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Storage Study on Partitioning Characteristics of β-Carotene in Emulsions with Different Surfactants and Solid Fat Contents

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ABSTRACT

A relatively less explored area of research in the encapsulation of palm-based phytonutrients is the location and partitioning of these bioactive compounds stabilised in emulsion system. Different food products with different chemical compositions and biological environment are to be fortified with certain bioactive compounds stabilised by emulsion systems, requiring different strategies to ensure success in fulfilling the purpose of the functional foods. Furthermore, considering the ultimate purpose of a functional food enriched with bioactive compounds encapsulated in an emulsion system, which is to give health benefits upon consumption, the knowledge of the partitioning characteristics of phytonutrients encapsulated in an emulsion is also undeniably relevant. The objective of this study was to determine the partitioning characteristics of phytonutrient (β-carotene) in emulsions when varying the surfactant and solid fat content of the oil phase upon storage. Emulsions were made by mixing two immiscible phases (oil phase and aqueous phase) together with surfactant, bioactive compound and xanthan gum using a mixer-homogeniser with a constant speed for 30 minutes. Partitioning analysis was done on the emulsion samples varying in surfactant and solid fat content of the oil phase. The analysis was repeated once every seven days for 28 days. The obtained results were then being analysed univariately. From this study, it showed that solid fat content increased the partitioning of bioactive compounds into the aqueous phase and ovalette retained the most of bioactive compounds in the aqueous phase.

Keywords: Carotene, bioactive, partitioning, emulsion, encapsulation

INTRODUCTION

Phytonutrients are plant nutrients that have many health benefits towards human health. There are a wide variety of plant sources containing different types of phytonutrients and palm oil is one of them. Palm oil differs from other plant and animal oil in that it is composed of 50% saturated fatty acids, 40% unsaturated fatty acids and

10% polyunsaturated fatty acids (Boateng et al., 2016). The fruits also include nutrient and health-promoting compounds that can be found in the oil. Carotenoids, tocopherols, and other phytonutrients like squalene, phenolic, and coenzyme Q10 are abundant in palm oil. Hitherto, most applications have focused on the usage of bulk fat in food products. A liquid fraction (palm olein) and a more solid fraction (palm stearin) can be separated from the refined, bleached and dioderised (RBD) palm oil (Wan Mohamad, 2018). RBD palm oil is refined from crude palm oil that undergo bleaching and deodorization process (Loganathan et al., 2010). Following the discovery that the phytochemicals in palm oil have health benefits, a food-grade red palm oil product rich in palm bioactive, primarily β-carotene, was developed. β-carotene is a plant-produced secondary metabolite that belongs to the carotenoids' unoxidised chemical group (Bogacz-Radomska & Harasym, 2018).

An emulsion system can be used to encapsulate bioactive compounds that are prone to oxidation, such as carotenoid groups in palm oil, and to avoid degradation (Shin et al., 2015, Zhang et al., 2019). Encapsulation through emulsification can improve the stability of phytonutrients during storage and processing. However, the ingredients in emulsion can affect the partitioning of the bioactive in emulsion. The partitioning characteristics of the bioactive component, which is either hydrophobic or lipophobic and enclosed inside the system, is particularly critical to stabilising the molecule in the emulsion system. Because the molecular environment determines the chemical stability of the lipophilic component, the bioactive partitioning within an emulsion affects the protection afforded against degradation (Cornacchia & Roos, 2011a). On the other hand, chemical and physical variables might limit the stability of bioactive compounds, resulting in degradation and losses (Cornacchia & Roos, 2011b).

In this study, food-grade oil-in-water (O/W) emulsions stabilised by different types of surfactants such as glycerol monostearate (GMS), ovalette, polysorbate 20 (Tween 20) and lecithin were produced. The principal function of a surfactant is to reduce surface and interfacial tension and stabilise the interface (Susanna Laurén, 2018). Besides surfactant, the solid fat content of the emulsions was also varied, and these two factors were evaluated for their effects on the partitioning characteristics and physical stability of β-carotene encapsulated in the emulsion systems upon storage. The knowledge on partitioning characteristics of phytonutrients within an emulsion is crucial in designing a stable encapsulation system in functional foods enriched with bioactive compounds that deliver various health benefits to the consumers.

MATERIALS AND METHODS

Materials

RBD palm olein and palm shortening were used in this study, which combination served as the dispersed phase of the O/W emulsion samples. β-carotene as the encapsulated bioactive compound was sourced from red palm oil. These palm-based products were purchased from a local supermarket in Besut, Terengganu. Different surfactants were used in this study including lecithin, GMS, ovalette and Tween 20, which were all food grade and purchased form Evacaely Enterprise. Xanthan gum was also incorporated into the samples as a stabilizing agent. For the extraction-spectrophotometric method, the following chemicals was used: dimethyl sulfoxide (DMSO), n-hexane and 100% ethanol. UV-Vis spectrophotometer UV-1280 (Shimadzu, Malaysia) was used for partitioning analysis.

Sample Preparation

The formulation for each sample is shown in Table 1 and Table 2. All samples were O/W emulsions prepared by homogenizing lipid phase with aqueous phase. First, all of the ingredients were weighed. For samples with different solid fat contents, the solid fat (shortening) was solubilised in palm olein by stirring at 140 °C for two minutes. Then, the red palm oil containing β-carotene was added to the lipid phase (i.e. palm olein with shortening) and mixed together using a magnetic stirrer for approximately 30 minutes at 140 °C in the dark. Since lecithin and GMS are oil soluble, each was added directly into the lipid phase and mixed to give a homogenous lipid phase, while ovalette and Tween 20 being water soluble, was each mixed with deionised water

until dissolved. Xanthan gum was added into the aqueous phase and thoroughly mixed by magnetic stirring. Macroemulsions were then formed by homogenising the lipid phase with the aqueous phase using a homogeniser (D-500 Homogeniser, Wiggen Hauser, Berlin, Germany) with a constant speed for 30 minutes under a preset processing pressure (Wan Mohamad, 2018).

Table 1. Formulation of O/W emulsions with different surfactants.

Ingredients	**Lecithin-stabilised emulsion (w/w%)**	**GMS-stabilised emulsion (w/w%)**	**Ovalette-stabilised emulsion (w/w%)**	**Tween 20-stabilised emulsion (w/w%)**
Red palm oil	1.0	1.0	1.0	1.0
Palm olein	24.0	24.0	24.0	24.0
Deionised water	74.0	74.0	74.0	74.0
Xanthan gum	0.5	0.5	0.5	0.5
Lecithin	0.5	-	-	-
GMS	-	0.5	-	-
Ovalette	-	-	0.5	-
Tween 20	-	-	-	0.5

Table 2. Formulation of O/W emulsions with different solid fat contents.

Ingredients	**0% (w/w %)**	**1% (w/w %)**	**2% (w/w %)**	**3% (w/w %)**	**4% (w/w %)**
Palm olein	24	23	22	21	20
Palm shortening	-	1	2	3	4
Red palm oil	1	1	1	1	1
Lecithin	0.5	0.5	0.5	0.5	0.5
Xanthan gum	0.5	0.5	0.5	0.5	0.5
Deionised water	74	74	74	74	74

Partitioning of β-carotene

Concentration of β-carotene within the emulsion droplets was determined using UV-Vis spectroscopy with wavelength of 450 nm following the method described by Wan Mohamad Fahmi et al. (2017). A standard curve for the concentration of β-carotene in red palm oil was plotted before conducting the analysis. The analysis was conducted both for whole emulsion and for oil phase.

For the extraction of β-carotene in whole emulsion, 200 mg of the emulsion sample was weighed into a 15-mL centrifuge tube. Then, 4 mL of dimethyl sulfoxide (DMSO) was added and hand-shakenly mixed. The tube was placed into a water bath at 75 °C for 5 minutes to dissolve the sample in the solvent. Then the tube was cooled at room temperature before extraction. The non-polar β-carotene compound in the emulsion was extracted using 4 mL of n-hexane, which was added into the tube and vortexed for 10 seconds. Then, it was left in the dark for 30 minutes to let the solution separated well. After that, a few drops of ethanol were added into the tube to prevent emulsion formation during the process. The top layer formed was pipetted into 10 mL volumetric flask using a glass Pasteur pipette. This step was repeated twice using 4 mL of n-hexane. The extraction obtained were combined and filled with n-hexane up to the flask level mark. Then, the extract was

filled into a glass cuvette and analysed using a UV-Vis spectrophotometer at the maximum absorbance wavelength for β-carotene (λ_{max} = 450 nm). A pure n-hexane solution was used as a blank.

The extraction of β-carotene in lipid phase was performed using the same procedures as above but preceded with the centrifugation of 10-g emulsion samples at 7000 g for 30 minutes at 25 °C, to allow separation between the lipid and aqueous phases. The concentration of β-carotene in the aqueous phase was then calculated using the difference in concentration of β-carotene in whole emulsion and lipid phase.

Storage study

The emulsion samples were kept at room temperature of 25 °C for 28 days. The partitioning of β-carotene was analysed once every 7 days throughout the storage period.

Univariate Analysis

Statistical analysis was carried out using SPSS 20.0 for Windows (SPSS Statistical software, SPCC Inc., USA). All samples were replicated twice and triplicate readings were taken for each sample for accuracy. One-way analysis of variance (ANOVA) was carried out to determine the presence of statistical significance and Dunken's range test was used as a post hoc test to examine significant differences between the samples. All data were presented as mean values ± standard deviations.

RESULTS AND DISCUSSION

Effects of different surfactants on partitioning of β-carotene in emulsions

Surfactants are typically employed to prevent emulsions from aggregating by forming a protective membrane around the droplets. Four different types of surfactants were used to examine their impact on partitioning of β-carotene in oil and aqueous phase. In this study, the difference in partitioning characteristic of β-carotene in oil-in-water emulsions using lecithin, GMS, ovalette and Tween 20 as the emulsifier, were compared towards each other.

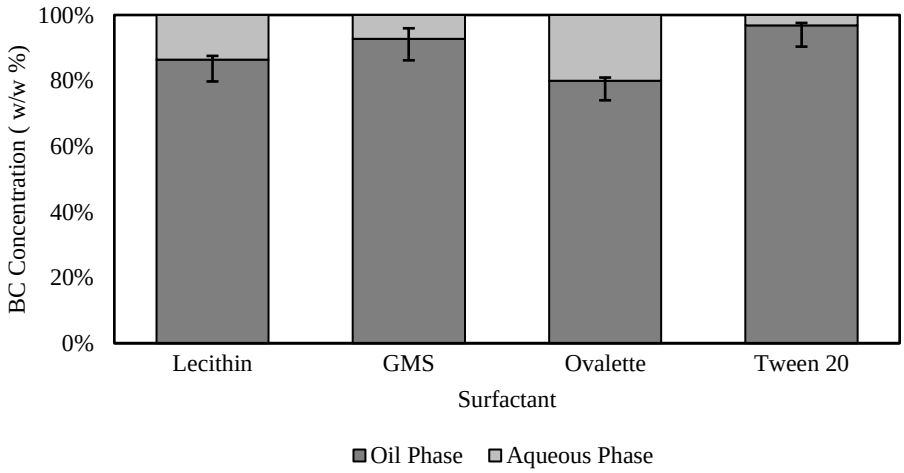

Fig. 1. Partitioning of β-carotene (BC) between the oil and aqueous phases of emulsions with different surfactants; lecithin, GMS, ovalette and Tween 20. Data are means ± standard deviations.

Based on Fig. 1, the use of ovalette results in a higher partitioning of β-carotene into the aqueous phase of emulsions (3.35 ± 0.65% w/w) than in lecithin (2.07 ± 0.13% w/w), GMS (1.10 ± 0.47% w/w) and Tween 20 emulsions (0.50 ±0.02% w/w), (1.11 ±0.47 % w/w), and (0.48 ± 0.09% w/w), respectively. The fact that partitioning was observed in all samples indicates the interactions between both surfactants and β-carotene in the aqueous phase.

In the dispersed and/or continuous phases, there is frequently enough free surfactant to form micelles or reverse micelles (McClements, 2004). Micelles can solubilise non-polar molecules in their hydrophobic interior, enhancing their affinity for the aqueous phase (Suratkar & Mahapatra, 2000). They may also solubilise amphiphilic molecules between the head groups of hydrophilic surfactants (Suratkar & Mahapatra, 2000). In the same way, reverse micelles in an oil phase can solubilise polar and amphiphilic molecules within their structures. According to McClements (2004), a hydrophobic surfactant with a low hydrophilic-lipophilic balance (HLB) number (3–6) dissolves preferentially in oil, stabilises W/O emulsions, and forms reverse micelles in oil. A surfactant with high HLB number (10–18) is predominantly hydrophilic, dissolves preferentially in water, stabilises O/W emulsions and forms micelles in water. The presence of micelles and/or reverse micelles in a pure liquid in an emulsion may thus alter the distribution of bioactive molecules among the oil phase, aqueous phase, and headspace regions, altering the system's perceived bioactive (McClements, 2004).

In this study, the interaction between each surfactant and β-carotene is expected due to the different emulsifier's molecular structures and conformations, which affect the different distributions of polar or non-polar groups as binding sites (Wan Mohamad, 2018). The reason behind the partitioning of β-carotene in aqueous phase is basically due to the attraction of the β-carotene towards the hydrophobic binding sites in the surfactant. Between all four types of surfactants, ovalette-stabilised emulsion resulting the highest partitioning of β-carotene, which may be due to the higher availability of the hydrophobic binding sites in ovalette (i.e. monoglycerides, polyglycerols and polysorbate). For lecithin, GMS and Tween 20, the partitioning characteristics follow their HLB values. Among these three surfactants, lecithin has the lowest HLB value (4), followed by GMS (4.8) and Tween 20 (16.7). The smaller the HLB value, the more hydrophobic is the surfactant.

Effects of different surfactants on partitioning of β-carotene in O/W emulsions upon storage

The β-carotene concentration changed with time for each O/W emulsion sample with different types of surfactant as shown in Fig. 2. Statistically, the concentrations of β-carotene in whole emulsions upon storage were significantly different ($p<0.05$) when different types of surfactant being used. However in general, Fig. 2 indicates the degradation of β-carotene within the different emulsion systems. Throughout the storage, ovalette sustain the highest concentration of β-carotene in the emulsion system followed by lecithin-stabilised emulsion, GMS and Tween 20. Low β-carotene degradation in lecithin-stabilised oil-in-water emulsions was also reported by (Helgason et al., 2009).

The higher concentration of β-carotene in ovalette-stabilised emulsion upon storage may be due to the structure of ovalette, which is made from a mix of emulsifiers such as monoglycerides, polyglycerols and polysorbate (Eva Rosenberg, 2021). On the other hand, for Tween 20-stabilised emulsion, it can be seen that the rate of degradation of the β-carotene concentration is quite high compared to others. This could be attributed to pre-existence of higher levels of peroxides in Tween 20 surfactant, which can oxidise the β-carotene present in Tween 20 micelles (Hejri et al., 2013).

According to McClements (2012), β-carotene is likely to be migrated and partitioned between the oil and aqueous phases of the emulsion system. A previous study shows that β-carotene degraded over the time and the degradation rate of the β-carotene in oil phase was much faster than in aqueous phase (Wan Mohamad, 2018). As shown in Fig. 2, for all emulsion samples, the concentration of β-carotene is higher in aqueous phase compared to the oil phase from day 14 and beyond. This may be due to the strong binding sites of the surfactant molecules in the aqueous phase of emulsions towards the bioactive i.e. β-carotene (Wan Mohamad, 2018). Overall, the ovalette-stabilised emulsion depicts the highest capability in providing a protective membrane

around the oil droplets and to form micelles in the aqueous phase of emulsion to solubilise the non-polar molecules of β-carotene.

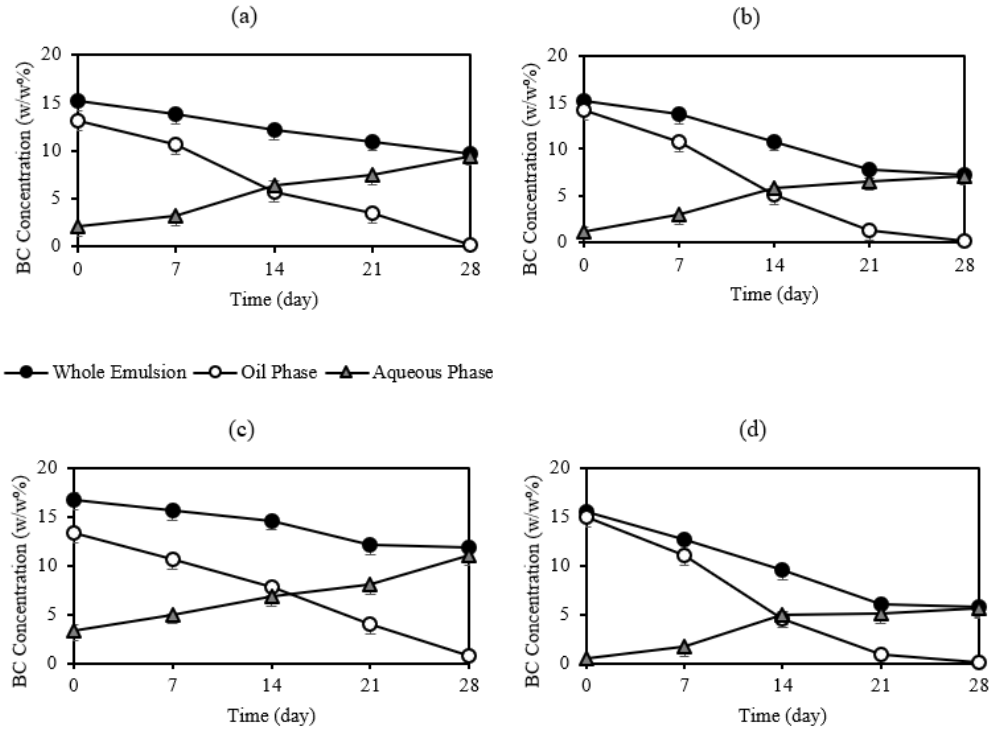

Fig. 2. Degradation of β-carotene (BC) in whole emulsions and its concentrations in oil and aqueous phases of emulsions with different surfactants; (a) lecithin, (b) GMS, (c) ovalette, (d) Tween 20, during 28 days storage at 25 °C. Data are means ± standard deviations.

Effects of different solid fat contents on partitioning of β-carotene in O/W emulsions

Fig. 3 shows the partitioning of β-carotene in oil and aqueous phases of the O/W emulsions with different solid fat contents in fresh samples (day 0). The solid fat contents of the samples were varied by varying the percentage concentration of palm shortening in the oil phase of emulsions. In general, increasing the concentration of shortening resulted in the increase of β-carotene partitioning into the aqueous phase. The sample with 0% shortening shows the least partitioning of β-carotene molecules into the aqueous phase, followed by those with 1, 2, 3 and 4% palm shortening in the oil phase. This is consistent with the study by Wan Mohamad et al. (2018) on β-carotene partitioning in nanoemulsions, where the β-carotene concentration in the aqueous phase escalated from 9.9 ± 1.7 to 53.0 ± 1.8 w/w % with the increase in the solid fat content of emulsions.

Although highly hydrophobic bioactive compounds such as β-carotene can be trapped within colloidal crystalline matrices such as the oil phase with higher solid fat contents, it is practically difficult to integrate the bioactive molecules into a highly structured crystalline phase, resulting in the expulsion of the molecules into the aqueous phase of emulsion (Wan Mohamad et al., 2018)

Besides that, lipid-based bioactive compounds can crystallise at ambient temperature, thus promoting partial coalescence and destabilising the emulsion system encapsulating them. Crystallising the carrier oil phase by increasing the solid fat content of the emulsion can reduce the partial coalescence and consequently stabilise the emulsion (McClements et al., 2007).

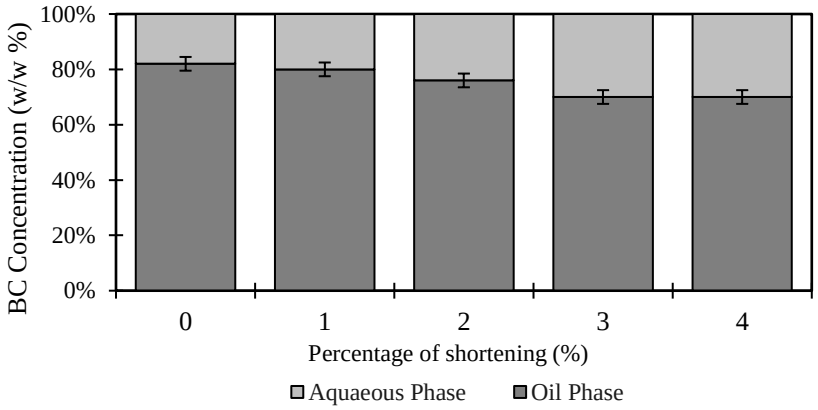

Fig. 3. Partitioning of β-carotene (BC) between the oil and aqueous phases of emulsions with different solid fat contents; 0, 1, 2, 3 and 4%. Data are means ± standard deviations.

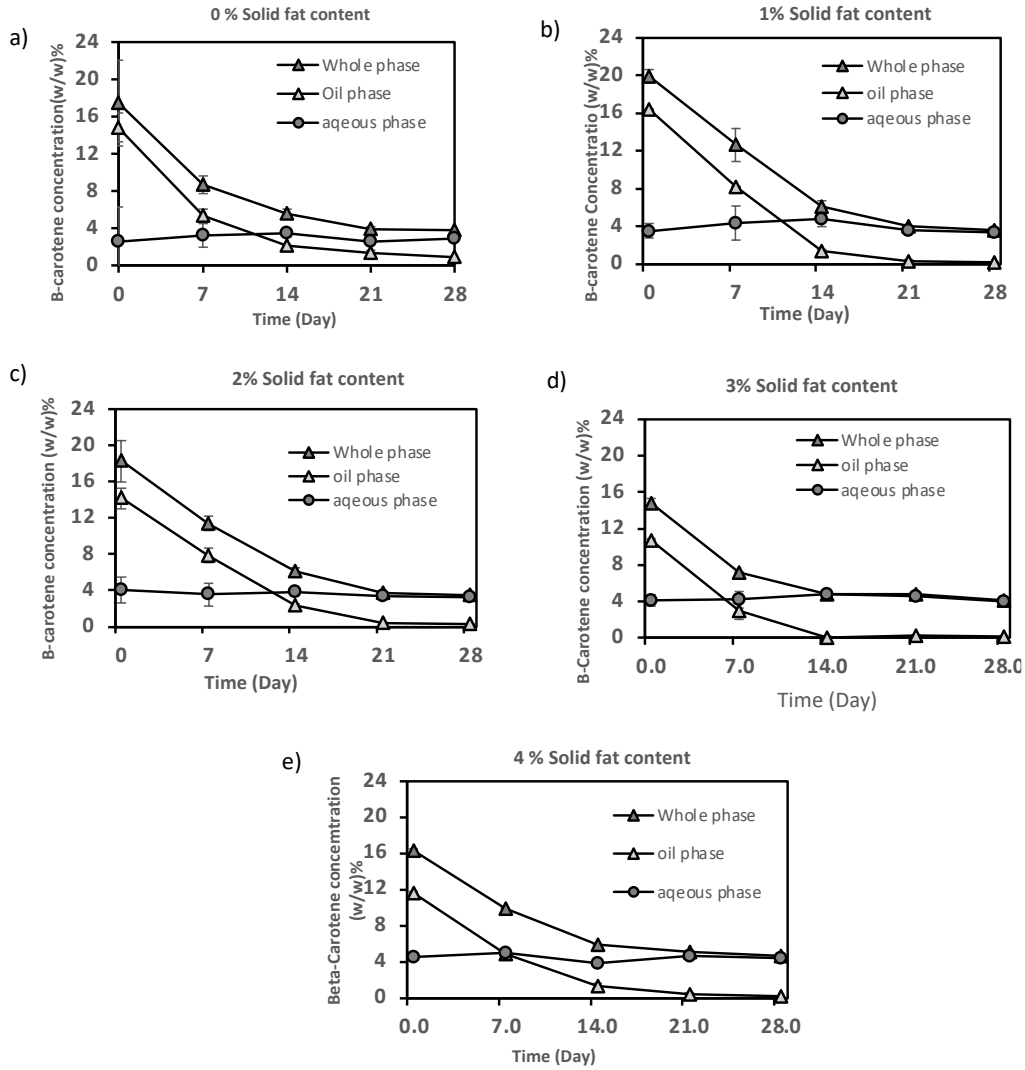

Fig. 4. Degradation of β-carotene (BC) in whole emulsions and its concentrations in oil and aqueous phases of emulsions with different solid fat contents; (a) 0%, (b) 1%, (c) 2%, (d) 3% and (e) 4%, during 28 days storage at 25 °C. Data are means ± standard deviations.

Effects of different solid fat contents on partitioning of β-carotene in O/W emulsions upon storage

Bioactive compounds contained in a food product get degraded with time, but this degradation can be delayed by several factors including type of surfactant, oil loading, type of bioactive compound and bioactive compounds co-encapsulation (Sayed Abdul Rahman et al. 2022). Fig. 4 depicts that in general, increasing the solid fat content in the oil phase had delayed the degradation of β-carotene in the whole emulsion samples. However, the solid fat content had caused a more rapid degradation of β-carotene in the oil phase of emulsion in comparison to that in the aqueous phase throughout the storage period. This resulted in the concentration of β-carotene being higher in the aqueous phase earlier as the solid fat content was higher in the oil phase. Fig. 4 (a) shows the partitioning of β-carotene into the aqueous phase of emulsion with 0% solid fat content exceeded that in the oil phase on the 11th day of storage, while Fig. 4 (e) with 4% solid fat content had the bioactive partitioned into the aqueous phase more than the oil phase on the 7th day. It can be hypothesised that the β-carotene molecules were expelled more easily from the oil phase into the aqueous phase upon increasing the solid fat content, hence the trend of more rapid degradation of β-carotene in the oil phase with time (Wan Mohamad et al., 2018).

CONCLUSION

In conclusion, food-grade O/W emulsions encapsulating β-carotene using different types of surfactants (lecithin, GMS, ovalette and Tween 20) and solid fat contents had resulted in partitioning of the bioactive compound between the oil and aqueous phases upon emulsification and throughout storage. The partitioning of β-carotene into the aqueous phase was the highest in the sample with ovalette due to the availability of binding sites on the surfactant micelles within the aqueous phase of emulsion. A higher concentration of β-carotene in the aqueous phase over the time was also observed, suggesting that oxidation of β-carotene occurred faster in the oil phase. The results of this study demonstrate that selection of emulsifier can significantly impact the oxidative stability of the encapsulated bioactive. Besides that, increasing the solid fat content had also increased the partitioning of β-carotene into the aqueous phase since the bioactive molecules were more difficult to retain in the increasingly structured crystalline lipid phase. This also resulted in the rapid degradation of β-carotene in the oil phase as compared to that in the aqueous phase of emulsion upon storage. In future, other factors affecting the partitioning characteristics of phytonutrients such as emulsion types, oil loadings, homogenisation methods and storage conditions can be studied towards designing stable and bioaccessible emulsion systems encapsulating bioactive compounds beneficial to human health.

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