

Development of reduced-fat and reduced-energy dark chocolate using collagen hydrolysate as cocoa butter replacement agent

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Summary

Dark chocolate contains high content of fat, which is directly related to cardiovascular diseases. A recent tendency in the food market is to offer foodstuffs with lower fat contents. So the effects of collagen hydrolysate (50–100 g·kg⁻¹) on the chemical, rheological and sensory characteristics of 372.2 g·kg⁻¹ fat dark chocolates were investigated by replacing varying levels (150–300 g·kg⁻¹) of cocoa butter (CB). Incorporation of collagen hydrolysate increased protein and moisture contents, but water activity and sensory characteristics did not show any significant difference. The amounts of hardness, thixotropy, plastic viscosity and apparent viscosity of the dark chocolate increased with the decrease of its fat content ($p < 0.05$). The yield stress had increasing trend with increase in the replacement percentage. Accordingly, it can be said that the substitution of CB not only influenced the rheological parameters, but also had effect on the mathematical fitting model. The chocolate formula with up to 150 g·kg⁻¹ replacement (D₁), containing 50 g·kg⁻¹ collagen hydrolysate and 280 g·kg⁻¹ CB, and the chocolate formula with up to 200 g·kg⁻¹ replacement (D₂), containing 66 g·kg⁻¹ collagen hydrolysate and 264 g·kg⁻¹ CB, indicated the least difference with the control. In this study, the best model determined was Herschel-Bulkley's model.

Keywords

dark chocolate; reduced-fat; reduced-energy; collagen hydrolysate; rheology; fat replacer

Dark chocolate is a dense suspension of solid particles, on average 650–750 g·kg⁻¹ saccharose, and non-fat cocoa solids dispersed in a fat continuous phase, mostly of cocoa butter (CB) [1]. Unlike high lipid and saccharose contents, chocolate consumption offers a contribution to human nutrition by means of antioxidants, mainly polyphenols and flavonoids such as epicatechin, catechin and procyanidins. There is a positive relation between the consumption of dark chocolate and consumer's health, since cocoa flavonoids could reduce blood pressure, decrease a type of low-density lipoprotein cholesterol and improve endothelial function [2]. However, dark chocolate has a high content of fat, which increases the risk of cardiovascular diseases. There is a tendency in the food market to offer the consumers low-fat foodstuffs [3]. The use of reduced-fat or non-fat

foods in preference to regular-fat foods could result in the reduction of fat intake and, consequently, loss of body weight [4]. The reduction of fat in chocolate is a difficult task, as CB plays an important role in the quality and processing of chocolate. The challenge is to produce reduced-fat alternatives with physical and sensory attributes that resemble as closely as possible the full-fat standard products and, at the same time, do not change the processing conditions [5].

LEE et al. [6] used β -glucan-rich hydrocolloid (C-trim30) as a CB substitute to evaluate its effect on the rheological and tribological properties of chocolate. They found that the Casson viscosity and yield stress were augmented with increasing content of C-trim30 in the chocolate. Moreover, substitution of CB with C-trim30 produced chocolates with softer texture [6]. ABBASI and FARZAN-

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MEHR [7], studying the possibility of fat reduction using inulin at three different levels (50, 100 and 200 g·kg⁻¹), found that by fat reduction, the moisture content of chocolates was decreased, while the hardness of the samples was increased. Besides this, the results of sensory evaluation did not show any significant difference [7].

DE MELO et al. [8] determined the sensory properties and acceptability of conventional diabetic (with no sugar), and diabetic/reduced-energy milk (no sugar and 25% energy reduction) chocolates containing high intensity sweeteners, sucralose and stevioside, and partial fat replacement with whey protein concentrate. There was no significant difference between the conventional diabetic and diabetic/reduced energy milk chocolates in brightness, cocoa aroma, CB aroma and cocoa flavour [8].

As already mentioned, chocolate is a product with a high nutritious and energy value and a high total fat content. Collagen hydrolysate Instant Gel Schoko (IGS, Gelita, Cotia, Brazil) may be used to reduce these values [9]. IGS can replace part of CB and minimize the impacts on chocolate viscosity, moisture, taste and mouthfeel [2].

The purpose of this study was to determine the effect of adding IGS, as a partial replacement of CB, on chemical, rheological and sensory characteristics of manufactured chocolates, and also to find the optimum level of replacement.

MATERIALS AND METHODS

The ingredients including alkalized cocoa powder (Schokinag, Mannheim, Germany), CB (Altinmarka, Istanbul, Turkey), IGS (Gelita, Cotia, Brazil), lecithin (Cargill, Kuala Lumpur, Malaysia), polyglycerol polyricinoleate (Beldem, Bijgaarde, Belgium), saccharose (Jahan, Karaj, Iran) and vanillin (International Flavors and Fragrances, Hilversum, Netherlands) were used for formulating the dark chocolate. Tab. 1 shows the composition of the raw materials used in the study.

Dark chocolate preparation

For preparation of dark chocolate samples, CB (the amount was calculated regarding the replacement levels of 150, 200, 250 and 300 g·kg⁻¹) was primarily melted in an oven at 60 °C. Other ingredients including cocoa powder, IGS (the amount was calculated according to different levels of replacement), saccharose, lecithin, polyglycerol polyricinoleate and vanillin were weighed according to the relevant formulation. All ingredients (except vanillin, which was added in the last step) were poured into the ball mill (CAO-B5; Caotech, Wormerveer, Netherlands), which is a shot mill that does both refining and conching processes together. This apparatus is capable of producing 2 kg chocolate and its tank contains about 25 kg stainless steel shots with 8 mm diameter. The temperature of the apparatus was set at 50 °C and then refining and conching processes were done using mixer at 60 × g for 2 h.

Afterwards, the chocolate samples were vacated and then tempering and moulding were done. In order to temper, temperature of the samples was decreased from 50 °C (melting to completion) to 32 °C (cooling to point of crystallization) and subsequently, it was decreased from 32 °C to 28.5 °C (crystallization) during 15 min. Then, the temperature was maintained steady at 28.5 °C for 10 min. After all, chocolates were casted into polycarbonate moulds and were kept cold at 5 °C in refrigerator. The moulded chocolates after packaging in aluminium foil were stored in refrigerator until the analysis. The control chocolate containing CB, without the addition of fat replacer, was prepared in the same manner. The amount of all ingredients, except for CB and IGS, remained constant. The formulae are shown in Tab. 2.

Chemical analysis, water activity and energy value

Moisture, lipids, protein and ash contents of the chocolates were measured using AOAC methods [10–13]. Water activity (a_w) of chocolate samples was determined using Novasina Labmaster apparatus (Novasina, Lachen, Switzerland).

Tab. 1. Proximate composition and pH of raw materials.

Raw material	Lipids [g·kg ⁻¹]	Moisture [g·kg ⁻¹]	Protein [g·kg ⁻¹]	Ash [g·kg ⁻¹]	pH
Cocoa powder	110	68.2	18.7	127.6	8
Skim milk powder	15	41.3	300	81	6.5
IGS	–	95.5	910	13.3	5.8

IGS – collagen hydrolysate (Instant Gel Schoko).

Tab. 2. Quantities of the ingredients used in the formulation of the reduced-fat and reduced-energy dark chocolates.

Formula	Ingredients [g·kg ⁻¹]						
	CB	IGS	Saccharose	Cocoa powder	Lecithin	PGPR	Vanillin
Control	330	0	375	300	7	5	1
D ₁	280	50	375	300	7	5	1
D ₂	264	66	375	300	7	5	1
D ₃	247.5	82.5	375	300	7	5	1
D ₄	230	100	375	300	7	5	1

Conventional Iranian-style dark chocolate was used as control.

D₁, D₂, D₃, D₄ – reduced-fat and reduced-energy dark chocolate formulae with varying levels of cocoa butter (150, 200, 250 and 300 g·kg⁻¹) replaced by equal amounts of IGS.

CB – cocoa butter, IGS – collagen hydrolysate (Instant Gel Schoko), PGPR – polyglycerol polyricinoleate.

Total energy values were calculated in relation to kilogram of the chocolates using the Atwater values [14] and expressed in joules. The energy value factors for lipids, protein and saccharides were calculated on the basis of EU Directive 90/496 [15].

Hardness

Hardness measurement was conducted using texture analyser Hounsfield-H5K5 (Hounsfield, Redhill, United Kingdom). Chocolate bars (dimensions 10 × 20 × 100 mm) were prepared and then stored in refrigerating incubator at 20 °C for 6 h. Based on several experiments with different probes, penetration probe number 1.6 mm was chosen, and the measurement was done at a speed of 1.5 mm·s⁻¹ in the depth of 6 mm. Maximum measured force was reported as the hardness index.

Rheological properties

In order to gain access to the best model for evaluating the rheological behaviour of the dark chocolate samples, their shear stress versus shear rate data were fitted with some well-known mathematical models including Bingham (Eq. 1), Casson (Eq. 2), Power Law (Eq. 3) and Herschel-Bulkley (Eq. 4):

$$\tau = \tau_0 + \eta_p \dot{\gamma} \quad (1)$$

$$\tau^{0.5} = \tau_0^{0.5} + k_1 \dot{\gamma}^{0.5} \quad (2)$$

$$\tau = k_2 \dot{\gamma}^{n_2} \quad (3)$$

$$\tau = \tau_0 + \eta_p \dot{\gamma}^{n_1} \quad (4)$$

where, τ is shear stress (in pascals), η_p is plastic viscosity (in pascalseconds), $\dot{\gamma}$ is shear rate (in reciprocal seconds), τ_0 is yield stress (in pascals), k_1 is Casson viscosity (in pascalseconds), k_2 is con-

sistency coefficient (in pascalseconds) and n is flow behaviour index (dimensionless).

The fitting of experimental data with models was evaluated on the basis of the coefficient of determination (R^2) value. Based on the statistical calculations, the Herschel-Bulkley model was best fitting for all chocolate formulations due to providing the highest R^2 . So all rheological properties (plastic viscosity, yield stress and flow behaviour index) were calculated using the Herschel-Bulkley's model.

Rheological measurements were performed by a Physica MCR301 rheometer (Anton Paar, Graz, Austria) using a double gap concentric cylinder geometry with a radius ratio of 1.035, internal gap of 0.43 mm, external gap of 0.47 mm and a gap of 1 mm between the rotational cylinder and the bottom of the cup. The temperature control was carried out with a Peltier plate system equipped with fluid circulator (Anton Paar). In order to melt the chocolate samples, they were incubated at 50 °C for 75 min. Then they were transferred to a cup and cooled down to 40 °C.

The measuring profile consisted of 4 intervals:

- pre-shearing for 500 s at 5 s⁻¹ to homogenize and control the temperature of the chocolate samples,
- shear rate ramp of 2 s⁻¹ to 50 s⁻¹ with 18 points in 180 s,
- constant shearing at 50 s⁻¹ for 60 s and
- shear rate ramp of 50 s⁻¹ to 2 s⁻¹, duration 180 s.

Mathematical models including Bingham, Casson, Ostwald-de Waele and Herschel-Bulkley were fitted to the collected data. The data were analysed using Rheoplus/32 V3.21 software (Anton Paar). The best fitted model was selected by statistical analysis based on the R^2 value. Moreover, the rheological parameters including viscosity and yield stress values of the selected

models were calculated. Other rheological parameters, such as apparent viscosity and thixotropy, were obtained from the data. Viscosity at a shear rate of 40 s^{-1} was considered as apparent viscosity, and the difference of viscosity during the ramp up and down represented thixotropy.

Sensory analysis

Chocolate samples were labeled with three-digit codes and, along with the questionnaire, were given to 15 experienced assessors who were staff of the Rezvan Chocolate Company (Karaj, Iran) with the ages ranging from 20 to 47 years. The assessors were asked to state their judgment of the samples individually in separate booths. All of the samples were evaluated for sensory characteristics such as colour, taste, mouthfeel, sweetness, hardness and texture using the ranking method (5 for liking extremely and 1 for disliking extremely). The order of presentation of the plates to each assessor was different in a random sequence. Bottled drinking water of room temperature was also provided to clean the palate between the assessments of each of the samples.

The assessors were also asked to fill another questionnaire for overall acceptability based on 5-point hedonic scale that ranged from very good quality to undesirable. The assessors recorded their responses on an evaluation sheet designed to indicate the score of the sample of each formula.

Statistical analysis

The research was done in triplicate and all determinations were carried out in duplicate. Data were analysed by one-way analysis of variance (ANOVA) test using SPSS 17 statistical software (SPSS, Chicago, Illinois, USA). Tukey's test

was used to determine the statistically significant differences among the means. The data obtained from sensory evaluation were subjected to Kruskal-Wallis test. The Mann-Whitney's U test was used to determine the statistical significance between the means. A 95% ($p < 0.05$) significance level was considered in all comparisons.

RESULTS AND DISCUSSION

Proximate composition

Tab. 3 lists the chemical composition and the data obtained on fat reduction, total energy and energy reduction of the manufactured chocolates. Lipids in chocolate (CB) were replaced with different levels of IGS. As a result, fat content was reduced and protein content (with regard to the protein-based nature of IGS) was increased. D₁ and D₂ yielded a fat reduction of 8.7% and 11.1%, respectively. The control had the highest level of total energy owing to its higher fat content. D₄ had lower total energy content and higher energy reduction percentage than the other formulae. This was due to the higher level of fat reduction in D₄. The total energy content of D₁ and D₂ was decreased significantly with the decrease of fat level ($p < 0.05$); for example, the decrease in fat content of 8.7% in D₁ led to a significant decrease in the energy value by 27.2% ($p < 0.05$) and the decrease in fat content of 11.1% in D₂ led to a significant decrease in the energy value by 35.8% ($p < 0.05$).

There were no significant differences in ash content among the formulae, indicating that these values were not affected by adding the IGS. The ash content also showed no significant increase in

Tab. 3. Chemical composition of the reduced-fat and reduced-energy dark chocolates.

Chemical composition	Formula				
	Control	D ₁	D ₂	D ₃	D ₄
Lipids [g·kg ⁻¹]	372.2 ± 13.6 ^a	339.9 ± 7 ^b	330.7 ± 9.9 ^b	307.2 ± 1.3 ^c	296.5 ± 15.3 ^c
Fat reduction [%]	–	8.7	11.1	17.5	20.3
Protein [g·kg ⁻¹]	66.2 ± 3 ^a	117.3 ± 4.4 ^b	121 ± 7.6 ^b	135.9 ± 18 ^{bc}	161.4 ± 0.7 ^c
Total energy [kJ·kg ⁻¹]	20666.8 ± 175.8 ^a	19530.2 ± 163.3 ^b	19166.3 ± 142.3 ^c	18791.2 ± 171.6 ^d	18393.4 ± 113.1 ^e
Energy reduction [%]	–	27.2	35.8	44.8	54.3
Protein increase [%]	–	77	82	105	143
Ash [g·kg ⁻¹]	33.6 ± 0.3 ^a	34.1 ± 0.1 ^a	34.3 ± 0.7 ^a	34.7 ± 1.7 ^a	34.9 ± 0.4 ^a
Moisture [g·kg ⁻¹]	15.8 ± 0.8 ^a	19.9 ± 2.1 ^{ab}	20.1 ± 2.5 ^{ab}	20.8 ± 0.3 ^b	22.7 ± 0.3 ^b
Water activity	0.26 ± 0.01 ^a	0.25 ± 0.01 ^a	0.25 ± 0.01 ^a	0.26 ± 0.01 ^a	0.26 ± 0.01 ^a

Variation of the means represents the standard deviations of duplicates for each treatment (six repetitions for each formula). Means in the same row (different formulae) with different letters are significantly different ($p < 0.05$).

any of the formulae with decreased levels of CB.

The chocolate formula with up to 300 g·kg⁻¹ replacement (D₄), containing 100 g·kg⁻¹ IGS and 230 g·kg⁻¹ CB, had the highest moisture content as compared with other formulae ($p < 0.05$); this could be related to the highest amount of IGS as a fat replacer and more moisture absorption by this component. Molten chocolate as a non-Newtonian fluid with an apparent yield stress [16] and a dense blend of phospholipid-coated saccharose and cocoa particles in liquid fat [17] typically has the moisture content of 5–15 g·kg⁻¹, mainly in the cocoa solids, which does not affect chocolate flow [18].

The increase in the moisture content of the chocolates could be due to the use of ball mill apparatus that does refining and conching operations together within a period of 2 h. Conching is the final operation of producing chocolate (both dark and milk chocolate) that eliminates the majority of the moisture from the product, being conducted at temperatures higher than 50 °C for at least 12 h. FARZANMEHR and ABBASI [19] added inulin, polydextrose and maltodextrin to low-sugar milk chocolate and reported the lowest value for moisture content in the samples with moderate polydextrose and maltodextrin ratios [19]. In the present study, the chocolate formulae containing high levels of IGS had higher moisture contents [7].

Water activity a_w is one of the factors that influences the growth of microorganisms and has great importance in preserving foods. Food stability, safety and other properties can be predicted on the basis of a_w more reliably than on the basis of the water content. As shown in Tab. 3, there were no significant differences between a_w values of individual formulae comparing with the control. Even though the moisture content was increased in the chocolates with higher content of the replacer, a_w did not show any significant difference with the control. This trend indicates that IGS binds water molecules, which causes free water reduction. Only limited is available in the literature regarding the effects of fat reduction or fat replacer addition on a_w value of chocolates, while this is a criterion for predicting the shelf-life of chocolates.

Hardness

The hardness values of the manufactured chocolates are shown in Tab. 4. As shown, there were no significant differences between D₁ and D₂ formulae and the control. For all formulae, the hardness increased as the levels of IGS increased. Consequently, D₁ and D₄ formulae had the lowest

hardness with 8.29 N and the highest hardness with 20.29 N, respectively.

LEE et al. [6], using β -glucan-rich hydrocolloid (C-trim30) as CB replacer, reported that the hardness decreased with the increase in the content of C-trim30. They explained that the replacement of CB with C-trim30 might have influenced the tempering process, which could have resulted in softening of the texture of the chocolates [6]. AFOAKWA et al. [20, 21] reported that the rheological characteristics of dark chocolate were very strongly related to the particle size distribution, fat, lecithin contents, and their key influencing factors. AFOAKWA [18] stated that fat content was inversely related to hardness at all particle sizes as well as lecithin levels. ABBASI et al. [7] found that the use of polydextrose and maltodextrin at different levels along with sucralose as sugar substitutes in milk chocolate caused an increase in the hardness.

Rheological properties

Plastic viscosity

As shown in Tab. 5, viscosity of dark chocolate significantly ($p < 0.05$) increased with the decrease in fat content (CB). The values ranged from 16.19 Pa.s in D₁ to 59.27 Pa.s in D₄. The increased moisture content, on one hand, and continuous phase (CB) reduction and increase in the content of total solids by adding IGS, on the other hand, could assumably be the reasons for the increase in plastic viscosity of the chocolates.

The effect of fat is proportionately much higher for the plastic viscosity than the yield stress. This is not too surprising as the extra fat will add to the free fat molecules and cause the particles, when they flow, to paste each other. This free fat has a large effect on lubricating the flow when it takes place, so the plastic viscosity decreases dra-

Tab. 4. Hardness of the reduced-fat and reduced-energy dark chocolates.

Formula	Hardness [N]
Control	8.05 ± 0.25 ^a
D ₁	8.26 ± 0.23 ^a
D ₂	8.92 ± 2.51 ^a
D ₃	13.66 ± 0.19 ^b
D ₄	20.29 ± 0.48 ^c

Variation of the means represents the standard deviations of duplicates for each treatment (six repetitions for each formula).

Means with different letters are significantly different ($p < 0.05$).

Tab. 5. Herschel-Bulkley's parameters of the reduced-fat and reduced-energy dark chocolates.

Formula	Plastic viscosity [Pa·s]	Yield stress [Pa]	Flow behaviour index
Control	3.43 ± 0.19 ^a	28.29 ± 1.28 ^a	0.88 ± 0.01 ^a
D ₁	16.19 ± 0.35 ^b	83.98 ± 2.26 ^{cd}	0.9 ± 0.32 ^a
D ₂	19.75 ± 1.64 ^c	69.73 ± 0.36 ^b	0.86 ± 0.83 ^a
D ₃	33.65 ± 0.9 ^d	92.55 ± 0.8 ^d	0.81 ± 0.006 ^a
D ₄	59.27 ± 0.05 ^e	79.78 ± 5.58 ^{bc}	0.82 ± 0.12 ^a

Variation of the means represents the standard deviations of duplicates for each treatment (six repetitions for each formula). Means in the same column (different formulae) with different letters are significantly different ($p < 0.05$).

matically [22]. Similar observations were made by LEE et al. [6]. They found that viscosity increased as CB was replaced by C-trim30.

Yield stress

Tab. 5 shows that the yield stress of the chocolate samples with different levels of IGS as a fat replacer were significantly different from the control ($p < 0.05$). The lowest and the highest levels of yield stress were related to D₂ and D₃, chocolate formulae with up to 250 g·kg⁻¹ replacement containing 82.5 g·kg⁻¹ IGS and 247.5 g·kg⁻¹ CB, respectively. Yield stress is affected by particle-particle interaction, the amount and specific surface area of the particles, emulsifiers and moisture. The trend observed for yield stress might be the result of high moisture content, particularly in the samples with high levels of IGS that caused increased content of total solids together with continuous fat phase (CB) reduction.

LEE et al. reported the increased Casson yield stress with increasing content of C-trim30 in the chocolates [6], which can be attributed to increased interactions between the solid particles due to replacement of CB by C-trim30.

Flow behaviour index

The results of the effect of CB replacement by IGS are presented in Tab. 5. As shown, there was no significant difference between the samples with different levels of CB replacement by IGS and the control. Flow behaviour index of all chocolate samples was in the range of 0.81–0.9, indicating the expected shear thinning behaviour of the chocolates.

Apparent viscosity

Apparent viscosity values were determined at 40 s⁻¹ shear rate (Tab. 6). SERVAIS et al. [23] noted that apparent viscosity could be represented by the value of viscosity at 30 s⁻¹, 40 s⁻¹ or 50 s⁻¹ depending on the product; however, he recommended the viscosity value of 40 s⁻¹ to represent the apparent

viscosity through relative reproducibility. In this study, the shear rate of 40 s⁻¹ was used to represent the apparent viscosity as obtainable from all formulations. As shown in Tab. 6, D₁ and D₂ formulae did not show any significant difference with each other unlike the rest of the formulations.

As the amount of IGS increased, the apparent viscosity increased substantially. The values of apparent viscosity were the same as the plastic viscosity results. AFOAKWA et al. reported that the trends of apparent viscosity were similar with those of Casson plastic viscosity [1]. Increasing of fat content had similar inverse relation with the apparent viscosity but this relation was less significant for 300 g·kg⁻¹ and 350 g·kg⁻¹ fat contents at all particle sizes. Also increasing of lecithin content from 3 g·kg⁻¹ to 5 g·kg⁻¹ caused further reduction in apparent viscosity for all particle sizes and fat contents. They found that the influence of fat and lecithin contents was more dependent on the apparent viscosity of dark chocolates [1]. Similar observations were made in the present study.

Thixotropy

Thixotropy occurs when apparent viscosity or shear stress decreases with the time of shear at

Tab. 6. Apparent viscosity and thixotropy of the reduced-fat and reduced-energy dark chocolates.

Formula	Apparent viscosity [Pa·s]	Thixotropy [Pa·s ⁻¹]
Control	6.75 ± 0.22 ^d	273.93
D ₁	12.93 ± 0.16 ^a	1785.42
D ₂	13.02 ± 0.4 ^a	2142.62
D ₃	19.27 ± 0.55 ^b	3897.11
D ₄	26.81 ± 1.02 ^c	6451.55

Variation of the means represents the standard deviations of duplicates for each treatment (six repetitions for each formula).

Means in the same column (different formulae) with different letters are significantly different ($p < 0.05$).

Tab. 7. Sensory characterization of the reduced-fat and reduced-energy dark chocolates.

Formula	Colour	Taste	Mouthfeel	Sweetness	Hardness	Texture
Control	3.87 ± 1.06 ^a	3.40 ± 1.30 ^a	3.60 ± 1.12 ^a	4.33 ± 0.99 ^a	3.87 ± 0.91 ^a	4.33 ± 0.90 ^a
D ₁	4.53 ± 0.64 ^a	3.67 ± 1.23 ^a	3.07 ± 1.10 ^a	3.47 ± 1.12 ^a	3.40 ± 1.24 ^a	3.73 ± 1.33 ^a
D ₂	4.60 ± 0.63 ^a	3.47 ± 0.99 ^a	3.33 ± 1.23 ^a	3.20 ± 1.01 ^a	3.53 ± 1.06 ^a	3.93 ± 1.16 ^a
D ₃	4.20 ± 0.86 ^a	3.33 ± 1.23 ^a	2.73 ± 1.16 ^a	3.33 ± 1.05 ^a	3.13 ± 1.19 ^a	3.64 ± 1.07 ^a
D ₄	4.09 ± 1.25 ^a	2.93 ± 1.33 ^a	3.09 ± 1.07 ^a	3.27 ± 1.16 ^a	3.27 ± 1.33 ^a	3.60 ± 1.06 ^a

Values are the mean ± standard deviation of ranking of each formula by 15 assessors (15 repetitions for each formula). Means in the same column (different formulae) with different letters are significantly different ($p < 0.05$).

a constant rate [18, 24]. The permanent decrease in apparent viscosity through shearing, and the following recovery of shear stress or apparent viscosity when the flow is ceased, produces a hysteresis loop. Thixotropy is counted from the area of loop or a specific point on the ramp curves of shear stress or apparent viscosity at a specific shear rate of usually 5 s⁻¹ or 40 s⁻¹. A specialized method has still to be denoted but a well conched chocolate should not be thixotropic. Difference between the yield stresses measured at a shear of 5 s⁻¹ during the ramp up and down in shear was used to represent thixotropy [1, 23].

Thixotropic behaviour was observed in all samples and there was a hysteresis loop between the shear stress increase and decrease diagrams. This function is one of the time-dependent thixotropic fluid properties. As can be seen in Tab. 6, CB reduction and its partial replacement by IGS had great effect on thixotropy at all replacement levels, in particular at the highest one (D₄). The main reason for the increase in hysteresis in the present study can be related to fat content reduction.

AFOAKWA et al. found that particle size distribution as well as fat and lecithin contents had significant effects on thixotropy [1, 20]; however, this observation was only made on the low-fat dark chocolate (250 g·kg⁻¹) [1]. The samples containing 300 g·kg⁻¹ and 350 g·kg⁻¹ fat contents showed little thixotropy, meaning that, regardless particle size distribution, and fat and lecithin contents, the chocolates of ≥300 g·kg⁻¹ fat were not thixotropic. They also reported that all 250 g·kg⁻¹ fat samples exhibited high thixotropic behaviour [1]. Finally, it is worth noting that the results of the present study are in agreement with AFOAKWA's findings even in the case of chocolates with the lowest fat content.

Sensory evaluation

As shown in Tab. 7, there was no significant difference between the samples in sensory attributes such as colour, taste, mouthfeel, sweetness, hardness and texture. Also no significant difference

Tab. 8. The scores of total acceptance of the reduced-fat and reduced-energy dark chocolates.

Formula	Overall acceptability
Control	3.47 ^a
D ₁	3.69 ^a
D ₂	3.33 ^a
D ₃	2.73 ^a
D ₄	3.60 ^a

Values of 5 and 1 were assigned to the highest and the lowest acceptances, respectively.

Values with same letter are not significantly different ($p < 0.05$).

was observed in the results of five-point hedonic scale shown in Tab. 8 and the sample D₁ showed the best acceptance in comparison with the other formulae.

Thus it is important to emphasize that, despite the results obtained from the mechanical and rheological properties that showed significant differences between the samples with IGS as a fat replacer and the control, no significant difference was observed in the sensory evaluation. Accordingly, this raises the possibility of using IGS as a fat replacer, as it may minimize the impact on sensory attributes.

FARZANMEHR et al. [19] reported similar observation. They found that the use of inulin as a fat replacer, at up to 50 g·kg⁻¹, in comparison with the control in low-energy milk chocolate, was possible without having undesirable textural and physiological effects on the product and consumers [19]. DE MELO et al. suggested that substitution of saccharose by high-intensity sweeteners, sucralose and stevioside, in conjunction with bulking agents and partial fat replacement by whey protein content, had potential as a palatable food in the formulation of diabetic/reduced-energy milk chocolates [8].

CONCLUSION

In this study, we developed reduced-fat and reduced-energy dark chocolates by replacing CB with IGS. The best results were obtained with 200 g·kg⁻¹ IGS, which resulted in improved chemical, rheological and sensorial properties of the manufactured chocolate (330.7 g·kg⁻¹ fat) as compared with the control chocolate (372.7 g·kg⁻¹ fat). The produced chocolate contained 11.1% less fat and 35.8% less energies (19166.3 ± 142.3 kJ·kg⁻¹) than the control chocolate. On the basis of these results, it is recommended that these chocolates can be consumed as an alternative to conventional chocolates. In addition, incorporation of IGS increased protein and moisture contents as the level of IGS increased, but water activity a_w did not show any significant difference in comparison with the control. The more the fat content was reduced, the more the hardness was increased.

Despite that the Casson model is recommended by the International Office of Cocoa, Chocolate and Sugar Confectionery (IOCCC), in this study, the best model was found to be Herschel-Bulkley's model. As a result, it can be said that the substitution of CB not only influenced the rheological parameters but also had effect on its mathematical fitting model. By CB replacement, the yield value of dark chocolates followed an irregular trend, as D₂ showed the lowest amount of it. Plastic viscosity, apparent viscosity and thixotropy showed increasing trends with decreasing of the CB content.

Acknowledgements

The data were obtained from the results of an approved research project (P/25/47/1036) of the National Nutrition and Food Technology Research Institute, Tehran, Iran. The authors would like to thank the Rezvan Chocolate Company (Karaj, Iran) for supplying the raw materials and technical help in the chocolate manufacturing process.

REFERENCES

1. Afoakwa, E. O. – Paterson, A. – Fowler, M.: Effects of particle size distribution and composition on rheological properties of dark chocolate. *European Food Research and Technology*, 226, 2008, pp. 1259–1268.
2. Shoorideh, M. – Taslimi, A. – Azizi, M. H. – Mohammadifar, M. A.: Study of effects of D-tagatose and inulin as sugar substitutes on the physical, chemical and rheological properties of milk chocolate. *Iranian Journal of Food Science and Technology*, 8, 2011, pp. 113–125.
3. Do, T. A. L. – Mitchell, J. R. – Wolf, B. – Vieira, J.: Use of ethylcellulose polymers as stabilizer in fat-based food suspensions examined on the example of model reduced-fat chocolate. *Reactive and Functional Polymers*, 70, 2010, pp. 856–862.
4. Kahkonen, P. – Tuorila, H.: Consumer responses to reduced and regular fat content in different products: effects of gender, involvement and health concern. *Food Quality and Preference*, 10, 1999, pp. 83–91.
5. Wang, H. X. – Wu, H. – Ho, C. T. – Weng, X. C.: Cocoa butter equivalent from enzymatic interesterification of tea seed oil and fatty acid methyl esters. *Food Chemistry*, 97, 2006, pp. 661–665.
6. Lee, S. – Biresaw, G. – Kinney, M. P. – Inglett, G. E.: Effect of cocoa butter replacement with a β -glucan-rich hydrocolloid (C-trim30) on the rheological and tribological properties of chocolates. *Journal of the Science of Food and Agriculture*, 89, 2009, pp. 163–167.
7. Abbasi, S. – Farzanmehr, H.: Optimization of the formulation of prebiotic milk chocolate based on rheological properties. *Food Technology and Biotechnology*, 47, 2009, pp. 396–403.
8. de Melo, L. L. M. M. – Bolini, H. M. A. – Efraim, P.: Sensory profile, acceptability, and their relationship for diabetic/reduced calorie chocolates. *Food Quality and Preference*, 20, 2009, pp. 138–143.
9. Chocolate with less fat. More than evolution, it's innovation. *Food Marketing and Technology*, 21, 2007, pp. 13–14.
10. AOAC 931-04. Moisture in cacao products (gravimetric method). In: *Official methods of analysis*. 18th ed. Gaithersburg : Association of Official Analytical Chemists, 2005. ISBN 0-935584-77-3.
11. AOAC 963-15. Fat in cacao products (Soxhlet extraction method). In: *Official methods of analysis*. 18th ed. Gaithersburg : Association of Official Analytical Chemists, 2005. ISBN 0-935584-77-3.
12. AOAC 939-02. Protein (milk) in milk chocolate (Kjeldahl method). In: *Official methods of analysis*. 18th ed. Gaithersburg : Association of Official Analytical Chemists, 2005. ISBN 0-935584-77-3.
13. AOAC 972-15. Ash of cacao products. In: *Official methods of analysis*. 18th ed. Gaithersburg : Association of Official Analytical Chemists, 2005. ISBN 0-935584-77-3.
14. Mohammadi, M. – Oghabi, F.: Development of low-fat and low-calorie beef sausage using modified starch as fat replacement agent. *Journal of The Science of Food and Agriculture*, 92, 2012, pp. 1291–1296.
15. European Council Directive 90/496/EEC of 24 September 1990 on nutrition labeling for foodstuffs. *Official Journal of the European Communities*, L 276, 1990, pp. 40–44.
16. Applications for laboratories and process control. In: *Rheotest Messgeräte Medingen* [online]. Ottendorf-Okrilla : Rheotest Messgeräte Medingen, sine dato [cited 3 May 2010]. <http://www.rheotest.de/html_en/applications.html>
17. Goncalves, E. V. – Lannes, S. C. S.: Chocolate rheology. *Ciência e Tecnologia de Alimentos*, 30, 2010, pp. 845–851.
18. Afoakwa, E. O.: *Chocolate science and technology*.

- Singapore : Wiley-Blackwell, 2010. 291 pp. ISBN 978-1-4051-9906-3.
19. Farzanmehr, H. – Abbasi, S.: Effects of inulin and bulking agents on some physicochemical, textural and sensory properties of milk chocolate. *Journal of Texture Studies*, *40*, 2009, pp. 536–553.
 20. Afoakwa, E. O. – Paterson, A. – Fowler, M. – Vieira, J.: Relationship between rheological, textural and melting properties of dark chocolate as influenced by particle size distribution and composition. *European Food Research and Technology*, *227*, 2008, pp. 1215–1223.
 21. Afoakwa, E. O. – Paterson, A. – Fowler, M. – Vieira, J.: Comparison of rheological models for determining dark chocolate viscosity. *International Journal of Food Science and Technology*, *44*, 2009, pp. 162–167.
 22. Beckett, S. T.: *The science of chocolate*. 2nd ed. Cambridge : Royal Society of Chemistry Paperbacks, 2009. 240 pp. ISBN 978-0-85404-970-7
 23. Servais, C. – Rance, H. – Roberts, I. D.: Determination of chocolate viscosity. *Journal of Texture Studies*, *34*, 2003, pp. 467–497.
 24. Chhabra, R. P.: *Bubbles, drops, and particles in non newtonian fluids*. 2nd ed. Boca Raton : CRC Press, 2007. 771 pp. ISBN 0-8247-2329-5.

Received 20 June 2013; 1st revised 21 July 2013; accepted 22 July 2013; published online 29 January 2014.