J Food Sci Technol (February 2024) 61(2):331–339
https://doi.org/10.1007/s13197-023-05844-7

ORIGINAL ARTICLE

Carnauba wax and adipic acid oleogels as an innovative strategy for cocoa butter alternatives in chocolate spreads

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Revised: 24 August 2023 / Accepted: 11 September 2023 / Published online: 4 October 2023

Abstract The aim of this study was to replace cocoa butter substitute (CBS) with structured sunflower oil in chocolate spread partially. Two types of oleogel, 6% carnauba wax (CWO) and 2% carnauba wax with 4% adipic acid (AD—CWO) were substituted (at 20%, 50%, and 70%), and chocolate spread characteristics were evaluated. Various properties of chocolate spread samples were investigated as peroxide value, firmness, oil binding capacity, moisture content, molecular interactions, and molecular conformation of fat crystals. The increasement of CBS substitution by oleogel in samples significantly reduced firmness. The samples with 20% replacement formulated by CWO and AD-CWO had the highest oil binding capacity, 97.48 ± 0.21% and 97.73 ± 0.02, respectively. Moreover, oxidative stability analysis showed a positive correlation with an increasing replacement level over 90 days of storage. Based on FT-IR analysis, the new intermolecular hydrogen bond formation in the oleogel-based spreads network has been confirmed. CBS replacement with oleogels revealed the presence of stable β´ polymorphs with low intensity. In conclusion, the carnauba-based oleogels have significant potential to substitute CBS in chocolate spread partially.

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Keywords Oleogel · Carnauba wax · Acid adipic · Chocolate spread

Introduction

Spreads are confectionery products consumed directly or used as filling in the formulation of cookies, cakes, etc. They can be defined as complex mixtures of solid particles (sugar, cocoa powder, milk powders, and nuts) in a continuous phase (Acan et al. 2021). Fats used as the continuous phase in spreads are significant components, consisting of approximately 50% lauric acid, and 20% myristic acid reaching up to 60% (Biswas et al. 2017). Their spreadability and sensory properties at room temperature depend on the amount of fat added to the solid ingredients (Espert et al. 2020), fatty acids, plant origin, and fatty acid composition. Depending on the aim of sensory and functional properties, various fatty acid mixtures (long and medium-chain) are used instead of cocoa butter. Cocoa butter substitutes (CBS) are commonly used in chocolate and confectionery industries to substitute cocoa butter (CB) completely. This alternative oil is a non-lauric fat derived from palm kernel, coconut, or babassu oils. They are also low-cost and more heart-stable alternatives to CB. Due to the origins of CBR and CBS, palm kernel oil or coconut oil contains high amounts of lauric acid (high in 12-carbon saturated fatty acids) or trans fats. Cocoa butter alternatives have similar melting behavior to CB but different molecular structures. β form of CBS crystals leads to a stable polymorphic form without tempering (Ghazani and Marangoni 2018). As well as, in spreads, the network of fat crystals usually affects various physicochemical properties of the final product (Espert et al. 2020; Fayaz et al. 2017).

The unique crystalline microstructure and colloidal network of solid fats in food products entrap oil and give suitable consistency to many foods, including spreads, whipped creams, chocolates, ice cream, and various bakery products (Pehlivanoğlu et al. 2018). On the other hand, solid fat processing conditions like hydrogenation cause isomerization and relocation of some double bonds, increasing trans-isomers and saturated fat content (Thirumdas 2022). From a health point of view, a high intake of CBR and CBS may increase the risk of cardiovascular, obesity, and other related diseases (Alvarez et al. 2021; Li and Liu 2019). The USA Food and Drug Administration (FDA) prohibited the use of partially hydrogenated oils in foods (the major source of artificial trans fat) from the GRAS list in 2015(Administration 2018). So, consumers' awareness of a balanced diet makes the chocolate industry develop the most appropriate optimized fat formulation, which does not negatively influence the quality characteristics of chocolates.

In particular, replacing saturated and trans fats with unsaturated fatty acids (FA) can significantly affect the chocolate spread's quality; however, achieving the desired quality is not easy (Fayaz et al. 2017). Oleogelation or organogelation is described as the gelation of high nutritional quality liquid oils in a three-dimensional network via different interactions while maintaining the chemical characteristics of oil (Co and Marangoni 2018). Structuring edible oils with organogels is a potential method for producing low-saturated fat products. Although mimicking solid fats' multiple roles (physicochemical structure, sensory characteristics, and flavor perception) with liquid oil is still a challenge (Li and Liu 2019).

Carnauba wax is heterogeneous, containing long-chain aliphatic esters with an inert and stable composition, with higher double and triple-bonded carbon atoms and branched methyl groups. It has potential advantages such as great oil binding capacity, a high melting point, and low solubility, which could form a gel at low concentrations (Adrah et al. 2022).

Several studies have been conducted on partially or wholly replacing solid fats to improve the physical and functional properties of spreads. Bascuas et al. (2021) used biopolymer-based oleogels in chocolate spreads. The spreads made with a mixture of oleogel and coconut fat at 50% showed significantly higher G' values and had an excellent structured system. Another study Fayaz et al. (2017) used pomegranate seed oil with different oleogelators to partially replace the fat phase in the chocolate spreads formulation. Spreads containing oleogels showed desirable mechanical parameters (rheology and texture) and oil-binding properties. To enhance the gelation properties of organogels, using a multi-component system could provide superior rheological properties through synergistic interactions; also, useful characteristics of different gelators could modify the structural organization of gelator molecules (Aliasl khiabani et al. 2020).

Adding more than one gelator in the oil phase could improve various physicochemical properties of the spreads. Firstly, carnauba wax forms gel via needle-like crystals, and secondly, it improves the mechanical properties via adipic acid. According to Aliasl khiabani et al. (2020) adipic acid could be a new exciting co-structuring agent in carnauba wax-based oleogel as a shortening and animal fat substitute in the cake and the beef burger, respectively. There is little information about the effects of carnauba-based oleogel on the properties of chocolate spreads. So, in the present study, the feasibility of CWO and AD-CWO as CBS substitutes in spreads formulation has been assessed.

Material and methods

Materials

CW was obtained from Sigma Aldrich (Germany), and AA was purchased from Tokyo Industry Co (Tokyo, Japan). Refined sunflower oil and powdered sugar were obtained from a local market (Tabriz, Iran).

Sample preparation

Oleogels

Two optimum sunflower-based oleogels (6% CW) and (4% AD + 2% CW) were prepared as described by a previous study (Aliasl khiabani et al. 2020). The appropriate masses of CW and AA were dissolved in hot (80–90 °C) sunflower oil, followed by magnetic stirring (400 rpm) until complete dissolution at 150 °C. Then, the mixtures were cooled to ambient temperature at 1 °C/min using a heating/cooling water bath (IKA, RH Basic 2, Germany). After the oleogel formation, the samples were stored at 4 °C for 24 h. The oleogel preparation was performed 1 day before the production of spreads.

Chocolate spread

Chocolate spreads (Table 1) were prepared by using three different replacements of the CBS with CWO (20%: CWO/20, 50%: CWO/50, and 70%: CWO/70) and AD-CWO (20%: AD-CWO/20, 50%: AD-CWO/50, and 70%: AD-CWO/70). The chocolate spreads were prepared according to the methodology reported by Fayaz et al. (2017) with minor modifications. The sugar particles were passed through a sieve with a mesh of 60 so that all the sugar particles were uniform (> 250 μm). Initially, the fat phase (oleogel-CBS mixture) was heated at 80 °C in a temperature-controlled water bath until completely melted. Then, half of the dry ingredients (sugar, skimmed powder milk, and cocoa

Table 1 The chocolate spreads formulations

g/100 g C							
Samples	Ingredients						
	CBS	Oleogel	Milkpowder	Sugar	leciThin	CocoaPowder	Vanillin
Control	32	–	5.5	46	0.4	16	0.1
CWO/20	25.6	6.4	5.5	46	0.4	16	0.1
CWO/50	16	16	5.5	46	0.4	16	0.1
CWO/70	22.24	9.6	5.5	46	0.4	16	0.1
AD-CWO/20	25.6	6.4	5.5	46	0.4	16	0.1
AD-CWO/50	16	16	5.5	46	0.4	16	0.1
AD-CWO/70	22.24	9.6	5.5	46	0.4	16	0.1

Control (cocoa butter substitute), CW: Carnauba Wax, AD: Adipic acid

powder) with lecithin were gradually dispersed in half of the molten fat phase and stirred with a mixer for 3 min at a speed of 200 rpm. The speed of the thermo-mixer was increased to 300 rpm and mixed for 5 min (at 80 °C). Afterward, the rest of the corresponding molten fat phases and ingredients were added, and the agitation continued for 15 min until the homogenous spreads texture was obtained. The chocolate spreads were prepared in triplicate and, after cooling (20 °C), stored at 5 °C for 24 h before analysis.

Chocolate spread analysis

Firmness

The hardness of the spread samples was measured by a texture analyzer (TA. XT Plus, Stable Micro Systems Ltd., Surrey, England). The samples were punctured with a 6 mm stainless-steel cylindrical probe up to 50% of the original height at a constant speed of 60 mm/min. The hardness was recorded as the maximum force required to puncture the sample at the defined penetrate distance using the software Automated Materials Testing System (version 5, Series IX, Instron Ltd., High Wycombe, UK). Every determination was performed in triplicate (Fayaz et al. 2017). *Moisture content.*

The moisture contents were determined with an oven (Behdad, Iran) according to Gargari et al. (2022). Approximately 5 g of chocolate spreads were heated at 105 °C for 24 h. The moisture content of the chocolates (%) was determined as follow:

$$\text{Moisture content}(\%) = \frac{\textit{Loss in moisture}}{\textit{Initial weight os sample}} \times 100 \quad (1)$$

X-ray diffraction (XRD) analysis

The molecular conformation of the spread samples was determined using XRD. XRD analysis was performed using an x-ray diffractometer (D5000; Siemens, Munich, Germany) with a Cu Kα source (λ = 1.54 Å) operated at 40 kV and 30 mA. A proper amount of the chocolate spreads were loaded onto the glass slide, and the diffraction angle was performed in the domain of the diffraction angle (2θ) within the range of 5–35 °C at room temperature with a step of 0.02 min (Zhanga et al (2020). The patterns of the samples were analyzed using Xpert Highscore Plus.

Oil binding

The oil binding capacity (OBC) of spreads was determined by the centrifugal method described by Sun et al. (2021) with a minor modification. 5 g spread samples were weighted in the centrifuge tube and centrifuged (Universal 320 centrifuge, Hettich, Germany) at 10,000 rpm for 15 min. Then, the drainage oil was removed by placing the tubes on filter paper inversely, and the remaining oil was weighted. Eventually, the oil binding capacity was determined using the following equations:

$$\%\text{Oil released} = \frac{\textit{Mass of expressed oil}(g)}{\textit{Totall mass of sample}(g)} \times 100 \quad (2)$$

FTIR The FT-IR spectroscopy (Equinox 55LS 101, Bruker, Germany) analysis was carried out to investigate the possible molecular interactions in the samples. The spectra were collected over the wave number range of 4000–400 cm^{-1} with a nominal resolution of 1 cm^{-1} and 100 scans (Aliasl khiabani et al. 2020).

Oxidation stability

The oxidative stability of the spreads was measured each month during 90-day storage by the peroxide value (Cd

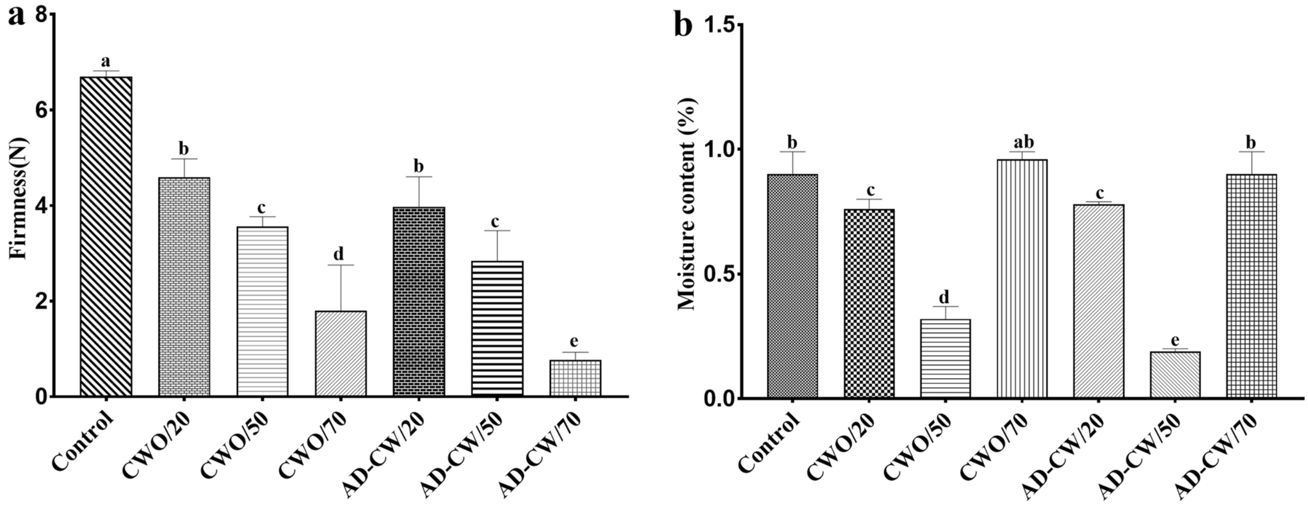

Fig1 **a** The firmness values of the chocolate spreads prepared with different OG: CBS blends, **b** moisture content, Control (cocoa butter substitute), CW: Carnauba Wax, AD: Adipic acid

8–53 method) measurement (AOCS, 1987). Peroxide values expressed as milliequivalents of active oxygen per kilogram of oil (Öğütcü and Yilmaz 2014).

Statistical analysis

GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA) was used for.

statistical analysis based on a one-way analysis of variance (ANOVA). The comparison of means and significant differences between replicates was conducted through Duncan's mean tests at $p < 0.05$.

Results and discussion

Firmness

Firmness is correlated with the degree of spreadability. Figure 1a summarizes the firmness results with different CBS: OG formulated system substitutions. A remarkable difference was found between the force values of the control spread (100% CBS) and the CBS/oleogel spreads. Control samples compared to all the CBS/oleogel showed greater firmness. A similar observation was also reported by Li and Liu. (2019) that the hardness of dark chocolates was higher than that of other chocolates containing oleogels as CB's saturated fatty acid content was higher than oleogel-containing samples. Chai et al. (2020) also determined that the higher content of saturated fatty acids creates a firmer structure owing to the higher level of densely compact crystalline fat in the dispersed phase.

As it is clear, the firmness is in line with the content of oleogel substitution in both CWO and AD-CWO. Since the replacement of oil substitution increased, the firmness values decreased ($p < 0.05$). Oleogels containing more unsaturated fat had a softening effect on the product. The higher amount of crystalline fat in the dispersed phase increases the strength of the network by forming mass-like structures in the crystals (Li and Liu 2019).

According to the results, a more rigid texture was obtained till 50% substitution. The fat phase (continuous phase) mixture of CBS/OG influenced textural properties. Alvarez et al. (2021) reported that there was a strong correlation between textural parameter values and the OG percentage level. By increasing the amount of oleogel, firmness was reduced, which depended on the arrangement of crystalline fats in cocoa butter. A stronger compact aggregation in fat crystals at 20 and 50% replacement led to higher firmness. The control sample had a more rigid texture than oleogel-based chocolates. It should be noted that the excessive softness of the 70% substitute samples is due to the weakening of the network strength, and the dispersed phase in the cocoa product needs to be adequately made. In addition, the reduction in firmness may be attributed to the polymorphism transition of β´ to β. There were no significant differences ($p > 0.05$) between the firmness of the chocolate spreads with the same replacement level of CWO and AD-CWO samples. The firmness significantly decreased in both 70% replacement samples ($p < 0.05$).

Kadivar et al. (2016) reported that adding more than 25 g of CBEs to a 100 g blend decreased the hardness of the fat and the control chocolate had a softer texture due to the differences in the TAG contents of CB and CBEs. In addition, a linear decrease was observed when more CB was replaced.

Moisture content

The moisture of the chocolate spreads ranged from 0.19 to 0.99% Fig. 1b. The AD-CWO /70 spreads presented the lowest moisture content results (0.19 ± 0.05), followed by CWO/70 (0.32 ± 0.05). Spreads with high moisture content levels exhibited relatively harder texture than the other spread samples. Water molecules contain hydroxyl groups generally involved in intermolecular hydrogen bonds. Therefore, the presence of moisture in chocolate spreads will influence particle–particle interactions. The stronger the particle–particle interaction, the higher hardness (Shourideh et al. 2012). According to Shourideh et al. (2012), spreads with high moisture content exhibited relatively harder texture than the other spread samples. In the study conducted by Yılmaz and Öğütcü. (2015), the fat content could significantly affect the moisture parameter of cookies. They concluded that the moisture content of cookies made with commercial bakery shortening (CBS) was higher than the other two cookies made with sunflower wax (SW) and beeswax (BW) oleogels. As it is clear from the texture results, the fluids samples showed the lowest moisture content.

XRD

XRD determined fats' polymorphic crystalline lamellar packing structures (Li and Liu 2019). The XRD results are summarized in Fig. 2. The diffractogram of control spread (100% CBS) showed a major one sharp peak at 4.2 A° conforming β' polymorphic form of triglyceride crystals and weaker peaks at 6.5, 4.25, 4.04, 2.59, 2.38, and 1.65 A°.

Adding oleogel to spread formulation in 20% concentration (CWO/20) showed three sharp peaks at 4.27, 3.41, 6.31 A°, and one weak peak at 4.44 A°, indicating the presence of β´ crystals. According to Zhang et al. (2020), CBS crystallizes as β´ polymorph without tempering. Due to melting temperature, the β′ is a favorable polymorph in chocolates. Also, it provides well dense crystal network structure and creaming properties (Rasouli et al. 2020). By increasing the oleogel content to 50% (CWO/50), the XRD patterns showed the same 4.27, 3.82, and 6.31 A° peaks

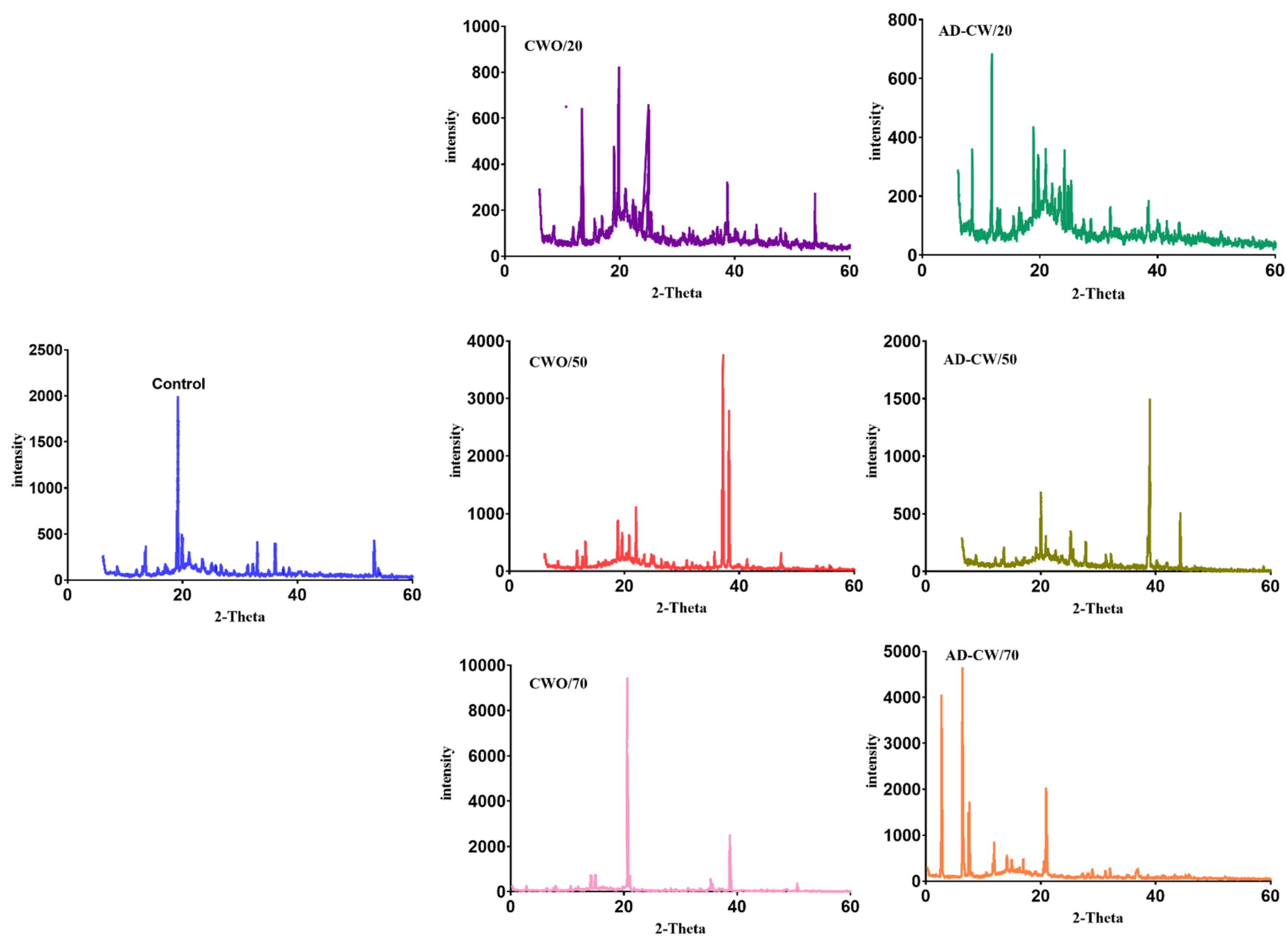

Fig. 2 The x-ray diffraction patterns of the different chocolate spreads, Control (cocoa butter substitute), CW: Carnauba Wax, AD: Adipic acid

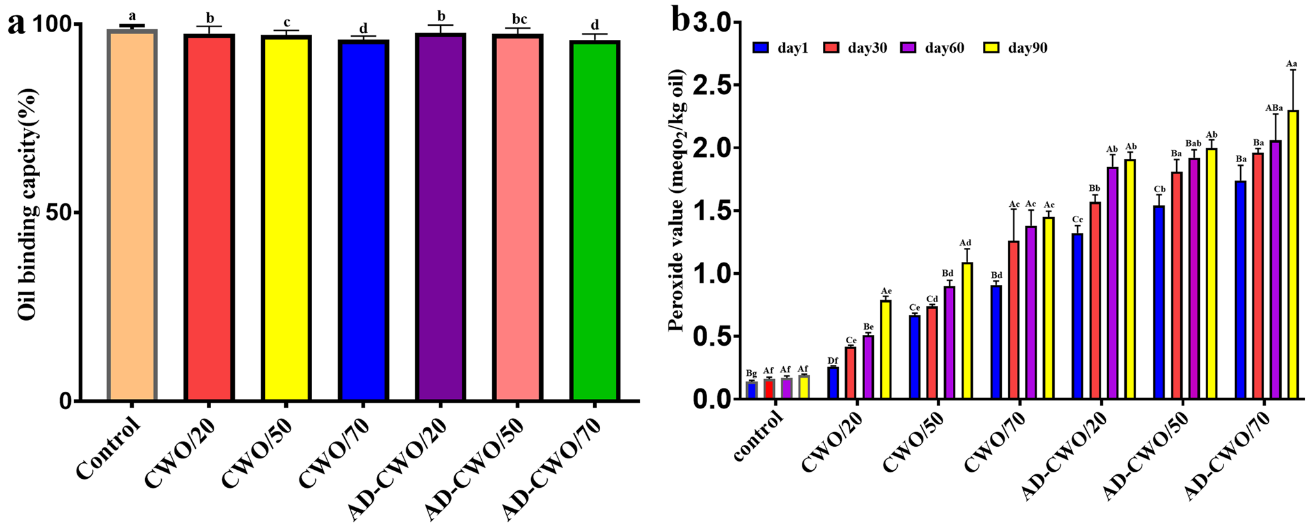

Fig. 3 The oil binding capacity (**a**) and Peroxide values (**b**) of different chocolate spreads. Data are presented as mean ± standard deviation (n = 3). Different lowercase letters indicate significant differences among the spreads (p < 0.05). Different capital letters indicate significant differences between storage times (p < 0.05). Control (cocoa butter substitute), CW: Carnauba Wax, AD: Adipic acid

(related to β´) at a lower intensity and two sharp 2.29 and 2.23 peaks (related to carnauba wax). It was also observed that the overall intensity of the peaks related to β´ polymorphism decreased. However, at (CWO/70) 70% replacement, there was just one sharp peak related to the formation of β´ (3.41 A°) polymorphism. This intensity reduction could be directly attributed to the replacement level or presence of other TAGs in oleogels. According to previous reports, Li and Liu. 2019 the intensity of peak around d = 4.58 Å (refer to β form) in 50% replacement of CB oleogels was mainly smaller than that of CB owing to the reduction of CB content. Also, a major diffraction peak around d = 3.72 Å in oleogel-based chocolates represented a mixture of β′ and β.

The same peaks in these regions (3.74 and 4.16 A°) by many studies (Dassanayake et al. 2009) showed orthorhombic perpendicular sub-cell packing representative of β′ polymorph crystals forms.

At spreads with AD-CWO/20 formulation, one sharp peak around 7.14 (relating to adipic acid) and small, medium peaks with low intensity 4.48, 4.31, 4.04, 3.52 A° were observed. AD-CWO/50 and AD-CWO/70 showed one medium peak at 4.29 A° and two medium peaks at 6.55 and 3.36 A°, respectively. As it is clear, the addition of oleogel has not affected the β´ crystalline form, So the presence of β′ crystal was still dominant. This could be explained by high unsaturated fatty acid content that could affect the fat crystalline phase and stable the β´crystals. XRD results showed highly consistent with texture and OBC.

Oil binding capacity

The OBC in the crystallized network of lipids plays a vital role by indicating the oleogel system's efficiency in immobilizing oil. The OBC values in the different CB: OG formulations and replacement are represented in Fig. 3a. At the same replacement of 20% and 50%, there was no significant difference in OBC values of the fat mixtures prepared with different concentrations of carnauba and adipic acid ($p > 0.05$). In addition, the control sample had the highest OBC. Among the spreads, samples CWO/20 and AD-CWO/20 showed great OBC after the control sample. According to the study conducted by Sun et al. (2021) the OBC of oleogels depends on textural properties, which dense gel crystallization network could maintain more liquid oil in the framework structure of the gel system. Results indicated that the efficiency of carnauba and adipic acid could create a stable intermolecular force to entrap more liquid oil in the gel network up to 50% substitution. XRD results can explain this. The decrease in OBC shows the polymorphic transition process, and the samples were not entirely in the β´ polymorphic form. In addition, the TAG composition of oleogels used in the formulation affects the stability of the spreads by giving more fluidness to spreads.

A similar result was also reported by (Alvarez et al. 2021b) that showed the percentage of replacement of CB by the OG has a significant effect on the OBC of the samples, demonstrating high oil retention capacity by the framework structure of HPMC-based OG up to 30:70 (CB: OG) content. According to the result of the previous study, carnauba wax could provide platelet-like crystalline in the oil matrix to reform the nucleation and crystallization process in the presence of AA up to 4% (Aliasl khiabani et al. 2020). It has been noted that these results appeared to be highly consistent with the texture and the XRD results.

FTIR

The FT-IR spectra of the spread samples are given in Fig. 4. The peaks at 1377 and 1460 cm^{-1} represented the CH3 and CH2 deformations. The peaks (1000–1200 cm^{-1}) relate to the C–C stretching region. In all samples peak at 1744 cm^{-1} is related to the stretching vibrations of carbonyl groups of esters (C=O) and the peak at 1650 cm^{-1} is related to the aromatic group (C=C). The peaks at 1743 cm^{-1} and 1744 cm^{-1}(Sun et al. 2021) are also related to C=O vibrations. According to the study (Aliasl khiabani et al. 2020), in CW6% pure oleogels sample exhibited the specified peak at 3006 cm^{-1}. All samples, except for the sample with the replacement of 20% (CWO, AD-CWO) new peaks 3007.6, 3009.42, 3008.78, and 3008.37 cm^{-1}, in CWO/50, CWO/70, AD-CWO/50 and AD-CWO/70 was observed, respectively. These peaks represent the C-H stretching vibrations. In addition, peaks 2343.37 and 2362.02, 2340.78 cm^{-1} in CWO/50 and AD-CWO/50 were seen, respectively. The variation observed in peaks in the wave number at approximately 725, 1450 cm^{-1}, and in 2800 to 3000 cm^{-1} regions related to C–H (and C=H) stretching. The new peaks included: (1) 3565.07 and 3749.31 cm^{-1} in the CWO/20 (2) 3933.19 and 3724.26 cm^{-1} in the CWO/50 (3) 3680.46 and 3892.11 cm^{-1} in the CWO/70 (4) 3333.40 cm^{-1} in the AD-CWO/50 (5) 3680.22 and 3888.95 cm^{-1} in the AD-CWO/70. Ristanti et al. (2018) studied beeswax-cocoa butter as an oleogelator, and their results showed that the region of 3200–360 cm^{-1} is related to a stronger area of alcohol O–H group at the formula of 50 and 70% replacement. Furthermore, they concluded that hydrogen bonding and Van der Waals interaction result in rigid and bulky fats interaction between oil and beeswax. However, broad absorbance disappeared in higher coca butter content ˃80% formulations.

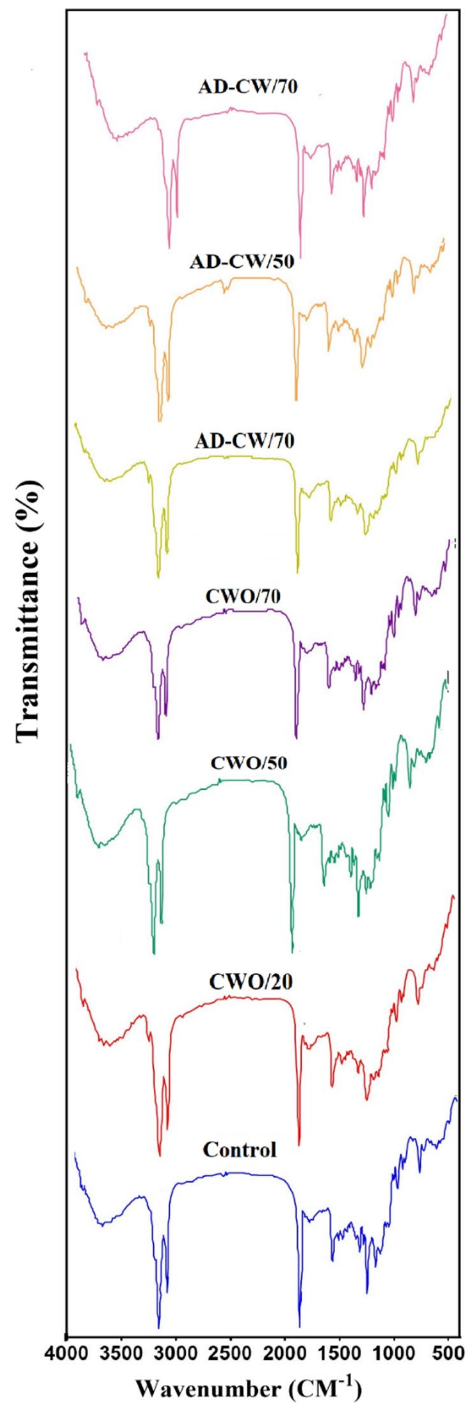

Fig. 4 The Fourier transforms infrared (FT-IR) spectra of different chocolate spreads, Control (cocoa butter substitute), CW: Carnauba Wax, AD: Adipic acid

Oxidative stability

The peroxide value (PV) of spread samples was monitored monthly during 90-day storage time. The lowest PV (0.14 meqO2·kg^{-1}) was determined in the control sample (100%CBS) for 1 day, and the highest PV (2.3 meqO2·kg^{-1}) was measured in the AD-CWO/70 replacement sample stored for 90 days. As shown in Fig. 3b, there was a significant difference between the treatments during the storage period ($p < 0.05$). After 90 days, the PV of AD-CWO was significantly higher than the spreads containing CWO. The PV of all samples showed an increasing trend during storage time. However, there was a gradual increase in the PVs of the control sample throughout the storage time. At both CWO and AD-CWO, the PV of the spreads was below the upper acceptance limits during 90 days of storage. Based on the OBC results, a solid-like structure of 20% replacement led to a compact, rigid structure protecting the oil against oxidation. It indicated that more excellent oxidative stability of carnauba wax-based spreads in comparison with a mixture of CW and AD formulation might attribute to the physical entrapment of oil in the platelet-like crystalline network created by CW.

As Yi et al. (2017) reported, carnauba wax oleogels increased oxidative stability compared to the control oil and had a lower rate of lipid oxidation. In addition, the peroxide values in the higher replacement showed an increasing trend during storage. In agreement with the previous study Öğütcü and Yilmaz. (2014), the oxidative stability of the developed oleogels showed that a solid-like structure strongly affected the oil against oxidation by bounding more liquid oil in the framework structure of the oleogel system. According to Lim et al. (2017), a solid oleogels structure seemed more effective in retarding the oil deterioration than soybean oil in a liquid state.

Conclusion

Using carnauba wax and adipic acid is a strategy to structure a large amount of healthy liquid oil and develop an OG with an improved lipid profile to be used as a CBS fat replacer. This study investigated two different oleogels (CWO and AD-CWO) with three different proportions as cocoa butter alternatives in chocolate spreads formulation. The current study revealed that a higher amount of substitution would increase the amount of unsaturated fat in oils that reduced the hardness. Replacement of CBS with oleogels resulted in lower oil binding capacity and oxidative stability due to the presence of high amounts of liquid-form unsaturated fatty acids. XRD results revealed the presence of β´ crystals in both (CWO and AD-CWO) at three substitutions. The results demonstrated that lower replacement up to 50% concentration positively affected the physiochemical properties. Thus, this study paved the way for the application of (carnauba wax and adipic acid) as a substitute for CBS or other unhealthy fats in the confectionary industry to develop healthy chocolate products with lower saturated and trans fatty acids.

Acknowledgements The authors gratefully acknowledge the supports of the Islamic Azad University, Tabriz Branch.

Authors's contributions LR: Conceptualization, supervision, project administration, writing—original draft. AHK: resources, methodology, investigation, project administration, writing—review & editing. NK: Conceptualization, supervision, writing—review & editing. ST: Methodology, resources. HRP: Methodology, data curation, formal analysis.

Funding The research was supported by the Islamic Azad university of Tabriz.

Declarations

Conflict of interest The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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