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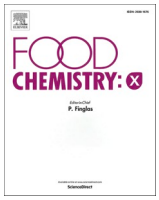

Effect of transesterified amaranth oil oleogel as a cocoa butter replacer on the physicochemical properties of dark chocolate

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ABSTRACT

In this study the possibility of replacing cocoa butter with transesterified amaranth oil oleogel (EAO; CBR 100 and CBR 40 at the concentration of 5, 10, and 15 % (*w*/w)) was evaluated. For this goal, the triacylglycerol composition of amaranth oil, cocoa butter and 2 type of replacer (monoglyceride (MG) oleogel with amaranth oil, and the combination of 40 % this oleogel with 60 % EAO) were determined. The pure oleogel (amaranth oil mixed with 10 % MG) was named cocoa butter replacer 100 (CBR 100) and 40 % of this pure oleogel mixed with 60 % enzymatically transesterified amaranth oil was named cocoa butter replacer 40 (CBR 40). Then the chocolate samples were prepared. The physicochemical properties of chocolates were measured using color and fat bloom tests, differential scanning calorimetric (DSC), X-ray diffraction and texture. Results showed that enzymatic transesterification can significantly reduce the amount of dominant triacylglycerols of cocoa butter in amaranth oil. The control cocoa butter chocolate showed higher whiteness index and the highest level of fat bloom. DSC showed that the thermal parameters were similar for the most chocolate samples, except replacer with 15 % of oleogel. The control chocolate and 5 % concentration of oleogel and EAO mixture, had more β' crystals, while chocolates containing replacers CBR 40/100 had more β type crystals, although hardness evaluation test showed no significant differences among the various types of chocolates. Accordingly, we consider the MG oleogel and its combination with EAO, especially in 5 % condensation, have potential as replacer for cocoa butter with no negative effect.

1. Introduction

Cocoa butter and sugar are considered the most important components of chocolate and contain about 70 % of the continuous phase. However, related to the special physical properties of cocoa butter in the formula such as triacylglycerol compositions and crystal form, use of it in formulations is inevitable (Simoes et al., 2021). The triacylglycerol organization plays a crucial role in melting features and oral sense of the products containing cocoa butter. On the other hand, cocoa butter and related products, such as chocolates are found in six different crystal polymorphisms known as γ, α, β'(III), β'(IV), β(V), and β(VI), which are named according to enhancement thermal stability and melting point order (Li et al., 2021). The β(V) is the best crystal form for a well-tempered chocolate, which is obtained through a controlled crystallization procedure during the tempering stage of chocolate production. In several studies it has been considered that the transition of the solid-state phase from β(V) to β(VI) is the main approved mechanism for the occurrence of fat bloom, which happens generally following a long storage time and/or temperature fluctuations (Ewens et al., 2021; Zhao & James, 2019).

The main fat of chocolate is cocoa butter and also stearic and palmitic acids contain more than 60 % of its fatty acid composition. These fatty acids are not easily absorbed in the body due to the long-chain length and their positions of 1 and 3 in the triacylglycerol molecule structure (78 % absorption versus 98 % for oleic acid). There are some problems for cocoa butter production such as high price, difficulty of cultivation, low efficiency, and pest attack (Toker et al., 2020). Nowadays, herbal and animal oils and fats are recommended for the cocoa butter replacement in the confectionery and chocolate industries.

Amaranth oil contains high levels of squalene (up to 10 %), phytosterols (2–3 %), tocopherols, carotenoids, and phospholipids (up to 10 %), which are natural organic materials involved in cholesterol metabolism and play an essential role in reducing blood cholesterol (Bozorov et al., 2018; Yarnia & Khorshidi Benam, 2017). It should be noted that the unsaturated fatty acids account for 77.1 % of total fatty acids in this oil (Bozorov et al., 2018). These studies also have shown that the high

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Table 1
Fatty acid composition of amaranth oil, MG and cocoa butter.

Samples	Monoglyceride	Amaranth oil	Cocoa butter
Palmitic acid (%)	55.4 ± 1.93[a]	6.5 ± 0.02[c]	26.8 ± 0.22[b]
Stearic acid (%)	34.9 ± 1.56[a]	3.3 ± 0.01[b]	36.3 ± 0.30[a]
Oleic acid (%)	6.0 ± 0.01[b]	30.2 ± 0.58[a]	32.7 ± 0.71[a]
Linoleic acid (%)	3.7 ± 0.00[a]	51.3 ± 1.78[b]	2.7 ± 0.00[a]

Each value represents the mean ± standard deviation of two replicates. Means (in the same column) with different letters are significantly different ($p < 0.05$). MG: Monoglyceride.

Table 2
Triacylglycerol's species in amaranth oil, cocoa butter, CBR 40 and CBR 100.

Triglyceride spices (%)	Amaranth oil	Cocoa butter	CBR 40	CBR 100
SUS				
POP	ND	19.1 ± 0.1[c]	7.9 ± 0.0[b]	2.0 ± 0.0[a]
POSt	ND	39.4 ± 0.5[c]	9.1 ± 0.1[b]	2.9 ± 0.0[a]
StOSt	ND	28.6 ± 0.1[c]	8.9 ± 0.0[b]	1.9 ± 0.0[a]
PLP	ND	0.9 ± 0.0[a]	6.6 ± 0.0[b]	15.3 ± 0.1[c]
Total	ND	88	32	22.4
SUU				
OOP	1.9 ± 0.0[a]	2.9 ± 0.0[b]	4.5 ± 0.1[c]	5.8 ± 0.0[d]
PLL	5.1 ± 0.0[a]	ND	9.3 ± 0.1[b]	10.2 ± 0.2[c]
POL	2.8 ± 0.0[b]	0.8 ± 0.0[a]	6.8 ± 0.2[c]	7.9 ± 0.1[d]
StOO	ND	3.7 ± 0.0[a]	ND	4.8 ± 0.0[b]
Total	9.8	7.4	20.6	28.7
UUU				
OOO	7.4 ± 0.0[d]	1.1 ± 0.0[a]	5.4 ± 0.0[c]	4.5 ± 0.0[b]
LLL	37.1 ± 0.0[c]	ND	18.4 ± 0.3[b]	17.5 ± 0.0[a]
LLO	25.3 ± 0.0[c]	ND	13.2 ± 0.2[b]	11.9 ± 0.1[a]
OOL	13.0 ± 0.0[c]	ND	6.9 ± 0.1[b]	5.3 ± 0.0[a]
Total	82.8	1.1	43.9	39.2

Each value represents the mean ± standard deviation of two replicates. Means (in the same column) with different letters are significantly different ($p < 0.05$).

content of tocopherols and squalene, which act as antioxidants, provides high oxidative stability for amaranth oil. The unique composition of amaranth seed oil makes it a useful ingredient in the food, pharmaceutical, and cosmetic industries. (Bozorov et al., 2018; Yarnia & Khorshidi Benam, 2017).

Favorable characteristics of cocoa butter such as melting behavior and crystallization are often not achieved by using pure oil in chocolate formulations. As a result, modifying methods including fractionation, hydrogenation, and transesterification are applied. Brüse et al. (2012) indicated that the replacement of 6.6 % of cocoa butter with enzymatically transesterified amaranth oil increased the melting temperature of cocoa butter about 16.5 °C (from 26 °C to 42.5 °C) without any changes in the appearance or oral sense. Kadivar et al. (2016) reported that the addition of 31 % of transesterified sunflower oil containing high levels of oleic or oleic and stearic acids has resulted in a lower melting point and hardness, as well as the waxy state in the mouth and release of flavor. In the literature, there are some studies on the enzymatic transesterification of cocoa butter using caprylic acid as a donor of the acyl group to produce replacers with lower calories for cocoa butter (Tran et al., 2015; Wu et al., 2014).

Because of the disadvantage of saturated fat and the limitations of direct use of liquid oils, it is recommended to use oleogels containing highly unsaturated oils as fat-like compounds. Oleogels are prepared by liquid oil getting trapped in a three-dimensional network without changes in chemical properties. Heat resistant chocolate, which is

Table 3
The color parameters and whiteness index of different samples after 20 days storing at 18 and 30 °C.

Samples	L*	a*	b*	Whiteness
After 20 days at 18 °C				
Chocolate	21.1 ± 0.45[a]	9.2 ± 0.01[a]	11.8 ± 0.14[a]	19.7 ± 0.01[a]
CBR 40 Chocolate				
5 %	21.1 ± 0.28[a]	9.1 ± 0.10[a]	11.7 ± 0.64[a]	19.7 ± 0.00[a]
10 %	21.1 ± 0.34[a]	9.1 ± 0.02[a]	11.6 ± 0.58[a]	19.7 ± 0.02[a]
15 %	21.3 ± 0.05[a]	9.0 ± 0.35[a]	11.3 ± 0.13[a]	20.3 ± 0.08[a]
CBR 100 Chocolate				
5 %	21.3 ± 0.10[a]	9.0 ± 0.29[a]	11.5 ± 0.00[a]	20.3 ± 0.00[a]
10 %	21.5 ± 0.15[a]	8.8 ± 0.11[a]	11.1 ± 0.10[a]	21.2 ± 0.05[a]
15 %	21.5 ± 0.07[a]	8.7 ± 0.33[a]	10.9 ± 0.05[a]	22.2 ± 0.13[a]
After 20 days at 30 °C				
Chocolate	30.2 ± 1.12[b]	6.1 ± 0.05[b]	7.0 ± 0.01[b]	29.8 ± 0.12[b]
CBR 40 Chocolate				
5 %	25.1 ± 0.43[a]	7.7 ± 0.20[a]	9.2 ± 0.02[a]	24.1 ± 0.08[a]
10 %	25.3 ± 0.21[a]	7.7 ± 0.11[a]	8.9 ± 0.02[a]	24.3 ± 0.03[a]
15 %	26.4 ± 0.09[a]	7.5 ± 0.25[a]	8.8 ± 0.00[a]	25.5 ± 0.06[a]
CBR 100 Chocolate				
5 %	27.1 ± 0.10[a]	7.6 ± 0.10[a]	8.8 ± 0.10[a]	26.1 ± 0.06[a]
10 %	27.4 ± 0.06[a]	7.5 ± 0.08[a]	8.5 ± 0.15[a]	26.5 ± 0.11[a]
15 %	27.3 ± 0.30[a]	7.5 ± 0.03[a]	8.5 ± 0.36[a]	26.4 ± 0.10[a]

Each value represents the mean ± standard deviation of three replicates. Means (in the same column) with different letters are significantly different (p < 0.05).

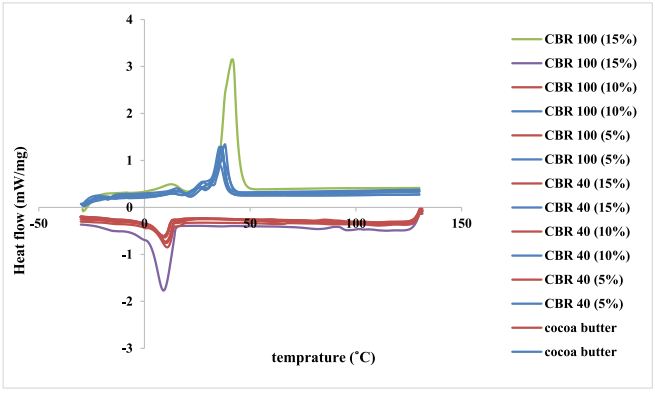

Fig. 1. Differential scanning of calorimeter's graphs from cocoa butter and cocoa butter replacements.

prepared by ethylcellulose oleogel can be considered as an example of using these compounds in chocolate products (O'Sullivan et al., 2016). Moreover, in another study the chocolate paste was produced by the complete replacement of oil binder and 27 % replacement of palm oil with Shellac oleogel. This product was stable during storage at 30 °C for several weeks (Rogers et al., 2014). Wendt et al. (2017) reported that the oil omission was reduced after the addition of β-sitosterol and gamma-oryzanol oleogels to the chocolate filled products.

Therefore, based on our previous study about physicochemical characterization of amaranth oil oleogel (Kamali et al., 2019), in the

Table 4
Result of differential scanning calorimetry for chocolate containing cocoa butter and butter replacers.

Temperature	Chocolate	CBR 40 chocolate			CBR 100 chocolate		
(°C)		5 %	10 %	15 %	5 %	10 %	15 %
Melting	35.69 ± 1.05[a]	35.74 ± 0.96[a]	35.66 ± 0.75[a]	35.89 ± 1.10[a]	36.61 ± 1.13[a]	36.6 ± 0.86[a]	41.71 ± 0.5[b]
Crystallization	9.35 ± 1.05[a]	8.91 ± 0.96[a]	8.87 ± 0.75[a]	8.94 ± 1.10[a]	8.05 ± 1.13[a]	8.1 ± 0.86[a]	8.01 ± 0.5[a]

Each value represents the mean ± standard deviation of three replicates. Means (in the same line) with different letters are significantly different ($p < 0.05$).

present study we aimed to investigate the effect of partial replacement of cocoa butter with interesterified and pure amaranth oil oleogel in chocolate.

2. Materials and methods

2.1. Materials

Amaranth oil was purchased from a Behave Company (Rietberg, Germany), and the monoglyceride and Malaysian CB were provided from Mino Chocolate Factory (Tehran, Iran). Triacylglycerol standards were purchased from Larodan Fine Chemicals AB (Malmo, Sweden). Lipozyme® TLIM (Thermomyces lanuginosus lipase, sn-1, 3 specific, specific activity 250 IUN/g: IUN = Interesterification Unit), a silica granulated thermomyces lanuginosus was donated kindly by Novozymes A/S (Bagsvaerd, Denmark). All materials were kept at 24 °C until further analysis.

2.2. Preparation of the amaranth oil oleogels

Oleogel was prepared by using amaranth oil (solvent) and a mixture of MGs (palmitic and stearic acids). MG oleogels were prepared by the addition of organogelator (MG) to the amaranth oil at a concentration of 10 % (*w*/w). The mixtures were heated along with stirred at 1200 rpm for 30 s (Heidolph MR 3001, Germany) to reach the temperature of ~65 °C to ensure the melting of the organogelator. MG oleogels were cooled and allowed to crystallize at refrigerator temperature (4 °C) for 24 h. Under a constant cooling process, the cold hard fatty phase with a maximum thickness of about 1 cm was produced (Patel et al., 2014). For interesterification, the amaranth oil was transferred to screw-capped sealed glass vials and mixed with lipase enzyme from *Thermomyces lanuginosus* at the concentration of 10 % (w/w) which was immobilized on the silica granules. The process was completed by heating the mixture at 60 °C (using an electrical heater) for 8 h at 700 rpm (Lupi et al., 2011). The pure oleogel (the mixture of amaranth oil and 10 % MG) was named cocoa butter replacer 100 (CBR 100). Also, 40 % of this pure oleogel was mixed with 60 % enzymatically transesterified amaranth oil and named cocoa butter replacer 40 (CBR 40).

2.3. Determination of triacylglycerol

The acylglycerol content of the samples was analyzed by an Agilent 7890 A GC, which applied a capillary column (100 m × 0.25 mm i.d. × 0.2 μm, BPX70; Hp_88, USA) and a flame ionization detector (FID). Nitrogen was used as the carrier gas at a flow rate of 4.41 ml/min. The temperature program of the oven was set as the initial temperature of 50 °C and then increased to 220 °C at 50 °C/min, augmented to 290 °C at 30 °C/min, and then increased to 330 °C at 50 °C/min, which was followed by an elevation to 380 °C at 50 °C/min and hold for 3 min. The injection was carried out in split mode with a split ratio of 20:1. The injector and detector temperatures were set at 380 °C. The samples were dissolved in hexane at a concentration of 10 mg/ml. Then, 2 μl of the samples were injected into the column. The level of monoacylglycerols, diacylglycerols, and triacylglycerols in oils were calculated using calibration curves and the TAG species were determined based on the retention time of standards. All the measurements were performed in triplicate (Wang et al., 2011).

2.4. Chocolate preparation

The control chocolate samples composition was as follows: 50 % ground sugar powder, 35 % cocoa butter, 14.5 % cocoa powder, and 0.5 % soy lecithin. For the preparation of samples with amaranth oil, cocoa butter was replaced with amaranth oil oleogel (CBR 100 and CBR 40) at the concentration of 5, 10, and 15 % (*w*/w). All the mentioned ingredients (except lecithin) were mixed at 60 °C and 200 rpm for 20 min. Then, the lecithin was added to the mixture and mixed under the same condition. The mixture was cooled at 27 °C for 20 min (1.5 °C/min) while stirring at 200 rpm to form the primary crystals. Then, the mixture was heated again to 29 °C to melt the unstable crystals. After completing the tempering process, the liquid chocolate was transferred to the plastic molds at 29 °C and then stored at 10 °C for 12 h. The solid chocolate samples with 3 replicates for each sample were wrapped in aluminum covers and kept at 4 °C for further experiments (Zhang et al., 2001).

2.5. Color evaluation

The color evaluation test was carried out to evaluate fat bloom formation in the samples, control, CBR 100 and CBR 40 in three ratios of 5, 10, and 15 %. Fat bloom formation was assessed using the accelerating bloom test. Whiteness index (WI) was measured during 20 days of storage at temperatures of 30 and 18 °C for 8 and 16 h, respectively. At these two temperatures and two times, at first the maximum content of β forms 1 to 5 and type 6 crystals melt, and then the type 6 β crystal increases, preparing the basis for the formation of chocolate fat bloom (accelerated bloom test) (Walter & Cornillon, 2001). Redness, yellowness, and brightness (a*, b* and L*) were measured every day using a colorimeter (Hunter Associated Lab Inc., Reston, Virginia, USA) and the following equation was applied for whiteness index (WI) calculation (Mitra et al., 2009):

$$WI = 100 - \left[(100 - L^{*})^2 + a^{*2} + b^{*2}\right]^{1/2}$$

2.6. Differential scanning calorimetry (DSC)

Thermal characteristics including profile of crystallization and melting of control and samples containing amaranth oil oleogel (CBR 100 and CBR 40) were determined using a differential scanning calorimeter (Maia 200 f3, Netzsch Scientific Instruments). Nitrogen was applied as the cooling gas at a flow rate of 100 ml/min. Briefly, 10 mg of each sample was placed in an aluminum empty pan and then closed by pressing the sample to be impenetrable. In addition, an empty dish was used as a reference. The samples were heated at 65 °C for 2 min to make them homogeneous and demolish any previously formed crystal. Then, the samples were cooled to −30 °C at the rate of 10 °C/min and stored for 3 min at the same temperature. Then, the samples were heated to 130 °C at the previous rate and kept again for 3 min at this temperature. The highest temperature, melting point, and crystallization were calculated by Netzsch software (Lohman & Hartel, 1994).

2.7. Polymorphism

The polymorphic transformations of the samples were determined using the X'Pert PRO MPD X-ray diffraction (PANalytical Company,

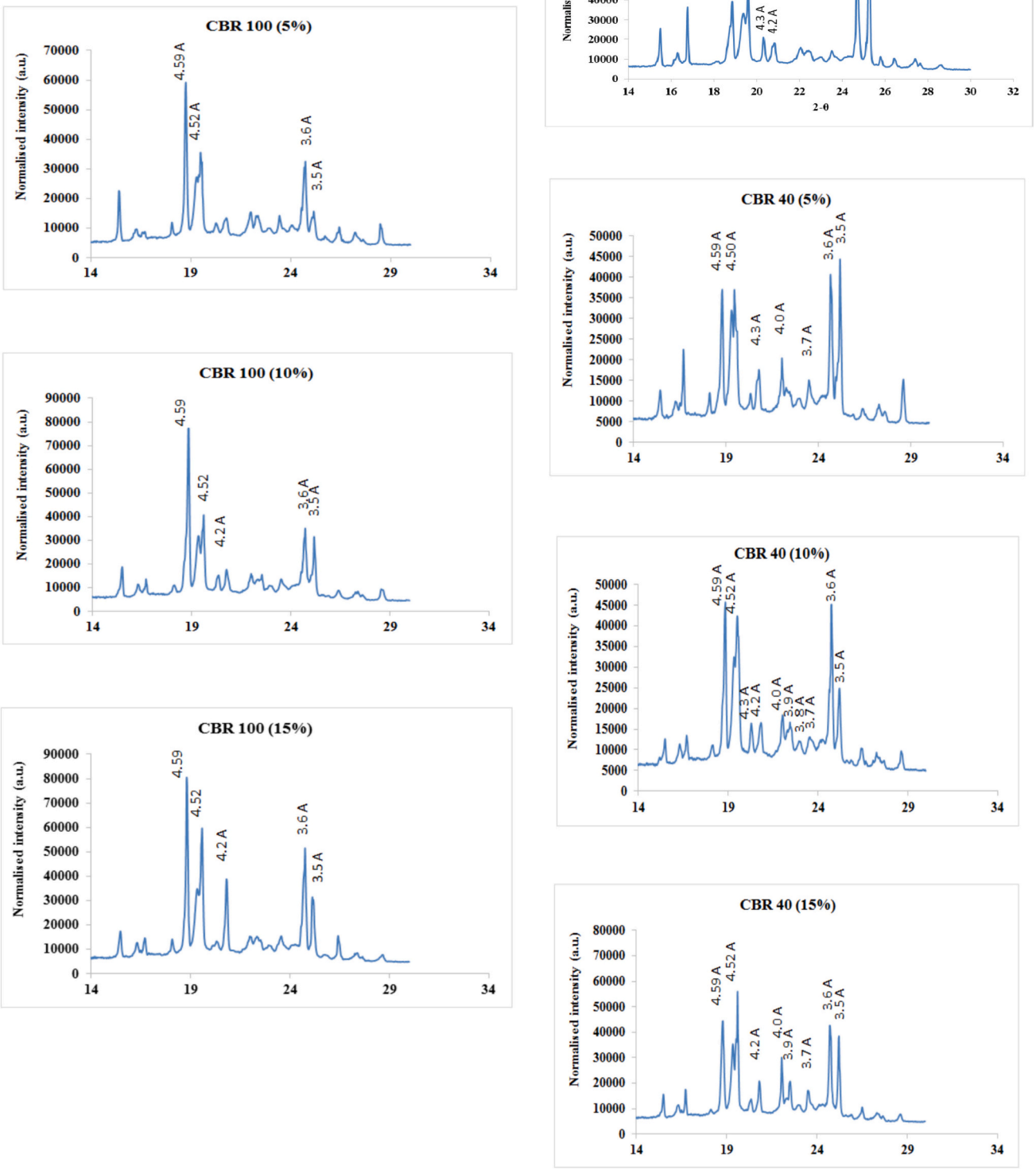

Fig. 2. X-ray pattern in chocolate containing cocoa butter and chocolate containing CBR 40 (5, 10 and 15 %) and CBR 100 (5, 10 and 15 %).

Table 5
Hardness (N) for chocolates containing cocoa butter and butter replacers.

Chocolate	CBR 40 chocolate			CBR 100 chocolate		
	5 %	10 %	15 %	5 %	10 %	15 %
11.5 ± 1.05[a]	12.0 ± 0.96[a]	12.6 ± 0.75[a]	12.9 ± 1.10[a]	12.7 ± 1.13[a]	13.0 ± 0.86[a]	13.2 ± 0.50[a]

Each value represents the mean ± standard deviation of three replicates. Means (in the same column) with different letters are significantly different ($p < 0.05$).

Poland) with Cu-Kα radiation (k = 1.5406 Å, current of 40 mA, and voltage of 40 kV) at room temperature. The samples were analyzed at 2θ angles of 1–30° and they were scanned at the rate of 5 °C/min. The X-ray diffraction patterns were analyzed by Hiscore Plus software. The polymorph of crystals was assigned according to the following features of short spacing characteristics for CB: α form (d = 4.15 Å), β' forms (d = 3.8–4.3 Å), and β forms (d = 4.5–4.6 Å). Before the measurements, the samples were kept at −35 °C for 24 h to inhibit polymorphism transition during the analysis (Sonwai et al., 2017).

2.8. Hardness measurement

The hardness of the chocolate samples was measured using a texture analyzer system equipped with a 1.6 mm smooth-end probe. The samples with the dimension of 50 × 25 × 10 mm was kept at 20 °C for 2 h and then penetrated at a rate of 1.5 mm/s. Finally, the maximum force at a depth of 6 mm of each sample was reported as the hardness (Osborn & Akoh, 2002).

2.9. Statistical analysis

All experiments and measurements were carried out in triplicate. The factorial test was carried out due to the completely random design. The data were subjected to analysis of variance (ANOVA) and the significant differences between means were determined by Duncan's multiple range test ($P < 0.05$) using SPSS statistical software (version 19.0; SPSS Inc., Chicago, IL).

3. Results and discussion

3.1. triacylglycerol content of amaranth oil, cocoa butter, CBR 100, and CBR 40

The fatty acid composition of amaranth oil was about 80 % unsaturated glycerides while MG consisted of about 90 % saturated glycerides based on the gas chromatography analysis (Table 1). The results of triacylglycerol species in amaranth oil, cocoa butter, CBR 100, and CBR 40 are demonstrated in Table 2. According to present findings, POP (1,3-dipalmitoyl-2-oleoyl-glycerol), POS (1-palmitoyl-2-oleoyl-3-stearoyl-glycerol), and SOS (1,3-distearoyl-2-oleoyl-glycerol) are the dominant triacylglycerols of cocoa butter (19.1, 39.4, and 28.6 %, respectively). Similar results were reported in a previous study by Zarringhalami et al., 2012. The present results also showed very low levels (almost zero) of POP, POS, and SOS in the amaranth oil. Using enzymatic transesterification in the CBR 40 these values increased to 7.9, 9.1, and 8.9 %, respectively. The present results were consistent with the results of the transesterification of tea seed oil and olive oil to produce a replacer for cocoa butter (Ali et al., 2001). However, these values are still significantly lower than that of cocoa butter. Moreover, these fatty acids are significantly lower in the CBR 100 compared to cocoa butter (CBR and cocoa butter total SUS triglycerides were 22.4 and 88 %, respectively). These low values might be resulted from adding stearic and palmitic acids (monoglycerides as the organogelator) to CBR 100 and CBR 40. On the other hand, the total unsaturated triglycerides (UUU) in cocoa butter, CBR 100 and CBR 40, were equal to 1.1, 43.9, and 39.2 %, respectively.

3.2. Color

The results of the color evaluation were given in Table 3. No significant differences were observed between the samples at 18 °C after production. The control sample had the highest brightness and the lowest level of color parameters. The color of samples containing cocoa butter replacers did not show significant differences after 20 days of storage at 30 °C. The control chocolate was less resistant to fat bloom formation under temperature fluctuations which is in line with the results of da Silva et al. (2017) who indicated that temperature fluctuations even in small ranges (24–25 ± 1 °C) increase the fat bloom formation. However, the lowest level of fat bloom was observed in the chocolate samples containing cocoa butter replacers, especially the CBR 40. This result can be attributed to the higher number of crystals, and more complex crystal structures in the chocolate prepared from 40 % transesterified oil (Li et al., 2021). Halim, Selamat, Mirhosseini, and Hussain (2019) reported that the addition of enzymatically transesterified oil as a cocoa butter replacer to the bitter chocolate led to a slower fat bloom formation compared to the control sample. Furthermore, in the optimum storage condition, no differences were observed between the control chocolate and chocolates containing the replacers regarding fat bloom. Moreover, fat bloom showed a lower level in the chocolate containing CBR 100. Masuchi et al. (2014) showed that the addition of 0.15 % of sorbitan monostearate might delay fat bloom formation in the dark chocolate. This result can be related to the capacity of this emulsifier (sorbitan monostearate) for forming a three-dimensional network that entraps the lipid crystals which increase thermal resistance in the lipid phase. These changes prevent the polymorphic transition, which is responsible for bloom formation (Zhao & James, 2019). It has been noted that solid emulsifiers such as sorbitan tristearate and monostearate hinder polymorphic lipid transition as a result of their role in crystalline assemblies, which causes imperfections in the triacylglycerol organization. Therefore, the transition from a less stable to a more stable crystal polymorphism is retarded (Lonchampt & Hartel, 2004). While the mechanism for fat bloom formation is not yet well defined, it has been reported that various factors may increase the possibility of converting the β-crystals type 5 to type 6 which has a higher melting point and results in the formation and development of fat bloom in chocolate products. Some of these factors are as follows: unsuitable thermal conditioning, rapid cooling after thermal conditioning and during casting, increasing temperature, and temperature fluctuations during chocolate storage (Bricknell & Hartel, 1998).

3.3. Differential scanning calorimetry (DSC)

As shown in Fig. 1 and Table 4, chocolate containing 5 %, 10 % and 15 % of CBR 100 and CBR 40 were almost the same to the chocolate containing cocoa butter regarding the thermal parameters, including initial temperatures, maximum melting point, and ending point the maximum melting temperatures of these samples were all in the body temperature range (35–36.6 °C). Consequently, the samples will melt in the mouth. Similar results were obtained using 1.5, 3, and 4.5 % replacement of cocoa butter with coconut oil (Halim et al., 2019). According to the findings of Clercq et al. (2014) the melting profile of the dark chocolate should have a narrow melting peak to melt the chocolate rapidly at the body temperature range (37 °C) and induce favorable oral sense. In the present study, the peak of melting was in the body temperature range. Furthermore, the favorable crystal in chocolate is β(V) with a melting point of 35–37 °C. The chocolate containing 15 % of pure amaranth oil oleogel had a higher melting point (41.7 °C). Moreover, the initial melting temperature for chocolate should be about 24 °C (a little higher than room temperature) to assure stability of the solid state at room temperature. Thus, the initial melting point of the chocolates was higher than 24 °C (27.7–32.8 °C) and was in an acceptable range.

3.4. Polymorphism of chocolates

The polymorphic structure of the chocolate samples with cocoa butter and mixture of cocoa butter and replacers are shown in Fig. 2. According to results, one peak with high intensity in the width distance of 4.5 A° (4.52–4.59 A°) and peaks with lower intensities in width distances of 3.5 A° and 3.6 A° were observed that might be associated with the presence of β(V)-crystals as reported by Afoakwa et al. (2007). Wang et al. (2011) indicated that all the samples had one peak with high intensity in width distance of 4.5 and six smaller peaks in distances of 4.3, 4.1, 3.9, 3.8, and 3.7. This pattern suggested the possible presence of a mixture of β'(III) and (IV), as well as β(V)-crystals in the samples. Biswas et al. (2017) confirmed that primary crystals of cocoa butter exist as β' types (III) and (IV) that convert to β(V) during crystallization under stable conditions. The evaluation of crystal formation in different samples of chocolate is indicative of a similarity between the sample containing 5 % of CBR 40 (probably due to the lower percentage of cocoa butter replacer) and the cocoa butter control sample (higher β' crystals). The level of β crystals increased compared to the β' crystal types by increasing the percentage of amaranth oil oleogel. Because in essence, the percentage of linoleic acid in amaranth was 2–5 times higher and so the bending of the double bonds is greater (TABLE 1).

3.5. Hardness measurement

As shown in Table 5, the hardness of the control cocoa butter chocolate was 11.5 N, while it increased to 13.2 N in chocolate containing 15 % of CBR 100. In general, no significant differences were observed in the hardness of the chocolate samples. These results were in agreement with the results of Fayaz et al. (2017) who reported no textural differences between the cookies prepared with canola oil and candelilla wax. They attributed the minor numerical differences in the mechanical parameters of chocolates containing monoglycerides to the presence of cocoa powder and sugar. These compounds increased the solid content of samples and consequently increased the interactions between OH groups of monoglyceride crystals and free OH groups of sugar crystals which resulted in the formation of more stable networks. In the present study, the solid fat content in the 40 % and 100 % CBR was 12.55 to 0.00 and 8.90 to 0.10 at 40–0 °C, respectively. The solid fat content in the 40 % was higher up to temperature of 20 °C. It seems to be due to the simultaneous effect of adding saturated fatty acids and modifying the triacylglycerols of the sample by enzymatic esterification.

4. Conclusions

In the present study, the control chocolate containing cocoa butter was compared with the chocolates containing 5 %, 10 %, and 15 % of cocoa butter replacers prepared from amaranth oil (100 % oleogel, 40 % oleogel +60 % enzymatically transesterified amaranth oil). The comparison between the cocoa butter triglycerides and two cocoa butter replacers showed a significant difference between the main triglycerides' species. However, enzymatic transesterification increased the three significant cocoa butter triglycerides in the amaranth oil. Our results demonstrated the lowest color parameters and the highest fat bloom formation in the control chocolate. The CBR 40 at all concentrations and CBR 100 at the concentration of 5 % amaranth oil oleogel did not cause a considerable alteration in maximum melting temperature compared to the control chocolate. In the control chocolate, the β' crystals were dominant but in the other samples (except for 5 % of CBR 40 probably due to the lower percentage of cocoa butter replacer) the β crystals, especially type 5 were dominant. There was no significant difference between the textural properties of the samples. Finally, it can be suggested that this CBR especially CBR 40 with a concentration of 5 % is an acceptable replacer for cocoa butter for the formation of chocolate.

Practical applications

This work provides information on the physicochemical properties of the transesterified amaranth oil oleogel as a cocoa butter replacer. Investigation of functional properties of chocolates containing transesterified amaranth oil oleogel showed that this oleogel can be used as a suitable replacer for cocoa butter in chocolates.

Ethical approval

This article does not contain any studies with human or animal subjects.

CRediT authorship contribution statement

Elahe Kamali: Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Mohammad Ali Sahari:** Writing – review & editing, Supervision, Project administration, Methodology. **Mohsen Barzegar:** Writing – review & editing, Supervision, Methodology. **Hassan Ahmadi Gavlighi:** Writing – review & editing, Supervision, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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