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Effects of CBD Oil Incorporation on Emulsion Stability

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Abstract **–** Products containing cannabidiol (CBD) has become popular because of its claims of health benefits. Oil-in-water (O/W) emulsions are used in delivery system and CBD could be incorporated in emulsions to make variety of products. However, as CBD is significantly viscous, there is concern over CBD emulsion stability and selection of carrier oils. Hence, this work investigated effects of addition of CBD on emulsion stability. Carrier oils used in the study were virgin coconut oil (VCO), fractionated coconut oil (FCO) and sunflower oil (SO) because these oils are suitable for used in cosmetics, available in Thailand and contain fatty acids and/or bioactive compounds beneficial for skin. Olivem, a natural emulsifier was used in preparation of the emulsion to investigate the possibility of making a natural grade CBD product. It was found that addition of CBD in VCO emulsion resulted in larger emulsion droplets whereas addition of CBD in FCO and SO made droplets of these emulsions became more defined and rounder. Nevertheless, no phase separation was observed in all emulsions stored at 4, 25 and 45°C for a 4-week period. No colour change was observed with all emulsions without CBD and all emulsions with CBD stored at 4°C. Emulsions with CBD stored at 25 and 45°C, however, became darker yellow; the changes were clearer at 45°C. This suggested that oxidation and degradation of CBD occurred. DPPH-scavenging activity of the emulsions stored at the 3 temperatures were also monitored during the 4-week period. Addition of CBD oil improved antioxidant activity stability of the emulsions. Although, the change in colour suggested degradation of CBD, DPPH-scavenging activity of all emulsions with CBD were quite stable. This could be because of the significantly large antioxidant activity of the CBD oil (IC50 = 45 μg/ml). Variation in DPPH-scavenging activity measurements were observed with SO emulsions and distribution and shape of droplets of these emulsions were less homogeneous and round compared to other emulsions. Nevertheless, the variation in DPPH-scavenging activity observed was only around 5% so the emulsion preparation method adopted in this study could be used to make O/W emulsions containing CBD.

Keywords**:** antioxidant activity, CBD, cosmetics, emulsions, stability

1. Introduction

Cannabidiol (CBD) is one of bioactive compounds found in cannabis plant [1]. Recently, use of CBD in various products has been widely discussed in many media. However, little scientific research is available resulting in misinformation and unsubstantiated health claims [2]. Anti-inflammatory and antioxidant effects of CBD have been reported [3]. In addition, CBD's anti-aging and anti-inflammatory benefits are clinically proven [4, 5].

Oil-in-water (O/W) emulsions are commonly used in delivery systems because of their compatibility with water [6]. Concerns regarding emulsions used in cosmetics is often involved stability of emulsions as emulsions are thermodynamically unstable systems with several undesirable physicochemical phenomena [7]. Emulsion droplet size and type of emulsifier or surfactant used have been reported to affect emulsion stability during emulsification and storage [8-10].

Vegetable carrier oils, which are derived mainly from seeds, are common constituent of O/W emulsions used topically [11]. Although the major reason for the use of carrier oils is as diluents, several studies have reported that carrier oils could enhance transdermal penetration for both hydrophilic and lipophilic drugs [12, 13]. Free fatty acids are key components of vegetable carrier oils. Some vegetable carrier oils also contain oil-dissolved vitamin and antioxidants. For example, sunflower oil is rich in linoleic acid and antioxidants and the oil has been reported to provide positive skin effects [14-16]. Virgin coconut oil (VCO) is rich in medium-chain fatty acids with antimicrobial and antiviral effects [17]. Moreover, it has been claimed that VCO has several beneficial health effects such as antioxidant capacity and high level of vitamin E [18, 19].

CBD oil is significantly viscous and is usually diluted in olive oil (in western countries) or coconut oil (in Asia). Although due to regulation, CBD is generally used in a small amount, the viscous oil could affect emulsion droplet sizes and hence emulsion's stability. Emulsion's stability could also affect stability of active ingredients in the emulsion [6]. Hence, this work aimed to study effect of addition of CBD in emulsions on their stabilities.

2. Materials and methods

2.1. Preparation of emulsions

Three carrier oils namely, virgin coconut oil (VCO), fractionated coconut oil (FCO), and sunflower oil (SO) were used in this study. The major reason these oils were selected because the objective of this work was to explore use of oils available in Thailand. In addition, VCO is commonly used to mix with CBD oil to make CBD oil-based products. FCO was also chosen because of its ease in application (lower viscosity compared to virgin coconut oil) and is commonly used in emulsions for cosmetics. Both VCO and FCO contain large amount of medium chain triglycerides (MCT) which has been reported to help enhancing stability of CBD [20]. Apart of the availability and price, SO is of high antioxidant activity and low viscosity [14, 15]. The carrier oils used were obtained from local supermarkets. CBD oil was supplied by Sweetcracker Co., Ltd (Thailand) and chemicals used were of analytical grade. Moreover, the ingredients used are allowed for making natural products.

To observe effects of CBD oil incorporation, two sets of emulsions were prepared: (i) emulsions without CBD oil and (ii) emulsions with CBD oil. Table 1 shows formula used in the emulsion preparation. When the emulsions with CBD oil were made, CBD oil was initially mixed with the selected carrier oil such that CBD composition would be at 1 wt% in the final emulsion; this was the maximum CBD oil could be used to maintain THC content within the allowable percentage Thailand (0.2 wt%).

Table 1: Emulsion formulation for 100 g emulsion

Ingredients	Amount (g)
Olivem	14
Carrier oil or carrier oil mixed with CBD	40
Cetyl alcohol	4
Deionized water	135.2
Glycerine	4
Xanthan gum	0.6
Vitamin E	0.2
Preservative	2

2.2. Optical images of emulsions

Optical images of emulsions were taken at x40 magnification to observe sizes and distributions of emulsion droplets.

2.3. Antioxidant activity measurements

Antioxidant activities of the emulsions were measured using DPPH method as reported by Yusoff et al. [21]. 2 ml of a solution of DPPH in methanol (0.1 mM) was mixed into 2 ml of emulsions. This mixture was placed in a dark place at room temperature for 30 min. Then an absorbance at 517 nm of this mixture was measured using a UV-visible spectrophotometer. This absorbance (A_s) was used to determine DPPH radical scavenging activity using Eq. (1) as follows:

$$\text{DPPH scavenging activity(\%)} = \frac{A_b - A_s}{A_b} \times 100 \quad (1)$$

Where A_b and A_s are absorbance values of a blank and the protein solution, respectively.

Antioxidant activities of the CBD and the carrier oils used in this work were also measured using the same protocol for various concentrations of the oils in methanol. IC_{50} of each oil then was extracted from this result. All measurements were done in triplicate and for each measurement, emulsion was sampled from different position in a container.

2.4. Stability test

Stability test of emulsions were conducted following the method reported by Smaoui et al. [22]. 100 g of each emulsion was placed in a closed plastic container covered with aluminium foil. Emulsion of each formula then was stored at 3 temperatures: 8±2°C, 25±2°C and 45±2°C. Physical changes of the emulsions were observed weekly for 4 weeks. Moreover, DPPH scavenging activity of the emulsions were also measured using the protocol described in 2.3.

3. Results and discussion

3.1. Antioxidant activity of CBD oil and carrier oils

Table 2 illustrates IC50 of CBD oil and the carrier oils used in this study. From the table, CBD had significantly high activity in relative to the carrier oils whereas FCO had the lowest antioxidant activity. From the literature, antioxidant activity of FCO has not been discussed. As the high antioxidant activity of VCO is from the phenolic compounds present in the oil [19], the process of FCO production could remove phenolic compounds from coconut oil or the high temperature used in the FCO production could destroy phenolic compounds or make them inactive.

Table 2: Antioxidant of the carrier oils (IC50)

Oil	IC50 (μg/ml)
CBD	45.0
SO	3,400
VCO	5,580
FCO	126,000

3.3. Optical images of emulsions

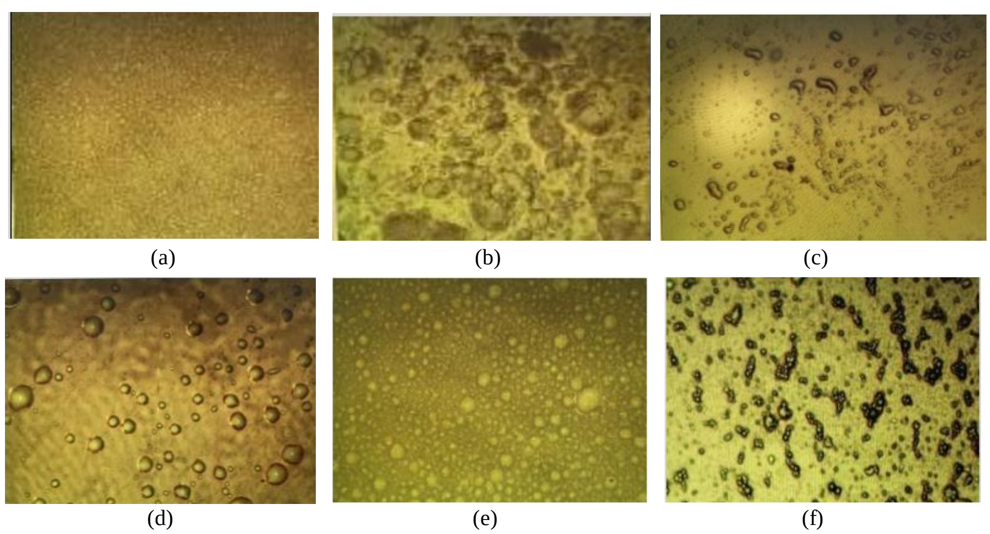


Fig. 1: Optical images of emulsions (x40) made with VCO, FCO and SO without CBD (a, b, c) and with CBD (d, e, f).

Optical images of emulsions (Fig. 1) showed that addition of CBD made VCO emulsion droplet sizes became larger. Emulsions made with FCO and SO, however, showed rounder emulsion droplets with addition of CBD. Addition of CBD could affect viscosity of FCO and SO which are less viscous compared to VCO. Further work must be done to confirm this.

3.3. Stability of the emulsions

Fig. 2: Emulsions containing CBD with FCO as a carrier oil at (a) week 0 and at week 4 at storage temperatures of (b) 4°C, (c) 25°C and (d) 45°

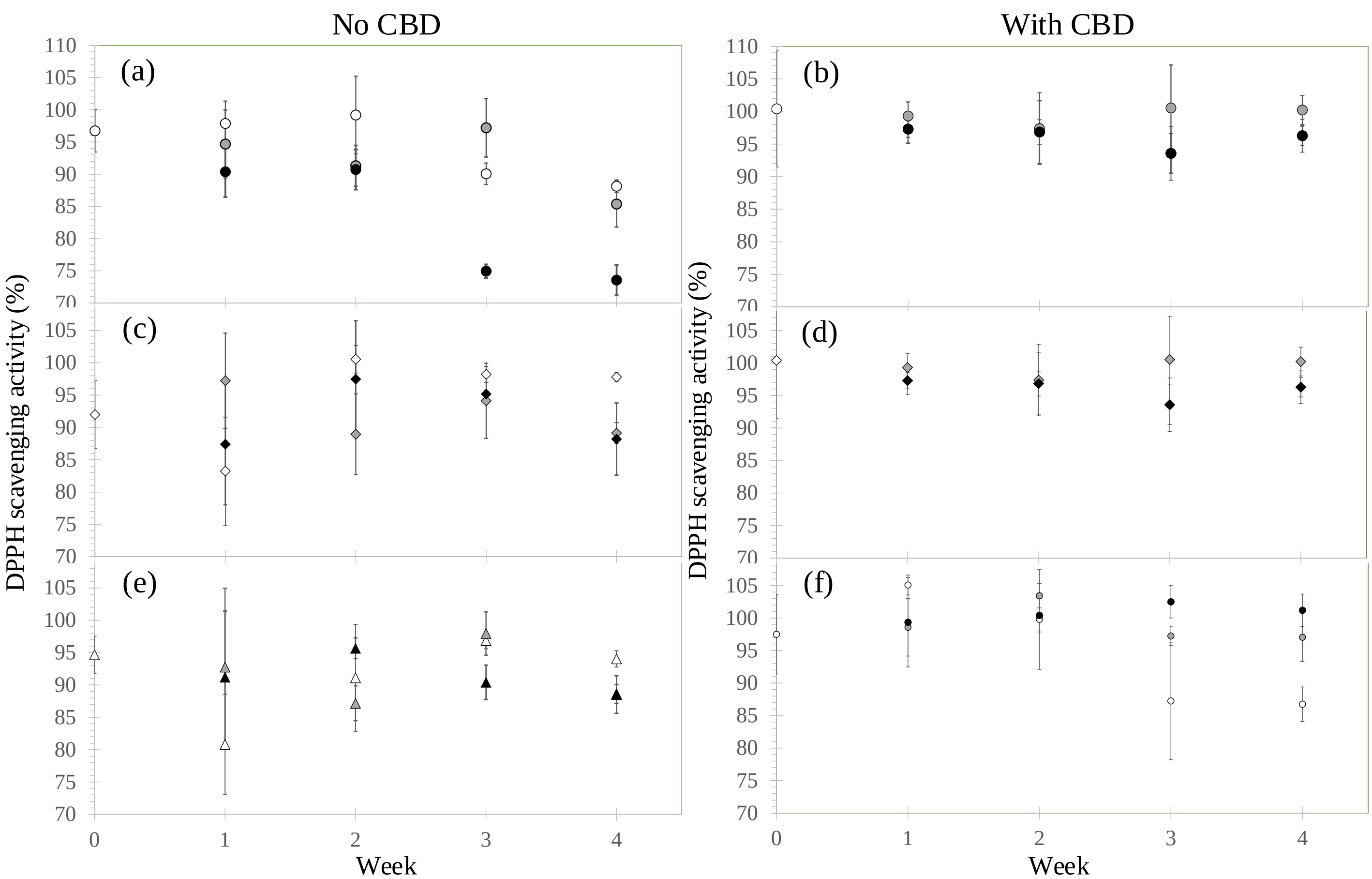
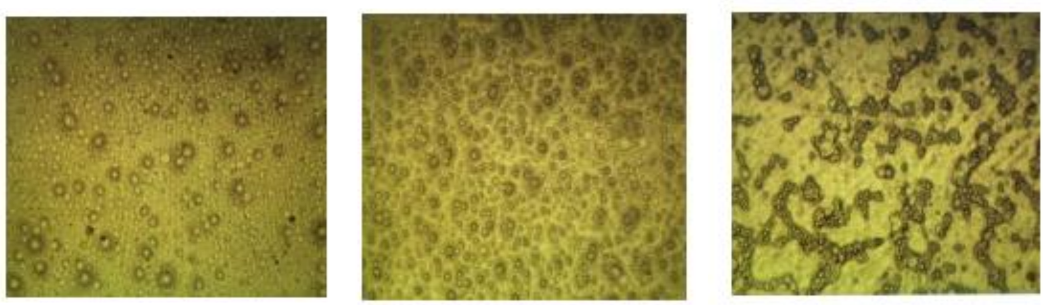


Fig. 3: DPPH scavenging activity (%) of the emulsions prepared with (a) VCO (circle symbol), (c) FCO (diamond symbol) and (e) SSO (triangle symbol) and with CBD and (b) VCO, (d) FCO and (f) SO stored at 4°C (open symbol), 25°C (grey symbol) and 45°C (black symbol) for 4-week period

No noticeable physical change (phase separation and colour change) of the emulsions prepared without CBD was observed during the 4-week period. The emulsions containing CBD also showed no phase separation even when addition of of CBD to VCO emulsion resulted in bigger emulsion droplets (Fig. 1). However, colour changes were observed. Similar colour changes were observed among the emulsions containing CBD with different carrier oils so only the results of the emulsion made with FCO are shown in Fig. 1. According to the figure, colour changes of the emulsions stored at 4°C and 25°C were slight whereas the change of the emulsion stored at 45ºC was significant; the emulsion became pale yellow after 4 weeks of storage. Slight change in colour of a mixture of CBD and sunflower oil (5mg CBD in 1 ml sunflower oil) was also observed when the mixture was stored at 40°C for longer than 180 days; the mixture became darker yellow [23]. The work also found degradation of CBD associated with storing the oil mixture at high temperature and the degradation was suggested to be a result of oil oxidation [23].

Stability results of antioxidant activity of the emulsions are shown in Fig. 3. According to Fig. 3 (a), (c) and (e), antioxidant activity of the emulsions made using FCO and SO stored at different temperatures did not change significantly with time. Similar observation was made with the emulsion made using VCO stored at 4°C and 25°C. However, antioxidant activity of the VCO emulsion stored at 45°C clearly dropped with increasing storage time. Since it has been reported that phenolic compounds could be responsible for antioxidant activity of VCO [19] and phenolic compounds in VCO decreased with time during its storage [24], the decrease in antioxidant activity of the VCO emulsion stored at 45°C suggested the drop of phenolic compounds present in the emulsion.

Nevertheless, with addition of CBD, antioxidant activities of all emulsions stored at all temperatures were stable (Fig. 3 (b), (d) and (f)). From Table 2, antioxidant activity of CBD oil used in this study was significantly larger than the activities of the carrier oils used. Hence, the results in Fig. 3(b), (d) and (f) suggested that even with oxidation that could occur during the storage of the emulsions, a large number of antioxidants from CBD could remained. Consequently, DPPH-scavenging activity of the emulsions measured using the protocol in this work did not change significantly.



(a) (b) (c)

Fig. 4: Optical images of emulsions (x40) made with SO with CBD addition stored at (a) 4°C, (b) 25°C and (c) 45°C.

Fig. 3(f) illustrates that the emulsion made with SO and CBD stored at 4°C showed larger variation compared to the same type of emulsion stored at higher temperatures. Such variation was also observed when SO emulsion was made without CBD and stored at 4°C (Fig. 2(e)). The variation came from taking emulsion samples from 3 different locations in the container. This suggested the uneven distribution of SO and SO and CBD. The uneven distribution of emulsion droplets in SO emulsion without CBD has been shown previously in Fig. 2. Nevertheless, it should be noted that variation in DPPH-scavenging activity measurements of SO emulsions with CBD stored at higher temperatures (25 and 45°C) was insignificant. Optical images of SO emulsions with CBD stored at the three temperatures at the end of week 1 of storage were taken and shown in Fig. 4. From the figure, there were redistribution of emulsion droplets; emulsion droplets became rounder and smaller. Coalescence of emulsion droplets were seen in Fig. 4(c). The redistribution of emulsion droplets could be due to mass transfer. As mass transfer rate increases with temperature, redistribution of emulsion droplets occurred more in the emulsions stored at 25 and 45°C. Optical images of SO emulsions with CBD addition at weeks 2, 3 and 4 were also The figures were not shown because there was no clear difference apart from more coalescence occurred. Nevertheless, differences in distribution of emulsion droplets of SO emulsions with CBD addition did not cause phase separation and variation in DPPH scavenging activity measurements observed with the sample stored at 4°C was only around 5%, the emulsion preparation method used in this study is appropriate for making O/W emulsions containing CBD.

4. Conclusion

Incorporation of CBD oil in emulsions could improve antioxidant activity stability of the emulsions. Although addition of CBD oil made emulsion droplets of the emulsion made using VCO as a carrier oil became larger, emulsion droplets of the emulsions made using FCO and SO as carrier oils were smaller and rounder. Nevertheless, all emulsions stored at 4, 25 and 45°C for 4 weeks showed no phase separation. Hence, Olivem which is a natural emulsifier is appropriate for making emulsions with CBD and there is possibility of developing natural grade cosmetics containing CBD. However, there was change in colour of emulsions containing CBD, particularly those stored at high temperatures. This change suggested that oxidation and CBD degradation occurred. However, this did not affect antioxidant activity of the emulsions. Addition of CBD also made antioxidant activity of all emulsions more stable. Variation in antioxidant activity of SO emulsions with CBD addition was observed and this could be due to the uneven distribution of emulsion droplets. Nevertheless, the variation was only around 5% and the uneven distribution of emulsion droplets did not cause phase separation.

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