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RESEARCH ARTICLE

Effect of Hydrocolloid and Red Palm Oil Oleogel Concentrations on Biphasic Gel Properties and Their Application in Chocolate Spread Formulation

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Abstract

In this study, red palm oil (RPO) rich in β-carotene was structured into biphasic gel to improve its application in the food system. The RPO biphasic gel was evaluated for its physicochemical properties and ability to replace cacao butter in chocolate spread. RPO biphasic gels were prepared at 5%, 7.5% and 10% total hydrocolloids (TH) and 30%, 40% and 50% oleogel concentrations. The results showed that both TH and oleogel concentrations significantly affected the physicochemical properties and stability of the biphasic gels. At TH 7.5% and 10%, adding 50% of oleogel could not produce stable biphasic gels. Higher TH concentration led to higher hardness and G'. Soft gels with hardness ranging from 0.46 - 0.98, water and oil holding capacity ranging from 91% - 99%, and β-carotene concentrations in the range of 140 - 170 ppm were obtained. The biphasic gels with the best physicochemical properties were obtained at 7.5% TH and 30% oleogel concentration. The biphasic gel had minimal influence on the texture of the chocolate spreads. However, the chocolate spread with 100% biphasic gels had high Casson yield stress and low viscosity, i.e.,: 68.59 ± 11.43 and 0.56 ± 0.14 Pa·s, respectively. Overall, the hedonic test revealed that the panelist's acceptance of the chocolate spread prepared with biphasic gel was comparable to that of the control. RPO was successfully used in chocolate spread by transforming it into a semisolid consistency using biphasic gelation.

Keywords: β-carotene, Biphasic gel, Red palm oil, Chocolate spread, Soy protein, Alginate

Introduction

Reducing the prevalence of non-communicable diseases can be done by providing healthy foods that are low in fat and calories as well as rich in active ingredients. β-carotene is a bioactive component that has activity as provitamin A and antioxidant. The benefits of β-carotene for health include preventing cancer, cardiovascular disease, cataracts, and increasing immunity [1]. There are different sources of β-carotene available in nature, one of them is red palm oil. Red palm oil (RPO) is a palm-derived product with a much higher β-carotene content than ordinary palm cooking oil [2]. Nonetheless, the utilization of RPO in food products has not been very extensive due to its liquid consistency at room temperature. Many food products require fats that have a semisolid consistency at room temperature. This property plays a crucial role in the physical characteristics, particularly the texture, of food products. Replacing semisolid or plastic fat with oil without structurization will influence the product's properties. Therefore, an effective method is essential for structuring RPO into a semisolid form at room temperature.

Structuring RPO into a semisolid form enhances its potential use as a fat substitute in food products. This can be achieved by hydrogenation, interesterification, and colloidal structuring. However, some of these methods adversely affect health, one of which is related to the formation of saturated and trans fatty acids due to partial hydrogenation [3]. Colloidal structuring through the formation of colloidal gels such as oleogel, emulsion gel (EG), and biphasic gel (BG) is quite simple and easy to do [4,5]. In addition, colloidal gels can provide a protective effect on the active ingredients inside. Whereas EG has been extensively studied as fat replacer in food system [6,7], BG has not yet been widely explored in food applications. The key difference between emulsion gels (EG) and biphasic gels (BG) is that, in EG, oil is emulsified with water and gelled using hydrocolloids and gelators [4]. In contrast, BG is created by structuring oil into oleogels with the aid of wax. Afterwards, the oleogel will be mixed with a hydrogel. Hydrogel consists of water trapped in a 3-dimensional hydrocolloid matrix. Thus, in one system, there are 2 types of gels [8]. Based on the ratio of hydrogel and oleogel in the system, 3 types of BG can be prepared, namely: Oleogel in the hydrogel, hydrogel in oleogel, and bi-continuous bigel [9]. In BG, water is trapped in the hydrogel, while the oil phase is immobilized by adding a gelator. This system is expected to have better stability against water and oil leaching than EG. BG combines the benefits of both structured phases, improving physicochemical stability and offering a lower fat content compared to animal fats or pure oleogels [10].

Biphasic gelation's potential to structure oil was explored in this study to convert RPO into a semisolid consistency. In this study, biphasic gel from RPO was prepared. Beeswax was used to stabilize the oleogel, while the hydrogel was prepared with the combination of soy protein and alginate. The biphasic gels were prepared with total hydrocolloid (TH) concentrations of 5%, 7.5% and 10%. Its properties and performance in the chocolate spread were evaluated.

Materials and methods

Materials

Red palm oil was obtained from a local market in Bandar Lampung. The RPO contained 633.46 ± 54.6 ppm β-carotene, peroxide value of 3.46 ± 0.40 meq O_2/kg, and Anisidine Value of 0.01 ± 00%. Refined sugar, beeswax, alginate, soy protein isolate, and skimmed powder milk were sourced from local vendor. Cocoa powder was obtained from the Cocoa Research Center, and cocoa butter was sourced from UGM Cocoa Teaching and Learning Industry, Batang, Central Java. Chemical analyses were carried out using analytical grade chemicals procured from Sigma Aldrich.

Red palm oil biphasic gel preparation

Biphasic gels were prepared following the method of Yang *et al.* [11] with modification. RPO oleogel was first prepared using 10% beeswax. Subsequently, the hydrogel phase stabilized by SPC-alginate at a ratio 4:1 (5%, 7.5% and 10% of TH) was prepared. A protein solution was prepared by heating it at 85 °C for 30 min. The heated protein solution was allowed to cool to room temperature. Subsequently, alginate was added to the protein solution. The solution was stirred with a magnetic stirrer at 400 rpm for 60 min and at room temperature to obtain hydrogel. The hydrogel and oleogel phases were mixed at 18,000 rpm for 2 min using Ultra-turrax (IKA T25, Germany) at an oleogel proportion of 30%, 40% and 50%. The biphasic gel was then allowed to stand for 24 h at 10 °C before analysis.

Formulation and preparation of chocolate spread

The formulation and preparation of chocolate spread referred to research performed by Bascuas *et al.* [12] with modification. The formulation of the chocolate spread can be seen in **Table 1**. The process of preparing chocolate spread was initiated with the preparation of all necessary ingredients, including water, powdered sugar, skimmed milk powder, cocoa powder, butter, and RPO biphasic gel. Subsequently, the ingredients were combined and mixed in the melanger (SANTA, USA). The mixture was then homogenized at a speed of 1,440 rpm for 15 min at 60 °C. Once the ingredients were homogenous and the optimal consistency for chocolate spread had been achieved, the mixture was transferred to a 175 mL glass jar. The sample was then stored for further use. In this study, CS referred to chocolate spread with 100% cocoa butter, CSB50 with 50% cocoa butter and 50% BG, and CSB100 with 0% cocoa butter and 100% BG.

Table 1 Chocolate spread formulations (based on 100 g total ingredients).

Chocolate Spreads	Ingredients (g/100g)						
	Sugar	Water	SMP	Cocoa powder	Kalium sorbate	Cococa Butter (CB)	Fat
CS	25	25	26	4	0.08	20	-
CSB50 (50% Cocoa butter replacement)	25	25	26	4	0.08	10	10
CSB100 (100% Cocoa butter replacement)	25	25	26	4	0.08	-	20

Physical and chemical analysis of the biphasic gels and chocolate spread

Gel hardness

The biphasic gel was evaluated for its hardness using a universal testing machine (UTM Zwick/Z0.5, Germany) equipped with a 0.01 N load cell, a probe diameter of 2.17 mm, and a measurement area of 3.67 mm^2. The gel was loaded into a tube (30 mL) and was compressed to a depth of 4 mm at a speed of 5 mm/s. The maximum force was recorded as the hardness value of the biphasic gel.

Water and oil holding capacity

For the determination of water and oil holding capacity, the method described in Lingiardi *et al.* [13] with modification was used. Initially, 5 g of BG was put into a centrifuge tube and centrifuged at 4,000 rpm for 30 min at 25 °C. The tube was then inverted to obtain the released liquid (water and oil) from the sample on a porcelain dish. The porcelain and liquid were then put into an oven at 105 °C until the water evaporated, leaving the oil fraction in the porcelain cup. Water holding capacity (WHC) was calculated as the percentage of water retained after centrifugation (Eq. (1)). Meanwhile, OHC was obtained by calculating the remaining oil in the matrix after centrifugation (Eq. (2)). In which w_1 is the weight of the porcelain cup with the total liquid (water and oil), w_2 is the weight of the porcelain cup after heating in the oven, w_3 is the initial weight of the sample, and w_4 is the weight of the porcelain cup. A similar method was used to determine the WHC and OHC of chocolate spread. However, 1 g of chocolate spread was used instead of 5 g for the chocolate spread.

$$\%WHC = 100 - \left(\frac{W_1 - W_2}{W_3}\right) \times 100 \quad (1)$$

$$\%OHC = 100 - \left(\frac{W_2 - W_4}{W_3}\right) \times 100 \quad (2)$$

Peroxide Value (PV) and Free Fatty Acid (FFA)

Determination of peroxide value was done by iodometric titration using sodium thiosulfate solution as a titrant, referring to the study of Mitrea, Teleky [14]. FFA was analyzed using the method described in AOAC [15].

β-carotene analysis

β-carotene analysis was performed using the method described in Biswas *et al.* [16], with some modifications. The first step was to make a standard curve (0 - 10 ppm) using pure β-carotene by dissolving pure β-carotene with a mixture of ethanol:hexane (2:3) in a 50 mL volumetric flask. The steps for testing emulsion gel samples were almost the same as making a standard curve by taking 0.5 g of emulsion gel sample and then diluting it using 5 mL of ethanol: hexane. The absorbance of the sample was read at 450 nm. The β-carotene content of the sample was calculated using the equation below. X was the β-carotene concentration obtained from the standard curve.

$$\beta - \text{carotene content} \left(\frac{\mu g}{g}\right) = \frac{X\left(\frac{\mu g}{mL}\right) \times volume\ of\ sample\ (mL) \times FP}{weight\ of\ sample\ (g)} \quad (3)$$

Fourier Transform Infrared Spectroscopy (FTIR) analysis

For FTIR analysis, Samples were freeze-dried first and then dissolved in KBr and then scanned at spectral lengths of 4,000 and 500 cm^{-1} at 25 °C. The results are presented as an average of 32 scans at 8 cm^{-1}.

Differential scanning calorimetry (DSC) analysis of BG and chocolate spread

Thermal properties of the BG were studied using the method described by Zhang *et al.* [17] using a DSC (DSC 60 Pus Simadzu, Japan). Approximately 2 mg of the sample was placed in an aluminum pan, and another empty aluminum pan was used as a reference. Analysis was performed in a nitrous atmosphere with a 50 mL/min flow rate at a temperature range of 20 - 150 °C with a heating rate of 5 °C/min.

Microstructure

The microstructure image of the BG and chocolate spread was obtained using a Scanning Electron Microscope (SEM) (Hitachi S-300 N, Japan) with 500× magnification and Polarized Light Microscope (PLM, Olympus BX51, Tokyo, Japan).

Texture analysis

TA-plus texture analyzer instrument (Lloyd Instruments, Australia) was used to evaluate the texture of the chocolate spreads. Samples were compressed using a cylindrical aluminum probe with (diameter: 50 mm) to a depth of 50% of the sample height with a test speed of 1 mm/s at a wait time of 0.5 s with a preload/stress and a preload/stress speed of 1 N and 300 mm/min, respectively. The data was processed using NEXYGEN Plus data analysis software.

pH

pH of the samples was analyzed using a pH meter. The pH test was carried out by dipping the electrode into the gel emulsion until it showed a constant number.

Rheology

A slightly modified version of Saputro *et al.* [18] approach was used to evaluate the chocolate spread's rheological characteristics. The Peltier plate diameters (20, 40 and 40 mm - degree (cone)) of a rheometer (TA instruments-DHR HR10, New Castle) were employed. In order to evaluate the chocolate spread characteristics, the sample was exposed to shear (2 to 50 s^{-1}). Casson rheology was utilized to model the data using the following industry-standard equation:

$$\tau^{\frac{1}{2}} = k\gamma^{\frac{1}{2}} + \tau_0 \quad (4)$$

where τ is shear stress (Pa), is the shear rate (s^{-1}), τ_0 is the slope, and k is the intercept. Cassonyield stress (τ_{0c}) (Pa) is the square of the intercept, and Casson plastic viscosity (ηca) (Pa·s) is the square of the slope. Angular frequency scanning was also performed to obtain the G' (storage modulus) and G" (loss modulus) profile of the chocolate spreads. The scanning was performed from 0.1 - 100 rad/s to evaluate the chocolate spread's structural integrity. These conditions were chosen to mimic the typical shear and oscillatory conditions that chocolate spreads undergo during handling and consumption.

Sensory evaluation

A hedonic test was performed with 46 untrained panelists. Participants were asked to assess the chocolate spread based on its appearance, aroma, taste, texture, spreadability, and overall acceptability. The hedonic test used a 9-point scale (1 = strongly dislike, 9 = extremely like).

Statistical analysis

Experiments were performed in triplicates. Data were analyzed using 1-way analysis of variance (ANOVA) with SPSS software version 25. Differences among samples were considered significant at $p < 0.05$ and further evaluated using multiple range tests. Non-parametric test (Kruskal-wallis and man whitney) was performed on the data of sensory analysis.

Results and discussion

Gels properties

In the first step of this study, the biphasic gels (BG) were prepared at RPO oleogel concentrations of 30%, 40% and 50% to obtain the suitable oleogel concentration in the biphasic gel. The evaluation of its properties is presented in **Table 2**.

Table 2 The properties of biphasic gels which were prepared at 5%, 7.5%, and 10 % of total hydrocolloids (TH) (SPC-Alginate) and 30%, 40% and 50% RPO oleogel concentration.

Parameter	Biphasic Gel								
	TH: 5%			TH: 7.5%			TH: 10%		
	RPO30	RPO40	RPO50	RPO30	RPO40	RPO50	RPO30	RPO40	RPO50
Hardness (N)	0.46 ± 0.05^{c}	0.53 ± 0.08^{c}	0.68 ± 0.07^{d}	0.72 ± 0.02^{d}	0.96 ± 0.04^{e}	NS	0.54 ± 0.08^{c}	0.98 ± 0.09^{e}	NS
WHC (%)	99.44 ± 0.44^{a}	92.77 ± 7.99^{a}	98.03 ± 2.42^{a}	99.79 ± 0.26^{a}	99.19 ± 1.04^{a}	NS	99.59 ± 0.66^{a}	97.42 ± 3.35^{a}	NS
OHC (%)	98.14 ± 2.11^{b}	97.37 ± 4.33^{b}	97.94 ± 1.65^{b}	98.31 ± 2.39^{b}	95.68 ± 6.15^{ab}	NS	91.22 ± 0.42^{a}	93.37 ± 3.20^{ab}	NS
PV (meq O^{2}/kg)	1.52 ± 0.19^{ab}	1.79 ± 0.17^{b}	1.85 ± 0.19^{b}	1.31 ± 0.00^{a}	2.80 ± 0.40^{c}	NS	1.40 ± 0.08^{ab}	2.50 ± 0.34^{c}	NS
β-carotene (μg/g)	114.57 ± 4.10^{a}	172.08 ± 13.05^{c}	160.46 ± 20.71^{bc}	114.87 ± 3.83^{a}	149.45 ± 0.30^{b}	NS	109.87 ± 1.31^{a}	151.52 ± 16.92	NS

Mean ± standard deviation (n = 3) followed by different superscripts in the same row are statistically different ($p < 0.05$). RPO30, RPO40, and RPO50 represented biphasic gel with 30%, 40%, and 50% of oleogel concentration in the biphasic gel.

Hardness

The hardness of the biphasic gels increased as their packing density increased [19]. The packing density is influenced by factors such as hydrocolloid concentration in hydrogel and oleogel to hydrogel ratio. In this study, biphasic gels were produced at 3 different total hydrocolloid concentrations (5%, 7.5% and 10%) and 3 RPO oleogel concentrations. At the same TH concentration, increasing the oleogel concentration led to higher gel hardness, as shown in **Table 2**. However, stable gels could not be obtained at 7.5% and 10% TH concentrations with 50% oleogel. Mixing 50% oleogel with 50% hydrogel at those TH concentrations resulted in a highly unstable gel. It could be that instead of forming oleogel droplets dispersed in the hydrogel, it formed bicontinuous gel [9]. A similar phenomenon at hydrogel:oleogel fraction ratio of 50:50 was also observed by Martins, Guimarães [20]. This might be due to poor incorporation of the oleogel droplets into the polymeric network of the hydrogel. Therefore, the properties of those gels were not measured. When well dispersed, a higher oleogel fraction produces a more compact and dense biphasic gel.

Increasing the concentration of TH in the hydrogel phase was observed to produce gels with higher hardness. This trend is especially clear in biphasic gels, with 40% oleogel concentration. The hardness of the gel increased from 0.54 N (5% TH) to 0.98 N (10% TH). However, biphasic gels with a 30% oil concentration exhibited lower hardness at 10%TH (0.54 N). Higher concentrations of hydrocolloids (protein and polysaccharides) might be responsible for the biphasic gels' hardness. Interaction between soy protein and alginate promotes the development of a stronger network, increasing the hardness of the biphasic gels. A positive correlation between the protein concentration and the hardness of the biphasic gel has been reported previously due to the presence of extra solid particles in the system [19,21].

Water and oil holding capacity

The biphasic gel consists of water in the hydrogel phase and oil in the oleogel phase. Therefore, to evaluate its stability, it is necessary to ensure that the gel has excellent WHC and OHC. Low WHC will cause the water to escape from the matrix of the gel (syneresis). Similarly, poor OHC will cause the oil to be released and found at the surface of the gels, which is undesirable. Gel with low OHC would result in poor sensory qualities due to the release of oil during chewing [22]. Based on **Table 2**, The WHC of the gels ranged from 97% - 99%, which was exceptionally good. In this study, the concentration of TH and RPO did not influence the WHC of the gels.

On the other hand, these 2 factors significantly influenced the OHC of the gels. At 5% TH, oleogel concentrations did not affect the OHC of the gels. Whereas at 7.5% TH, higher RPO oleogel concentrations decreased the OHC. Nevertheless, at 10% TH, biphasic gel with 30% oleogel concentration had a comparable OHC with that prepared with 40% oleogel concentration. Surprisingly, higher concentrations of TH led to lower OHC. At 30% oleogel concentration, the OHC of biphasic gels with 5% TH was comparable to that of 7.5% TH. However, when the TH concentration was increased to 10%, the OHC decreased (91.22%). A similar trend occurred in biphasic gels prepared with 40% RPO oleogel. Biphasic gels with 30% RPO oleogel seemed to produce gels with good WHC and OHC. Saffold and Acevedo [23] reported that as the proportion of oleogel increased, the mean droplet size and the packing density of the droplets increased. This phenomenon might lead to the coalescence of the oleogel phase, which might induce the release of the oil from the biphasic gel; hence, the low OHC was observed.

β-carotene and peroxide value

The β-carotene content of the gels as influenced by TH and oleogel concentration can be seen in **Table 2**. β-carotene content was influenced by the oleogel concentration. Increasing the oleogel concentration from 30% to 40% was shown to increase the β-carotene content of the gel, which was expected. However, in the gel prepared with 5% TH, increasing the oleogel concentration from 40% to 50% resulted in a comparable β-carotene content. This was suggested due to the degradation of β-carotene during the preparation of the gels. It was reported that emulsion gel containing a higher concentration of RPO underwent higher β-carotene degradation than that containing a lower concentration of RPO [24]. TH concentration did not significantly affect the β-carotene content of gels with 30% oleogel concentration. However, at 40% of oleogel concentration, increasing the TH from 5% to 10% reduced the β-carotene content of the gels. A high peroxide value might be caused by oxidation of the oil, which is undesirable. Higher RPO oleogel concentrations at all TH concentrations were shown to have increased the PV of the gels. This could be related to the quality of the RPO used in this study instead of the effect of gel preparation methods. However, the peroxide values of the gels remained low and can be considered within safe limits.

The data shows that biphasic gels prepared at higher oleogel concentrations provided a higher content of β-carotene. However, it was also revealed that higher oleogel concentration resulted in lower OHC, which is critical. Considering these results, biphasic gels prepared with 30% RPO oleogel concentration were chosen for further experiments. Therefore, studies on the functional group and thermal properties of the biphasic gel, as influenced by TH concentration, were performed on biphasic gels containing 30% oleogel concentration.

Microstructure and FTIR spectra of the biphasic gels

Figure 1 shows the microstructure of the biphasic gels observed using SEM. The figure shows the droplet of oleogels embedded in the gel network. The droplet of oleogel seemed to be interconnected, which contributed to the integrity of the matrix. It appeared that biphasic gels with 10% TH had smaller oleogel droplets dispersed in the matrix. However, the droplets of the oleogel seemed to be more clustered than those of lower TH concentrations (5% and 7.5%). In addition, at 5% TH, the gel seemed to have pores, which explains the lower hardness of the gel. The microstructure of the biphasic gel is important for determining the gel's functional properties, such as texture and stability. The spectra of FTIR shown in **Figure 1(d)** exhibited the presence of different functional groups from RPO, SPC, and Alginate. In this part, only the prominent functional groups are discussed. SPC had 1,656 and 1,545 cm^{-1} peaks representing Amide I and Amide II, respectively [25]. The amide I peak shifted to a lower wavenumber in the gels. At 5%, 7.5% and 10% TH, the peak was found at 1,638, 1,640, and 1,645 cm^{-1}. Furthermore, the higher the concentration of TH, the lower the intensity of these peaks. This could be due to greater interaction between SPC and Alginate [26].

Furthermore, the peak representing Amide II was not found in the gels. These phenomena could be due to the interaction of SPC with alginate [26]. In alginate, the bands appeared at 1,613, 1,417 and 1,030 cm^{-1}, representing the -COO- asymmetric and symmetrical telescopic vibration and stretching vibration of C-O, respectively. These peaks were almost undetected in the gels, which could also be due to the electrostatic interaction interaction of alginate with SPC. The peak at 3,000 - 3,500 cm^{-1} of the gels was also shifted, indicating the presence of hydrogen bonds [27]. This could be due to intermolecular hydrogen bonding between the C=O and $-NH_2$ groups of the proteins. The

O-H stretching vibrations, which indicate hydrogen bonding between the SPC-alginate hydrogel matrix and the polar head groups of fatty acyl chains in the oleogel, were observed within this spectral range [28]. This might suggest that the interactions involved in stabilizing the biphasic gel were hydrogen bonds and electrostatic interaction [29]. In addition, the very minor peak at 2,960 cm^{-1} could also indicate that minor hydrophobic interaction might also be involved [29]. In RPO, there were peaks at 2,924 and 2,853 cm^{-1} representing the asymmetric and symmetric stretching of CH_2 groups, respectively. These peaks were only slightly shifted in the gels. On the other hand, the peak observed at 1,746 (stretching vibration of the carbonyl group (C=O) of the triglycerides) was less intense. This could be due to the involvement of this functional group in hydrogen bond formation [30]. In addition, it could also be due to the physical entrapment of the RPO oleogel in the hydrogel. As can be seen, the higher the TH concentration, the less intense this peak was. The absence of new peaks in the FTIR spectra of all bigel samples suggests that there were no chemical interactions between the hydrogel and the oleogel phases [27].

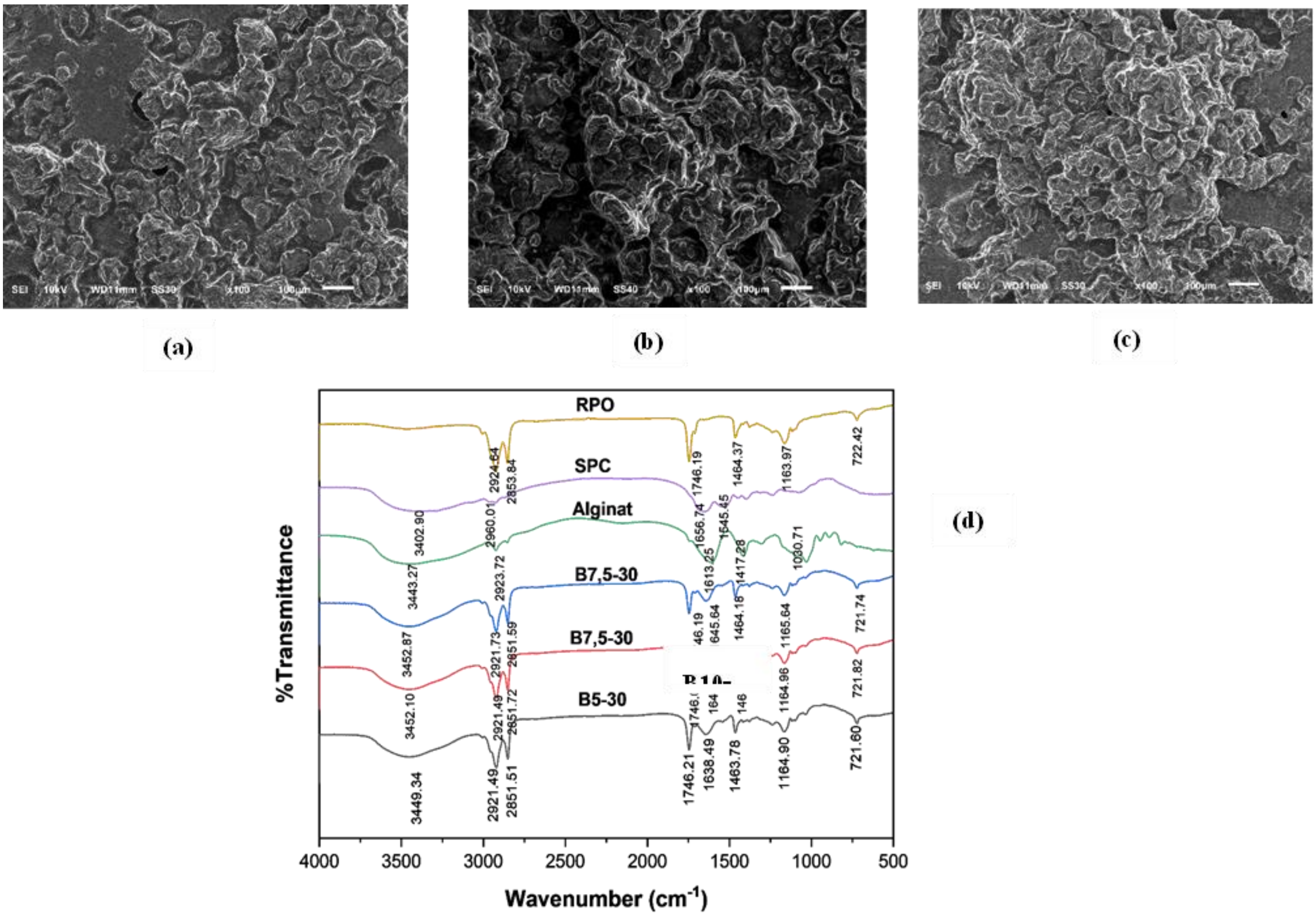

Figure 1 SEM of biphasic gels prepared with 30% of RPO oleogel at 5% (a), 7.5% (b) and 10% (c) of TH and FTIR Spectra of RPO, SPC, Alginate, and Biphasic Gels with 30% RPO oleogel stabilized with 5% (B5-30), 7.5% (B7.5-30), and 10% (B10-30) TH (d).

Rheology of the biphasic gels

Figure 2 illustrates the viscoelastic properties of biphasic gels (BG) formulated with 5%, 7.5%, and 10% total hydrocolloid concentrations, as represented by their storage modulus (G') and loss modulus (G'') over a frequency range of 0.1 to 100 rad/s. All samples demonstrated typical gel-like behavior, where G' was greater than G'' across the entire frequency range, indicating that the elastic (solid-like) response dominated over the viscous (liquid-like) response. As the frequency increased, both G' and G'' also increased, reflecting enhanced resistance to deformation at higher oscillation rates. An increase in total hydrocolloid concentration led to a significant rise in both moduli, with BG10 exhibiting the highest values, followed by BG7.5 and BG5. These results suggested that higher total hydrocolloid content strengthens the gel network, resulting in more rigid and structured biphasic gels [31]. On the other hand, biphasic gel prepared with a low TH concentration (5%) had a relatively weak structure and was less affected by the frequency. Therefore, the total concentration of hydrocolloids plays a crucial role in determining the rheological strength and structural integrity of the biphasic gel system. In addition, no crossover point between G' and G" was observed in all samples, indicating that the biphasic gels can be classified as a gel [23]. This also demonstrated that the biphasic gels could maintain their structure integrity during the measurement, proving their stability [23].

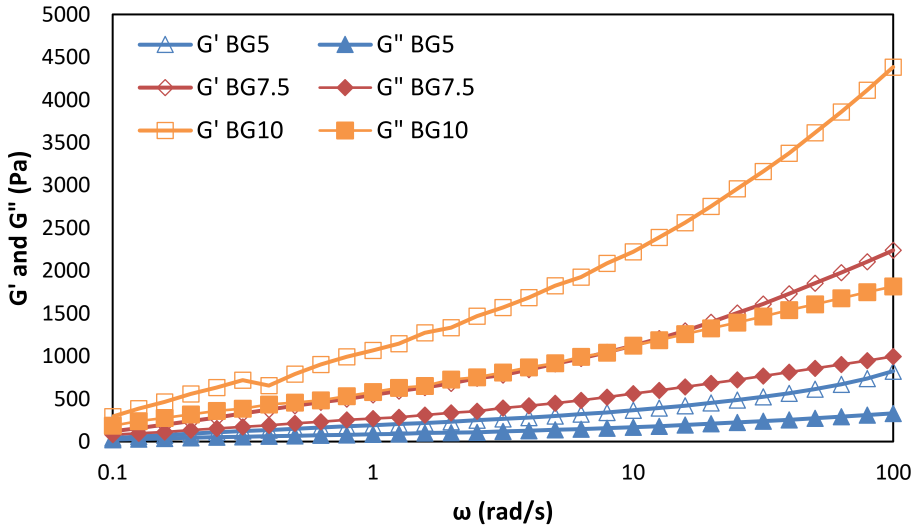

Figure 2 Frequency sweep curves of storage modulus (G') and loss modulus (G'') for biphasic gels (BG) containing 5%, 7.5%, and 10% total hydrocolloids.

Thermal properties

The interaction between the oil phase, gelator, and hydrocolloids in hydrogel influenced the thermal stability of the gels [32]. It can be seen from **Figure 3** that TH concentration influenced the thermal behavior of the gels. Higher TH concentration resulted in a peak with higher temperatures. All gels generally showed good thermal stability with melting points ranging from 100 - 121 °C. The peak observed could be due to denaturation or degradation of the protein-polysaccahrides, a transition from gel to sol, or water evaporation [27,33,34]. A high melting point biphasic gel prepared from a combination of beeswax, a plant sterol, gelatin, and whey protein was also previously reported [27]. On the other hand, free and bound water can be evaporated from biphasic gels during heating [27,33]. Higher water evaporation temperatures in the biphasic gel indicated higher thermal stability, which is important for its commercial application [32]. A higher concentration of TH might provide better water binding capacity for the gel; thus, a higher peak temperature appeared. Therefore, higher concentrations of TH were observed to contribute to the higher thermostability of the biphasic gels. The presence of these hydrocolloids improved and delayed the decomposition of the gels. This phenomenon could be due to the stabilization of the biphasic gel by a combination of soy protein and alginate [27]. Furthermore, the soy protein and alginate were initially microparticulated via heat treatment. Microparticles of soy protein and sodium alginate were reported to have denaturation temperatures in the range of 85 - 117 °C depending on the pH [29].

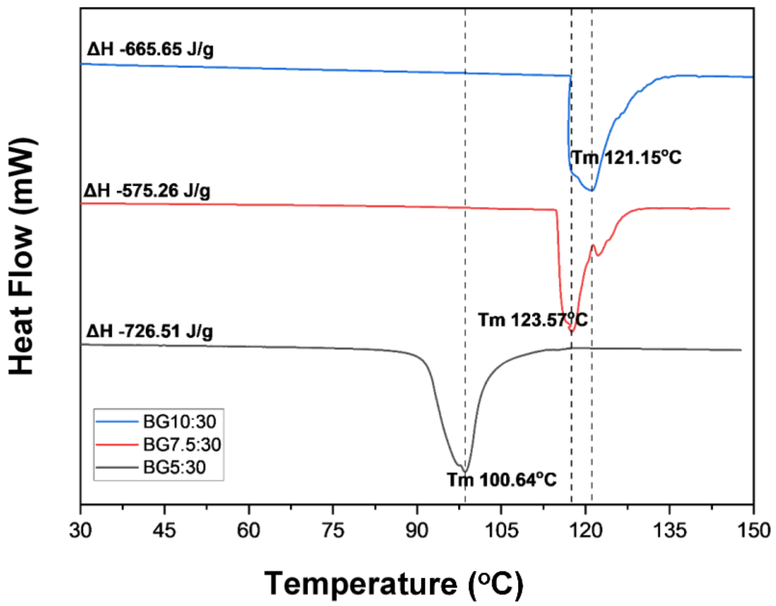

Figure 3 DSC Melting curve of the biphasic gels prepared at different TH oncentrations and RPO oleogel concentrations of 30%.

Applications in chocolate spread

Based on the results presented above, biphasic gel prepared at 7.5% TH and 30% RPO was selected as the best one since it had the highest hardness, good WHC and OHC, good retention of β-carotene, and good thermal stability than gels prepared at 5% and 10% TH. Even though biphasic gels prepared at 10% TH and 30% RPO oleogel concentration had the highest thermal stability, the gel itself had the lowest OHC. Low values of OHC of the gels might cause instability of the chocolate spread, such as oiling off, which is undesirable. The chosen biphasic gel was then used to substitute (50%) and replace (100%) cocoa butter in a chocolate spread. In this study, cacao butter was replaced with biphasic gel (BG) at a 1:1 ratio. Chocolate spreads with biphasic gel were formulated with 50% cocoa butter: 50% biphasic gels (CSB50), and 0% cocoa butter:100% biphasic gels (CSB100).

Table 3 Texture and chemical properties of chocolate spread prepared with biphasic gels (7.5% TH and 30% RPO oleogel) and cocoa butter.

	CSB50	CSB100	CS
Texture			
Hardness (N)	8.88 ± 2.05^{ab}	6.64 ± 0.39^{b}	9.31 ± 1.62^{a}
Cohesiveness	0.17 ± 0.03^{a}	0.15 ± 0.00^{a}	0.16 ± 0.04^{a}
Adhesiveness	30.80 ± 0.32^{a}	28.29 ± 2.62^{a}	54.17 ± 4.33^{b}
Chemical Properties			
pH	7.01 ± 0.06^{c}	6.89 ± 0.07^{b}	6.63 ± 0.05^{a}
PV (meq O_2/kg)	1.52 ± 0.38^{a}	1.63 ± 0.33^{a}	2.02 ± 0.26^{a}
FFA (%)	0.19 ± 0.01^{b}	0.14 ± 0.05^{a}	0.20 ± 0.02^{b}
β-carotene (µg/g)	34.57 ± 0.76^{a}	57.01 ± 5.42^{b}	25.57 ± 1.51^{a}

Mean ± standard deviation (n = 3) followed by different superscripts in the same row are statistically different ($p < 0.05$).

Texture

Replacing 50% of the cocoa butter with biphasic gel did not influence the chocolate spread's hardness (**Table 3**). CSB50 had a comparable hardness to CS. However, further replacing cocoa butter with 100% BG (CSB100) significantly lowered the hardness of the chocolate spread. This could be due to the presence of water in the BG. Higher water content, and thus lower fat content, was suggested to reduce the hardness value. Hardness is related to the spreadability of a spread. Hardness that is too low or too high provides undesirable spread properties. When the hardness is too low, the chocolate spread will not be able to form a nice layer on the surface of the bread. On the other hand, when it's too high, chocolate spread becomes hard to spread on the surface of the bread. The spread properties of the chocolate spread were evaluated using panelists in sensory analysis.

Cohesiveness was not influenced by the substitution of cocoa butter with biphasic gel. This value reflects the strength of internal bonds between particles in chocolate spread [35]. The observed phenomenon could be due to the addition of hydrocolloids in biphasic gels. These gels could interact with other ingredients of the chocolate spread, providing comparable internal bonds to that of the control chocolate spread. On the other hand, adhesiveness was significantly influenced by the substitution of cocoa butter. Substitution of cocoa butter with BG reduced the adhesiveness of the chocolate spread compared to the control. This could be due to the presence of lower fat and higher water fractions. It should be noted that BG contained a 70% concentration of hydrogel and 30% of oleogel concentration. Therefore, the fat content of biphasic gel was lower than that of cocoa butter. In addition, a lower concentration of saturated fat in CSB50 and CSB100 might have contributed to the lower hardness and adhesiveness of the chocolate spread. It was reported that reducing the fat content of a spread resulted in lower adhesiveness [36].

β-carotene, peroxide value, anisidine value, and free fatty acids

Based on **Table 3**, cocoa butter seemed to contain some β-carotene. Substituting 50% of cocoa butter with BG resulted in a chocolate spread with comparable β-carotene to control. However, replacing cocoa butter with 100% BG produced chocolate spread with significantly higher β-carotene content (57 µg/g). 25 g CSB100 could provide 1.4 mg of β-carotene. The presence of 2.1 mg of β-carotene can fulfill the 50% RDA for β-carotene for children aged 7 - 10 years [37]. Thus, 25 g of CSB100 can meet the 30% RDA for β-carotene.

The analysis of peroxide value showed that very limited oxidation occurred in the chocolate spread. The presence of water in the biphasic gel could promote hydrolysis of triacylglycerol. However, our results showed that the FFA value was very low, which was desirable. Thus, the chocolate spread produced exhibited good quality parameters.

Polarized light microscopy

Figure 4 shows the image of the chocolate spread taken using PLM. It can be seen that biphasic gel existed as the microgel in the chocolate spread with a bright center, as highlighted in the image above. The bright color at the center of the microgel is the oleogel concentration. At the same time, the dark color surrounding them could be gel matrix consisting of hydrocolloids. More microgel was found in the chocolate spread prepared with 100% biphasic gel than that prepared with 50% BG. The shearing applied during the preparation of the chocolate spread might cause the continuous biphasic gel to transform into microgel-particulate [38]. The microgel was then dispersed into the matrix of the chocolate spread. In addition, in **Figures 4(b)** and **4(c)**, there were more voids and open areas, indicating the presence of water. This may be related to the increased water content in the network's gaps. A similar observation was obtained by Tirgarian *et al.* [7]; Francis and Chidambaram [39].

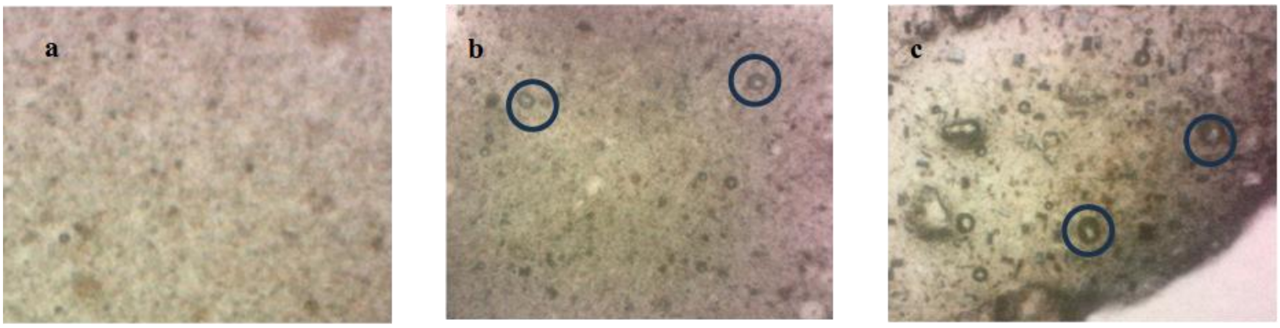

Figure 4 Polarized Light Microscopy of Chocolate spread prepared with 100% cocoa butter (CS) (a), 50% BG (CSB50) (b), and 100% biphasic gel (CSB100) (c).

Flow properties of chocolate spread

The rheological properties of chocolate are influenced by its composition, structure, and processing The flow behavior of chocolate is determined by its composition, structure, and processing. The viscosity profile of the chocolate spreads can be seen in **Figure 5(a)**. Replacement of cocoa butter in chocolate spread with biphasic gels did not alter the flow properties of the chocolate spread. Both SCB100 and SBG100 exhibited shear thinning behavior. The flow profile of the chocolate spreads fitted well with the Casson model, as can be seen by its high R^2 (**Table 4**). From the Casson model, Casson yield and Casson viscosity can be obtained. Casson yield stress represents the stress required to initiate flow, while Casson viscosity shows the energy needed to keep fluid in motion. The Casson viscosity of SCB100 was higher (2.10 Pa·s) than SBG100 (0.56 Pa·s). This was related to the fat content of the chocolate spread, in which higher fat content resulted in higher Casson viscosity. A similar trend was observed by Tirgarian *et al.* [7] in which an increase in water content decreased the Casson viscosity and Casson yield stress. However, in this study, the opposite phenomenon for Casson yield stress was observed. SCB100 required much lower stress to start flowing. Nevertheless, Homayouni Rad and Rasouli Pirouzian [40] reported that an increase in moisture content and the presence of hygroscopic macromolecules could cause particle aggregation, leading to a rise in Casson yield. In addition, this could also be attributed to the presence of solid hydrogel and oleogel particles, which created resistance against the initial flow of the chocolate spread [39]. This means that even though SBG100 required higher energy to start flowing, the chocolate had lower resistance to flow once it did. Nevertheless, the Casson yield for both SCB100 and SBG100 fit within the range of Casson yield stress (10 - 200 Pa) of chocolates.

SCB100 exhibited relatively low G' and G'', with G' slightly higher than G'', indicating a predominantly elastic behavior (**Figure 5**). However, the graph shows that the structure was rather weak, as seen by the slightly higher G' than the G". On the other hand, SBG100 exhibited significantly higher G' and G'' values than SCB100. The big gap between G' and G" reflected a well-developed gel network with strong elastic characteristics. These findings are consistent with the Casson yield value of SBG100, which was much higher than that of SCB100.

Table 4 Casson yield, Casson viscosity, and thixothropy of chocolate spread prepared with cacao butter (SCB100) and biphasic gel (SBG100).

Paramater casson	**SCB100**	**SBG100**
Casson yield stress (Pa)	27.63 ± 3.19^a	68.59 ± 11.43^b
Casson viscosity (Pa·s)	2.10 ± 0.66^a	0.56 ± 0.14^b
R^2	0.95 ± 0.02^a	0.95 ± 0.01^a

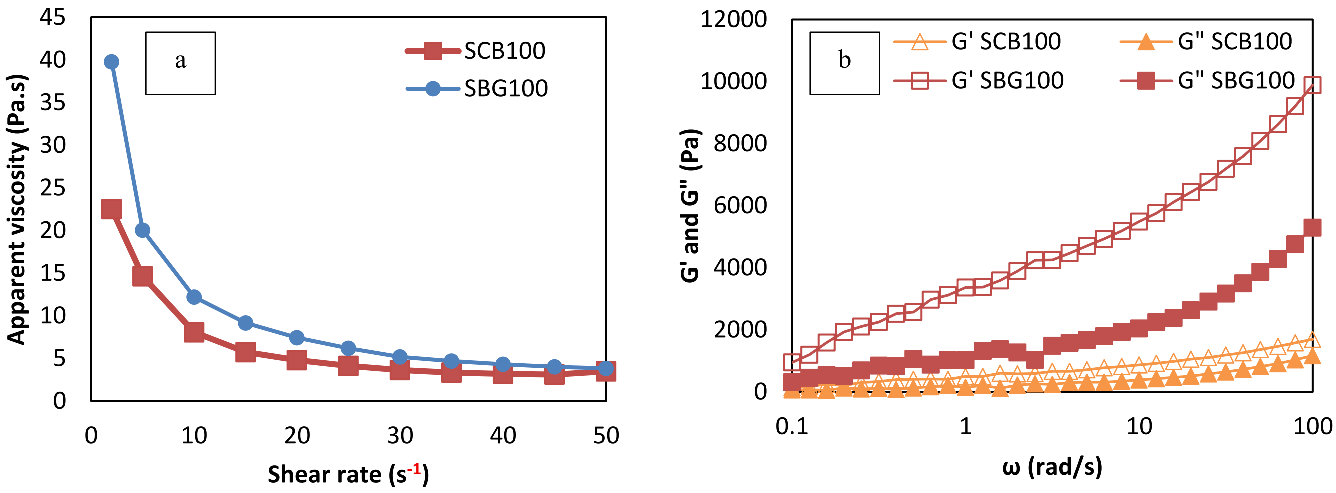

Figure 5 Flow Properties (a), G'and G" profile (b) of chocolate spread prepared with 100% cocoa butter (SCB100) and 100% biphasic gel (SBG100).

Thermal properties

Replacing cocoa butter with BG seemed to influence the thermal behavior of the chocolate spread (**Figure 6**). The thermogram of CS (100% cocoa butter) shows a wide and shallow peak at temperatures of approximately 113 °C. On the other hand, CSB100 had a narrow and sharp peak at higher temperatures (124.37 °C). However, the peak related to the melting temperature of the beeswax in the oleogel phase was not observed in the thermogram. This could be due to the temperature increment applied to the sample during analysis or the effect of the food matrix [41]. It was possible that smaller temperature intervals were required to observe the phenomenon. Principato *et al.* [41] and Alam *et al.* [42] found peaks at temperatures above 100 - 150 °C in hazelnut spread and chocolate filling thermograms, respectively. They suggested this could be due to the melting of sugar in the spread. However, Cropotova *et al.* [43] also reported the presence of a peak at 124.3 - 133 °C in the different formulations of fruit jam filling when studying their thermal properties. The author mentioned that the phenomenon could be attributed to the boiling and evaporation of water. The chocolate spreads in this study all contained water, with CSB100 having more water than CS. The sharp peak at high temperatures in CSB100 could indicate that the water removal from the chocolate spread required more energy since the water was more tightly bound to the biphasic gel. This result is in agreement with the thermal evaluation of the gel. Thus, this chocolate spread has the potential to be used as a filling for biscuits or bread since there is a possibility that it can withstand high temperatures. Fruit filling with higher total solid and contain hydrocolloids had better bakery stability than those with lower total solid and hydrocolloids content [43]. This way, the chocolate spread could maintain its consistency during baking.

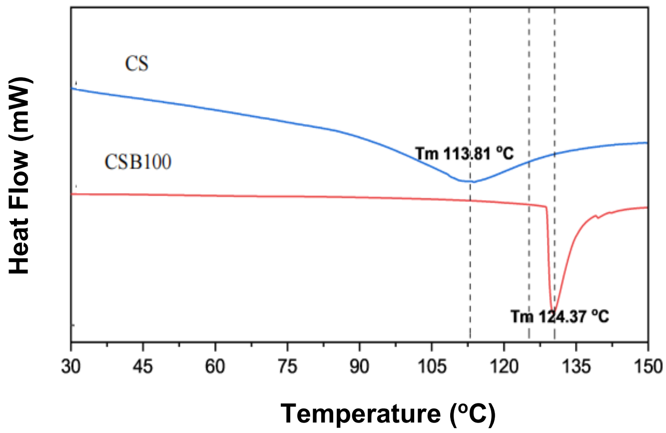

Figure 6 DSC Thermogram of chocolate spread prepared with 100% Cocoa Butter (CS) and 100% BG (CSB100).

Sensory analysis

Sensory analysis was performed to study the preferences of the panelists. The sample tested was chocolate spread control and CSB100 (100% BG). The results can be seen in **Table 5**. Based on **Table 5**. It was observed that the preference of the panelists for CSB100 in terms of appearance, aroma, flavor, texture, and spreadability was comparable to that of CS. Replacing cocoa butter with other types of fat/oil replacer influenced the physical properties of the chocolate spread, as shown by texture analysis in **Table 3**. However, the hedonic test showed that the preference of the panelists for the 2 samples was comparable, meaning that the changes were still in the acceptable range for the panelists. In addition, spreadability, an important parameter of a spread product, was also shown to have a good score. Nevertheless, the overall preference for CSB100 was slightly lower than that of CS. Some panelists mentioned the palmy aftertaste in the samples. Therefore, some masking method, such as adding flavoring agent and essential oil such as vanilla, orange oil, etc., can be performed in the future. Those essential oils have been studied and applied in a chocolate bar, providing a pleasant flavor [44,45].

Table 5 The results of hedonic test of chocolate spread prepared with 100% cocoa butter (CS) and 100%BG (CSB100).

	Score	
	CS	**CSB100**
Appearance	7.12 ± 1.73^a	7.20 ± 1.65^a
Aroma	7.02 ± 1.66^a	5.74 ± 1.79^b
Flavor	7.12 ± 1.71^a	5.22 ± 2.16^b
Texture	6.26 ± 1.94^a	6.80 ± 1.81^a
Spreadability	6.84 ± 1.86^a	7.04 ± 1.77^a
Overall	6.96 ± 1.75^a	6.10 ± 1.89^b

Mean ± standard deviation (n = 3) followed by different superscripts in the same row are statistically different ($p < 0.05$).

Conclusions

Biphasic gelation was able to convert RPO into semisolid consistency. The properties of the biphasic gels were significantly influenced by the concentration of TH in the hydrogel phase. Hardness, oil holding capacity (OHC), and β-carotene content were affected by both TH concentration and RPO oleogel concentration. In the range of TH concentration and oleogel concentration, WHC of all gels was comparable. The gels had a good retention of β-carotene. Furthermore, the biphasic gel of RPO exhibited enhanced thermal stability at higher TH concentrations, which is crucial for its potential commercial application. The stabilization of the biphasic gel was attributed to the interaction of hydrocolloids and oil in the gel, as shown by FTIR analysis. Rheology evaluation showed that higher TH concentration resulted in a stronger structure integrity. However, biphasic gels prepared with 10% TH had the lowest OHC. The replacement of 50% of cocoa butter with biphasic gel in the chocolate spread had minimal impact on its texture. However, a complete replacement of the cocoa butter had more effect on the textural properties, thermal properties, and β-carotene content. In addition, chocolate spread prepared with 100% biphasic gel had higher Casson yield and lower Casson viscosity than chocolate spread with 100% cocoa butter. Nevertheless, sensory analysis revealed that the preference score for SCB100 was comparable to CS for almost all sensory attributes, such as appearance, flavor, aroma, texture, and spreadability. Based on this study, the biphasic gel of RPO worked well in replacing cocoa butter in chocolate spread. These results expand the potential applications of RPO in both food and pharmaceutical products via biphasic gelation.

Nevertheless, this research has not yet explored the long-term stability of the β-carotene provided by the biphasic gelation system. Therefore, the long-term stability of the β-carotene during storage is suggested for evaluation in the future since the delivery system and matrix type highly influence its stability. In addition, some remarks mentioned the palmy aftertaste from the sample contained RPO biphasic gel; thus, careful formulation is needed to mask the palmy aftertaste. Therefore, detailed sensory analysis with a trained sensory panel or quantitative descriptive analysis (QDA) is needed to provide more detailed sensory attributes. Thus, a suitable method to improve the sensory properties of the chocolate spread prepared with RPO-based biphasic gel can be obtained in the future.

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Ethical approval and consent to participate

Ethical committee approval number: Ref. No KE/FK/1626/EC/2024

Declaration of Generative AI in Scientific Writing

The authors acknowledge the use of generative AI tools (e.g., Grammarly) in the preparation of this manuscript, specifically for language editing and grammar correction. No content generation or data interpretation was performed by AI. The authors take full responsibility for the content and conclusions of this work.

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