketogenic diet the mechanism is different. Ketones (produced by fat break in liver) generate acetyl coenzyme A (acetyle CoA) during kerbs cycle and energy would be supplied (Clanton et al., 2017). Ketogenic diet is different from low carbohydrate diet. Low carb is kind of diet which was introduced by William Banting in 1863. Carbohydrate amount should be consumed up to 130 g per day in this diet (Watanabe et al., 2020).

The first step to produce a keto-friendly chocolate is sugar substitution. Sugar should be replaced by a low energy sweetener and keto compatible bulking agent. Stevia and alcoholic sugars are the main substitution of sugar in confectionary industry (Sabbaghi, 2021). One such sweetener is the stevia which is a natural sweetener with 300 times sweetness compared to sucrose. Due to its low glycemic index, it can be safe for ketogenic diet (Shah et al., 2010). European Food Safety Authority stated that the stevia consumption is permitted up to 4 mg per day and it is generally recognized as safe (GRAS) (Aidoo et al., 2013). The soybean hull is waste of soybean oil extraction. The hull consists of 8 to 10 percent of whole bean weight. The soybean hull is a rich source of dietary fiber including cellulose, hemi cellulose, lignin, and pectin. It lacks some specific compounds such that the glycogenesis metabolic pathway is not followed (Poore et al., 2012).

Increasing fat content of the chocolate helps it to be more compatible to ketogenic diet. However, different variations of fat amount in chocolate formulation are limited by the dominance of solid's fraction on textural and also other properties of the chocolate (Aidoo et al., 2017). Rezende et al. (2015) declared a direct relationship between fat amount and hardness of the chocolate due to its higher Solid Fat Content (SFC). Azevedo et al. (2017) reported that high fat chocolates were sensed less bitter and also easily melted than low fat chocolate. Aidoo et al. (2015) found out that increasing fat content of sugar free dark chocolate decreased the hardness significantly but the peak temperature was not influenced by this variation. Guinard & Mazzucchelli. (1999) also stated that high fat chocolate samples were faster melting in the mouth according to hedonic sensory test.

Increasing fat amount is not sufficient for the purpose of this study. Ketogenic diet should be enriched with 60- 90% of fat amount (Clanton et al., 2017). In fact, the consumption of food is both for satiety and palatable sense (Holt et al., 1995). There is a reverse relationship between satiety and fat content of food. In other words, the ketogenic diet as a high fat diet is less satiation. Among all macronutrients, protein has more potential action on satiety (Gerstein et al., 2004). So other macronutrient compounds like fiber and protein are needed to be added into ketogenic base formulation to supply satiation sufficiency.

Casein is a type of milk protein existing in 4 types including κ-Casein, β-Casein, αs1-Casein, and αs2-Casein. Sodium caseinate can be immediately dispersed in the fat phase. It is usually used in dairy, meat, and coffee industries (Modler, 1985). Some researchers studied the effect of increasing total protein in the chocolate. Zarić et al. (2015) investigated the effect of increasing soymilk powder in chocolate formulation. They declared that addition of 20% of soymilk powder changed the optimal producing procedure. These samples would produce in longer milling time with less pre-crystallization temperature. Also, the interaction between soy protein and glycine made more stable gel and T_{index} enhancement. Ashrafie et al. (2014) evaluated the effect of cocoa butter substitution by collagen hydrolysate. Samples with 15 and 20% substitution were the best ones according to textural and sensory experiments.

Therefore, there are limited researches in which the effect of increasing both total fat and total protein were studied. The main purpose of this study was to investigate the effect of increasing total fat and protein and decreasing cocoa powder on physicochemical, textural, thermal and sensory properties of chocolate. Additionally, the acceptable amount of each material will be reported.

Materials and Methods:

Various ingredients include cocoa powder (Altinmarka, Turkey– with 10-12% fat), cocoa butter substitute (Sime-Darby, Malaysia), stevia ketogenic powder (Below, Iran-containing inulin, stevioside and isomalt), soybean hull (Toos-soya, Iran-with 83.7% carbohydrate, 0% sucrose, 1.2% fat, 5.5% ash and 9.2% protein), Polyglycerol polyricinoleate (PGPR) (Kerry, Belgium), the soy lecithin and sodium caseinate (92% protein, 2.9% carbohydrate,0.1% sucrose, 0.9%fat and 4.1% ash) (Toos-Argan, Iran).

Chocolate production

Dark chocolate samples were formulated in factorial design as shown in Table 1. Samples were prepared by semi-industrial ball mill refiner (made by Arman-kherad-toos, Iran) equipped with steel balls 0.92 mm in diameter. First, the melted cocoa butter substitute and emulsifiers were added to ball mill which was rotated at 10 rpm for 5 minutes in 50°C. Then, other mixed ingredients were gradually added to ball mill refiner. The instrument was set at 100 rpm for 4 h. Finally, the mixture was placed into molds with cube shapes and vibrated to remove the bubbles. After cooling the samples to 4°C for 30 minutes, they were wrapped in aluminum foils and stored at 25°C in the incubator for the analysis.

Table 1- The percentage of ingredients in the nine chocolate samples

Sample	Cocoa powder (%)	Cocoa butter substitute (%)	Stevia ketogenic powder (%)	Sodium caseinate (%)	Soy hull (%)	PGPR (%)	Lecithin (%)
1	37	30	25	0	7	0.7	0.3
2	32	30	25	5	7	0.7	0.3
3	27	30	25	10	7	0.7	0.3
4	32	35	25	0	7	0.7	0.3
5	27	35	25	5	7	0.7	0.3
6	22	35	25	10	7	0.7	0.3
7	27	40	25	0	7	0.7	0.3
8	22	40	25	5	7	0.7	0.3
9	17	40	25	10	7	0.7	0.3

Analytical methods

Proximate chemical analysis

The moisture was measured by oven (AOAC, 2000). The water activity analyzer was also used to measure water activity at 25°C (Novasina, Switzerland). The crude protein content was estimated by the Kjeldhal method (AOAC, 2000) and the fat content was measured by Soxhlet extraction (AOAC, 2000). Total minerals were measured as the residue after ashing at 550°C overnight. The carbohydrate content was calculated by subtracting the sum of moisture, fat, ash and protein content from 100.

Hardness

Textural parameters of the chocolate samples (1.1× 1.1× 1.1 cm^3) were evaluated by TA plus texture analyzer (Lloyd Instruments Ltd, UK) connected to the Nexygen software (version 4.5.1). A trigger force was set on 5 g. The puncture test was done with flat ended probe, 2 mm in diameter, and a 50 N load cell. The probe penetrated each sample to a depth of 5 mm at a constant speed of 1 mm/s at the ambient temperature (Aidoo et al., 2013).

Melting properties

The parameter was evaluated by DSC 214 Polyma (Netzsch, Germany). Five mg of each sample was placed in the instrument's pan. The samples were then heated from 0 to 60°C at a rate of 10°C/min in N_2 stream. Onset temperature (T_{onset}), maximum temperature (T_{max}) and offset temperature (T_{off}) were calculated.by software (Genc Polat et al., 2020).

Sensory evaluation

Chocolate samples (2.5 g) were randomly coded in three-digit cod numbers. Fifteen trained panelists (25- 40 years old including 4 men, 11 women) were selected according to being interested in participating and absence of aversions, allergies or intolerance against chocolate. The evaluations were done at the specific time of 9- 12 A.M. According to Table 2, the sensory attributes of these chocolates including color, hardness, melting rate, bitterness, mouth coating, and casein flavor and overall acceptance were evaluated using a hedonic test 9-point rating scale. The panelists were asked to consume water and cracker between each sample (Rezende et al., 2015).

Table 2- Definition and the main references used in the hedonic test

Attribute	Definition	References
Color	Dark color without any white spots on the surface	Weak: Milk chocolate Strong: 80% Dark chocolate
Hardness	The force required to bite the chocolate using front teeth	Weak: Probiotic cheese Strong: Carrot
Melting rate	The time required for the conversion of solid phase of chocolate into liquid one in the mouth	Weak: Probiotic cheese Strong: Toffee
Bitterness	Characteristic taste of caffeine	Weak: Milk chocolate Strong: 80% Dark chocolate
Casein flavor	Characteristic taste of casein	Weak: Skim milk powder Strong: 99% Dark chocolate
Mouth coating	Sensation of a fat layer in the mouth after the swallowing of chocolate	Weak: Water Strong: Cocoa butter substitution

Ketogenic validation of optimized sample

Reducing sugar content was measured by Lane-Eynon Method (AOAC, 2000). The calorific value was calculated by summing up the samples of multiplication of carbohydrate, protein and fat content by 3.7, 4.36, and 9.02 respectively (Sai et al., 2016).

Oil stability evaluation of optimized sample

The peroxide value was measured for the optimized treatment immediately after production and also after two months (AOAC, 2000).

Data analysis

The effect of cocoa powder, fat and protein amount on the quality of the chocolate samples was analyzed by two-way ANOVA at a significance level of 95% significance with SPSS version 25 software. All experiments were conducted in triplicates and then mean values were considered. Mean values were compared with Duncan's test at $p<0.05$. Also principal component analysis (with pearson correlation coefficient) was used to determine the relationship between all sensory variables by using SPSS software.

Results and discussion

Proximate chemical analysis

According to Table 3, the moisture content of all samples was in acceptable range of 0.5-1.5 % as Afoakwa mentioned (Afoakwa, 2010). Also, by increasing sodium caseinate, the moisture content is significantly increased ($p<0.05$). This is due to the interaction between protein and vapor molecules which came to the structure during counching. Hydrophilic part of sodium caseinate like carboxyl and phosphoseryl (in B-Cn monomer) creates the hydrogen bond with water and then moisture content would increase (Post et al., 2012; Modler, 1985). As a result of increasing moisture, less water was available in the structure and then water activity was reduced. Ashrafie et al. (2014) found that collagen addition in the chocolate formulation influenced the moisture of chocolate due to the interaction between steam and collagen hydrophilic part but this variation of collagen amount didn't significantly ($p>0.05$) affect the water activity.

Cocoa is a rich source of minerals like Calcium, cupper, etc. (Afoakwa et al, 2007). Analysis of variance showed that both CBS and sodium caseinate had significant impact on ash amount ($p<0.05$). According to Table 3, as the cocoa powder substitution increased, the ash amount is reduced. This reduction was due to the more amount of minerals which existed in the cocoa powder. Onwuka & Abasiekong (2006) substituted the cocoa powder with Bambara Groundnut and Treculia Africana. As cocoa substitution increased, ash amount became more. Because the bambara groundnut and treculia Africana had more ash than cocoa powder.

Total fat content of the chocolate depends on CBS amount and rare quantity of fat in other ingredients. According to Table 3, total fat content of samples with 35 and 40 g of CBS increased 5.54 and 11.72 percent respectively in comparison with 30 g CBS sample. Also, total protein amount of samples ranged between 5.42 and 14.60 gram in 100 g of chocolate. Samples 3, 6, and 9 had the considerable amount of total protein than others. According to Table 3, the carbohydrate amount was differed from 40.81 to 5.66 percent. In fact, samples with less cocoa powder substitution had less carbohydrate amount.

Table 3- The chemical composition of chocolate samples

Sample	Carbohydrate (%)	Ash (%)	Protein (%)	Fat (%)	Water activity (%)	Moisture (%)
1	$55.66\pm 0.55^{Aa\lambda1}$	$5.61\pm 0.04^{Aa\lambda1}$	$7.40\pm 0.10^{Aa\lambda1}$	$30.34\pm 0.44^{Aa\lambda1}$	$0.21\pm 0.00^{Aa\lambda1}$	$0.95\pm 0.01^{Aa\lambda1}$
2	$52.11\pm 0.29^{Aa\lambda2}$	$5.19\pm 0.62^{Ab\lambda2}$	$11.32\pm 0.60^{Ab\lambda12}$	$30.38\pm 0.34^{Aa\lambda2}$	$0.20\pm 0.00^{Ab\lambda12}$	$0.97\pm 0.04^{Ab\lambda12}$
3	$48.66\pm 0.41^{Ac\lambda3}$	$5.09\pm 0.60^{Ac\lambda3}$	$14.60\pm 0.04^{Ac\lambda12}$	$30.66\pm 0.38^{Ac\lambda23}$	$0.18\pm 0.00^{Ab\lambda123}$	$0.98\pm 0.04^{Ac\lambda123}$
4	$51.29\pm 0.27^{Bc\lambda2}$	$5.16\pm 0.11^{Ba\lambda2}$	$6.34\pm 0.06^{Ba\lambda12}$	$36.23\pm 0.28^{Ba\lambda12}$	$0.21\pm 0.00^{Ab\lambda12}$	$0.95\pm 0.02^{AB\lambda1}$
5	$48.13\pm 0.43^{Bb\lambda3}$	$4.57\pm 0.11^{Bb\lambda3}$	$10.38\pm 0.10^{Bb\lambda12}$	$35.93\pm 0.34^{Ba\lambda23}$	$0.20\pm 0.00^{Ab\lambda123}$	$0.96\pm 0.01^{ABb\lambda12}$
6	$45.25\pm 0.31^{Bc\lambda3}$	$4.27\pm 0.03^{Bc\lambda3}$	$13.63\pm 0.04^{Bc\lambda12}$	$35.86\pm 0.31^{Ca\lambda23}$	$0.19\pm 0.00^{Ab\lambda23}$	$0.97\pm 0.03^{AB\lambda12}$
7	$47.00\pm 0.81^{Ca\lambda3}$	$4.44\pm 0.04^{Ca\lambda3}$	$5.42\pm 0.63^{Ca\lambda12}$	$42.16\pm 0.75^{Ca\lambda23}$	$0.20\pm 0.00^{Ab\lambda123}$	$0.96\pm 0.05^{Ba\lambda12}$
8	$43.28\pm 0.44^{Cb\lambda4}$	$4.06\pm 0.05^{Cb\lambda4}$	$9.27\pm 0.05^{Ca\lambda12}$	$42.41\pm 0.08^{Ca\lambda34}$	$0.20\pm 0.00^{Ab\lambda23}$	$0.97\pm 0.06^{Bb\lambda23}$
9	$48.81\pm 0.40^{Cc\lambda5}$	$3.62\pm 0.34^{Cc\lambda4}$	$12.61\pm0.40^{Ca\lambda2}$	$41.96\pm0.11^{Ca\lambda4}$	$0.19\pm0.00^{Ab\lambda3}$	$0.98\pm0.02^{Bc\lambda123}$

***** Means ± standard deviations from analysis, means within same column with different letters are significantly different ($P<0.05$) from each other for each measured parameter. Uppercase and lowercase letters represent CBS and sodium caseinate, respectively. The letter λ shows the cocoa powder variable.

Hardness

Hardness shows the amount of force needed to penetrate the chocolate structure. It depends on ingredients, polymorphism structure of cocoa butter, and the production method (Rezende et al., 2015). ANOVA showed that both CBS and sodium caseinate significantly ($p<0.05$) influenced the hardness of chocolate samples. According to Fig 1, as cocoa substitution increased, the hardness was reduced. Samples 1 and 9 were the hardest and softest samples, respectively. Addition of CBS to chocolate formulation made more space for solid particles to move. Consequently, the interactions became less and the structure would be softer. Also, as sodium caseinate increased, the cocoa powder and its fiber content were decreased. The less fiber made the texture softer.

There are two kinds of studies about the effect of increasing fat amount on the hardness. The first one relies on increasing solid fat content as a result of increasing fat phase. This SFC increasing would lead to stronger network and harder structure of chocolate. Do et al. (2007) and Rezende et al. (2015) investigated different amount of fat content on the hardness of chocolate. They stated that samples containing more fat content showed more hardness due to the SFC increase and its effect on hardness. The other studies reported an inverse relationship between fat amount and hardness. They rely on the fact that the addition of fat led to increase the fat phase and then solid particles and their concentration would reduce. As a result, the hardness became less as fat amount is increased. Afoakwa et al. (2008) and Aidoo et al. (2017) declared that high fat chocolate samples had less hardness at any particle size or bulking agent concentrations.

The hardness ranged between 30.61- 58.46 N in our study. This amount is near to Do et al. (2007). They investigated the effect of fat increase at 3 levels of 22, 25 and 30% cocoa butter equivalent (CBE) on the hardness of chocolate. The hardness in that study was between 30- 55 N. It can be concluded that the presence of 40% CBS didn't change the minimal hardness in this study. Dewi et al. (2020) reported that hardness was between 15-20 N at 34% CBS in the formulation which was so lower than the current study. The presence of sodium caseinate and soybean hull might be the reason for the suitable hardness even at low fat samples. Also, there was a significant ($p< 0.05$) correlation between hardness and ash ($r= 0.97$), fat ($r= -0.89$) and carbohydrate ($r= 0.95$). It means that as fat content of samples reduced and ash or carbohydrate increased, the hardness would increase.

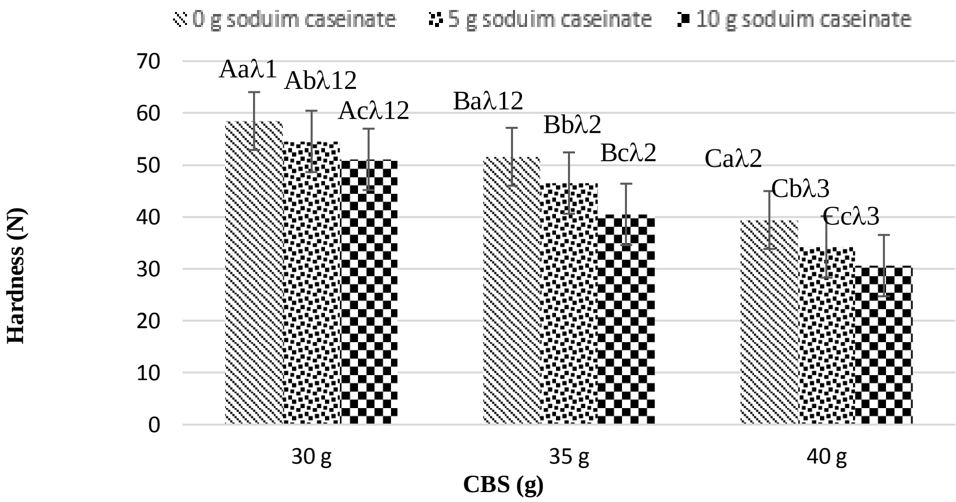

Fig. 1. The effect of CBS and sodium caseinate on hardness of chocolate samples.

*Uppercase and lowercase letters represent CBS and sodium caseinate, respectively. The letter λ shows the cocoa powder variable.

Melting Properties

In the current study, the effect of cocoa powder substitution on thermal properties of chocolate samples was studied. In Table 4, the thermal properties of samples heated from 0 to 60°C were reported. T_{onset}, T_{Peak} and T_{off} values varied between 23.06– 25.43°C, 27.60-29.40°C and 33.40- 29.93°C, respectively. ANOVA showed that increasing CBS from 30% to 40% significantly ($p < 0.05$) reduced all thermal parameters. In fact, the fat phase is responsible for melting behavior of chocolate. When more CBS was added to the formulation, it covered the solid particles. Consequently, the solid's interactions became less and free moving plastic flow is increased. Also, more fat could easily dispersed in salvia and melting procedure could be facilitated. (Afoakwa et al, 2008; Glicerina et al., 2013). Afoakwa et al. (2008) declared that increasing fat content significantly affected only offset temperature. Also, they reported all the parameters above 30° C which were higher than this study.

There is a significant ($p < 0.05$) correlation between peak temperature and ash ($r = 0.84$), fat ($r = -0.90$), carbohydrate ($r = 0.81$), and hardness ($r = 0.89$). It means that as fat content of samples reduced and ash or carbohydrate increased, the peak temperature also increased. As mentioned before, by increasing fat amount, more spaces are created in the structure and as a result particle-particle interactions are reduced. This reduction both facilities first bite (hardness) and melting of the chocolate samples.

Table 4- The effect of CBS and sodium caseinate on melting properties of chocolate samples

Sample	T_{onset} (°C)	T_{max} (°C)	T_{off} (°C)
1	25.20±0.36$^{Aa\lambda1}$	29.10±0.26$^{Aa\lambda1}$	32.96±0.45$^{Aa\lambda1}$
2	25.43±0.41$^{Aa\lambda1}$	29.46±0.10$^{Aa\lambda1}$	33.40±0.26$^{Aa\lambda1}$
3	25.43±0.31$^{Aa\lambda1}$	29.00±0. 17$^{Aa\lambda1}$	33.26±0.56$^{Aa\lambda1}$
4	23.60±0.43$^{Ba\lambda2}$	28.96±0.43$^{Ba\lambda2}$	30.56±0.50$^{Ba\lambda2}$
5	23.80±0.66$^{Ba\lambda2}$	28.40±0.26$^{Ba\lambda2}$	29.93±0.11$^{Ba\lambda2}$
6	23.56±0.23$^{Ba\lambda2}$	28.38±0.45$^{Ba\lambda2}$	30.43±0.35$^{Ba\lambda2}$
7	23.26±0.28$^{Ca\lambda3}$	27.76±0.20$^{Ca\lambda3}$	30.70±0.36$^{Ca\lambda3}$
8	23.06±0.30$^{Ca\lambda3}$	27.60±0.34$^{Ca\lambda3}$	29.96±0.61$^{Ca\lambda3}$
9	23.10±0.36$^{Ca\lambda3}$	27.63±0.15$^{Ca\lambda3}$	30.03±0.51$^{Ca\lambda3}$

***** Means ±standard deviations from analysis, means within same column with different letters are significantly different ($P < 0.05$) from each other for each measured parameter. Uppercase and lowercase letters represent CBS and sodium caseinate, respectively. The letter λ shows the cocoa powder variable.

Sensory Analysis

ANOVA showed that all sensory parameters were significantly affected by the cocoa powder substitution ($p<0.05$). As cocoa substitution increased, the panelists' score on the color decreased. It means they prefer darker chocolate samples. Guinard and musschelli, (1999) reported the same result. They stated that higher score was given to dark samples by panelists. Addition of CBS to samples significantly influenced the hardness and melting rate ($p < 0.05$). Generally, samples with 35 and 40% CBS had less hardness and the melting rate scores than samples with 30% CBS. In fact, the panelists prefer softer and easily melted samples. Farzanmehr and Abbasi, (2008) reported the same result. The cocoa substitution had a positive effect on bitterness and casein flavor perception. The panelists liked the dairy flavor of casein. Perception of chocolate bitterness intensity is kind of diversity matter in people. However, this study revealed that panelists liked less bitter chocolate samples. Puchol Michel et al. (2020) reported the same result. They stated that samples containing 70% cocoa were more acceptable in sweetness than 95% cocoa powder. Mouth coating is the layer of fat which covers mouth surfaces (Tobrica et al., 2016). Mouth coating of sample 7 and then 5 and 6 were the best. According to Table 5, samples with high fat t had more overall acceptance scores. Samples 5 and 7 had the highest overall acceptance scores. In fact, overall acceptance had a significant correlation with the fat content (r= 0.39). In other words, as fat content of samples increased, overall acceptance scores also increased. Rezende et al. (2015) reported the same result in which panelists gave more scores to high fat samples.

Comparing sensory and instrumental data showed an acceptable correlation. The correlation coefficients of hardness and melting variables were 0.88 and 0.70, respectively. Mahdavian and Mazahei (2015) also found high correlation between sensory and instrumental results in chocolate enriched with silver skin of coffee.

Table 5- Sensory characteristics of the chocolate samples

Sample	Overall acceptance	Mouth coating	Casein Flavor	Bitterness	Melting rate	Hardness	Color
1	$5.57\pm0.14^{Aa\lambda1}$	$5.11\pm0.40^{Aa\lambda1}$	$4.33\pm0.21^{Aa\lambda1}$	$5.16\pm0.25^{Aa\lambda1}$	$6.02\pm0.14^{Aa\lambda1}$	$5.38\pm0.26^{Aa\lambda1}$	$6.67\pm0.19^{Aa\lambda1}$
2	$5.32\pm0.27^{Aa\lambda1}$	$5.13\pm0.77^{Aa\lambda1}$	$5.44\pm0.60^{Ab\lambda1}$	$5.22\pm0.17^{Aab\lambda12}$	$6.07\pm0.60^{Aa\lambda1}$	$5.47\pm0.17^{Aab\lambda12}$	$6.46\pm0.87^{Aa\lambda1}$
3	$5.38\pm0.21^{Aa\lambda1}$	$5.15\pm0.30^{Aa\lambda1}$	$5.58\pm0.19^{Ab\lambda1}$	$5.40\pm0.16^{Ab\lambda2}$	$6.18\pm0.16^{Aa\lambda1}$	$5.45\pm0.19^{Ab\lambda2}$	$6.32\pm0.05^{Ab\lambda1}$
4	$6.26\pm0.17^{Ba\lambda2}$	$5.74\pm0.13^{Ba\lambda2}$	$5.01\pm0.10^{ABa\lambda12}$	$5.99\pm0.37^{Ba\lambda23}$	$7.40\pm0.48^{Ba\lambda2}$	$5.42\pm0.46^{Ba\lambda2}$	$6.38\pm0.13^{Ba\lambda2}$
5	$6.70\pm0.33^{Ba\lambda2}$	$5.79\pm0.80^{Ba\lambda2}$	$5.41\pm0.38^{ABa\lambda2}$	$6.37\pm0.14^{Bab\lambda3}$	$7.53\pm0.13^{Ba\lambda2}$	$5.73\pm0.14^{Bab\lambda23}$	$6.30\pm0.18^{Ba\lambda2}$
6	$6.46\pm0.14^{Ba\lambda2}$	$5.93\pm0.18^{Ba\lambda2}$	$5.73\pm0.183^{ABb\lambda2}$	$6.39\pm0.12^{Bb\lambda3}$	$7.36\pm0.31^{Ba\lambda2}$	$5.70\pm0.10^{Bb\lambda3}$	$6.27\pm0.34^{Bb\lambda2}$
7	$6.67\pm0.31^{Ca\lambda3}$	$6.04\pm0.33^{Ca\lambda3}$	$4.76\pm0.69^{Ba\lambda23}$	$6.38\pm0.21^{Ca\lambda4}$	$7.63\pm0.15^{Ca\lambda3}$	$6.14\pm0.69^{Ca\lambda4}$	$6.34\pm0.10^{Ca\lambda3}$
8	$6.45\pm0.22^{Ca\lambda3}$	$5.92\pm0.40^{Ca\lambda3}$	$5.78\pm0.27^{Bb\lambda3}$	$6.16\pm0.38^{Cab\lambda4}$	$7.57\pm0.10^{Ca\lambda3}$	$6.13\pm0.15^{Cab\lambda4}$	$6.23\pm0.15^{Ca\lambda3}$
9	$6.36\pm0.75^{Ca\lambda3}$	$5.96\pm0.14^{Ca\lambda3}$	$5.89\pm0.14^{Bb\lambda3}$	$6.47\pm0.12^{Cb\lambda4}$	$7.08\pm13^{Ca\lambda3}$	$6.21\pm0.07^{Cb\lambda4}$	$5.83\pm0.24^{Cb\lambda3}$

*Means± standard deviations from analysis, means within same column with different letters are significantly different ($P< 0.05$) from each other for each measured parameter. Uppercase and lowercase letters represent the comparison within the columns in CBS and sodium caseinate content, respectively. The letter λ shows the cocoa powder variable.

Principal Component Analysis (PCA)

In this study, principal component analysis (PCA) was applied to determine the most effective sensory parameters. The component 1 and component 2 had the most variance of 4.13 and 1.54, respectively. These two components could explain about 81% of total variance (eigenvalue >1). Also, Kaiser-Meyer-Olkin index (KMO) was 0.79 which showed sufficiency of analysis.

According to Table 6, as coefficient of each variable became more, the variable was more effective. Melting, bitterness, mouth-coating, and overall acceptance are the most effective parameters in PC1 and color and casein taste were the most effective variables for PC2.

The parameters which were close to each other, had strongly positive relationship. We observed that the hardness, mouth-coating, bitterness, melting an overall acceptance had propensity to be inversely proportional to the color parameter. Due to closer differential of hardness, bitterness, mouth-coating, and melting properties to overall acceptance, they were the most effective parameters for panelist’s overall acceptance.

Dewi et al. (2020) investigated the sensory and instrumental properties of sugar free chocolate samples. They declared that particle size distribution, melting, hardness, luminance (L*) and b* were strongly related to each other and inversely related to moisture and a*. Yeganeh Zad (2012) found that the first three components consisted 94% of total variance. Also, the sweetness, milk taste, hardness, melting, and then bitterness, soy taste, PSD, mouth-coating and at last color were the most effective variables in PC1, PC2 and PC3, respectively

Table 6- Rotated component matrix of first two main components

Sensory Parameter	Component	
	1	2
Color	0.21	0.91
Hardness	0.57	0.60
Melting Rate	0.91	0.24
Bitterness	0.86	0.42
Casein Taste	0.05	0.76
Mouth-Coating	0.84	0.39
Overall Acceptance	0.83	0.37

Selection of the best sample

According to the results, the moisture and a_w of all samples were in acceptable range. The peak temperature and the hardness of samples containing 30 and 35% CBS were closer to defined amount. Also, the overall acceptance showed that samples 5 and 7 had the highest score. By considering these results, sample 5 was the best among other samples.

Calorie amount

Sample 5 contained about 48.13 g carbohydrate, 10.38 g protein and 35.93 g fat. According to Table 7, the total calorie for 100 g of this sample is calculated as follows:

Consuming 100 g of this chocolate can supply 27% of daily calorie of an adult person (assuming 2000 kcal per day for adults). Consuming this 100 g can also supply 17% and 40% of classic and atkins keto diet.

Table 7- Total calorie of sample 5 (35% CBS and 5% sodium caseinate)

Macronutrient	Total amount (g)	Total calorie (kcal)
Fat	35.93	-
Protein	10.38	-
Carbohydrate	48.13	-
Total calorie	-	547.41

Total sugar amount

As mentioned before, the commercial chocolate contains 40- 50% sugar which is not appropriate for ketogenic diet. Reducing sugar content of optimized sample was 2.17%. There is no official standard about legal percent of reducing sugar in keto chocolate products. Famous keto-chocolate brands have at most 3 g reducing sugar in 100 g chocolate.

Peroxide value

The Chocolates may be affected by lipid oxidation. Oxygen which comes from conching process can interact with triglycerides. As shelf life of chocolate is increased, there would be more time for their interaction. Then, aldehyde and ketones are produced and the special odor would be sensed. Measuring peroxide value during shelf life of products help producers to investigate the quality (Williamson, 1998). According to Figure 4, the more shelf time led to more peroxide value. Also, peroxide value was acceptable even after two months. Pandey et al. (2010) and Selamat (1998) reported the same result.

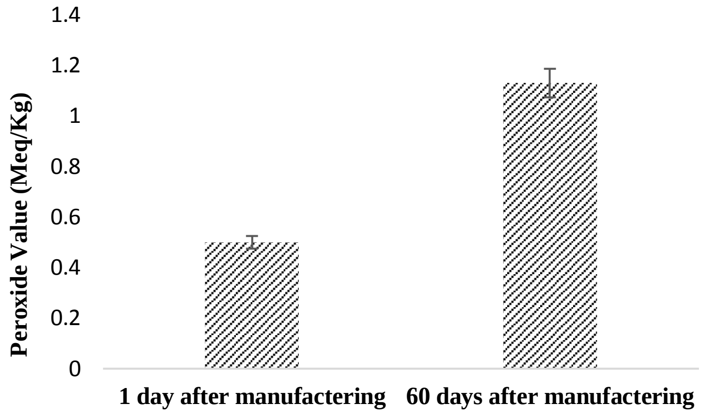

Fig. 4. Peroxide value of optimized chocolate sample

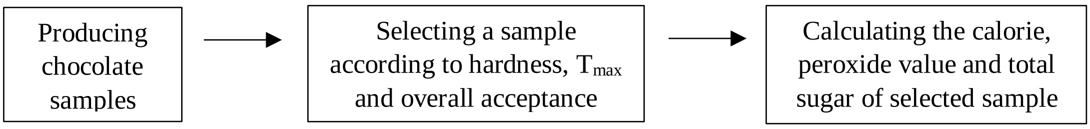

Fig. 5. Configuration map for choosing the best sample

Conclusions

There is a challenge in producing a portion compatible to ketogenic diet with sufficient satiety. This matter led us to produce sugar free chocolate by increasing total fat and protein for ketogenic diet. Increasing both total fat and protein significantly affected all physicochemical and sensory parameters. High fat chocolate samples were softer and easily melted. Panelists gave more scores to sample which contained 35% CBS and 5% sodium caseinate. There was a suitable correlation between sensory and instrumental data. Also, PCA showed a great relationship between overall acceptance of chocolate with hardness, bitterness, mouth-coating and melting properties. The optimized sample was selected by overall acceptance of hedonic test and also by comparing textural and thermal parameters to reliable references. Sugar and calorie content of optimized sample were close to commercial keto-chocolate. The optimized sample could be kept for two months with acceptable peroxide value. These results showed the possibility of producing chocolate which is compatible to ketogenic diet. However, clinical assessment of optimized sample should be done in future researches.

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بهینه‌یابی فرآورده شکلاتی تلخ بدون شکر سازگار با رژیم کتوژنیک و بررسی خصوصیات فیزیکوشیمیایی، بافتی، حرارتی و حسی آن

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چکیده

یکی از چالش‌های طراحی رژیم کتوژنیک، حفظ مقدار کالری عمده هر وعده از چربی و همچنین حفظ مقدار پروتئین مناسب جهت حس سیری می‌باشد. این تحقیق جهت امکان‌سنجی تولید شکلات بدون شکر و با مقدار چربی و پروتئین بالا در مقدار ثابت فیبر سویا و پودر استویا برای افراد ملزم به رژیم کتوژنیک صورت گرفت. پودر کاکائو توسط جایگزین کره کاکائو و سدیم کازئینات هر کدام در سطوح (صفر، ۵ و ۱۰ درصد) جایگزین شد. نتایج نشان داد که جایگزینی پودر کاکائو به‌طور معنی‌داری ($p< ۰/۰۵$) موجب افزایش رطوبت، فعالیت آبی، مقدار چربی و پروتئین کل شد اما مقدار خاکستر و کربوهیدرات کاهش یافت. همچنین افزایش مقدار جایگزین کره کاکائو و سدیم کازئینات به‌طور معنی‌داری ($p< ۰/۰۵$) موجب شد تا همزمان سختی و دمای ذوب نمونه‌های شکلات کاهش یابد. نتایج آزمون هدونیک نشان داد که نمونه‌های با چربی و پروتئین بیشتر، امتیاز بالاتری را کسب کردند. همچنین تجزیه به روش مولفه‌های اصلی نشان داد که دو مولفه اصلی اول ۸۱ درصد کل واریانس داده‌های حسی را تشکیل می‌دهند. در نهایت، با توجه به نتایج آزمون‌های حسی و دستگاهی، بهترین ترکیب شکلات انتخاب شد. این ترکیب حاوی ۳۵ درصد جایگزین کره کاکائو و ۵ درصد سدیم کازئینات بود. مقدار قند کل و کالری نمونه بهینه به‌ترتیب ۲/۱۷ درصد و ۵۴۷/۴۱ کیلوکالری به ازای ۱۰۰ گرم بود. مقدار اندیس پراکسید ۱ روز پس از تولید ۰/۵ میلی‌اکی‌والان در کیلوگرم بود که پس از ۶۰ روز این مقدار به ۱/۱۳ میلی‌اکی‌والان در کیلوگرم افزایش یافت. مصرف ۱۰۰ گرم نمونه بهینه می‌تواند ۲۷ درصد کالری روزانه یک فرد بزرگسال را تامین کند. این نتایج نشان داد که فرمولاسیون بهینه، حداقل شرایط رژیم کتوژنیک را داراست و می‌تواند یک میان وعده مناسب برای افراد ملزم به این رژیم باشد.

واژه‌های کلیدی: بدون شکر، شکلات، رژیم کتوژنیک، استویا.

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