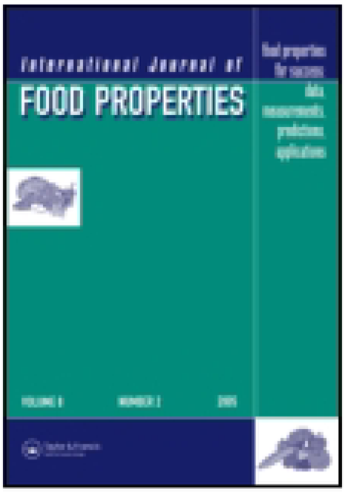
Stability of Palm Carotenes in an Organic Solvent and in a Food Emulsion System

by Tatas Hardo Panintingjati Brotosudarmo



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Stability of Palm Carotenes in an Organic Solvent and in a Food Emulsion System

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Palm carotene has potential as an application for a natural food colorant with bioactivity as pro-vitamin A and an antioxidant. However, during processing and storage, the palm carotene encounters excessive treatments. In this study, the stability of palm carotenes was observed in organic solvent and a food emulsion system in order to learn its molecular behavior, as well as to evaluate the color stability due to exposure to light and temperature. Spectroscopy and chromatography measurements proved the formation of *cis* isomers and even colorless compounds after the treatment of its acetonic solution at 90°C (11 h) or under excessive illumination (11,470 lux). An application of 0.050–0.500% concentrate of palm carotene into emulsion system gave diverse shades of yellowness, which remained without any obvious color difference ($\Delta E \leq 2$) after 28 days at 4°C (dark) or 8 days at 30°C under average illumination of most display racks at stores (2500 lux).

Keywords: Carotenoid, Color, Emulsification, Pigment, Food stability.

INTRODUCTION

The production of palm oil has undeniably been an economic importance as one of the major export commodities for Indonesia. The Indonesian Directorate General of Estate reported that the increased export volume of palm oil has reached to an average of 13% per year during 2003–2012, and it is accompanied by a 22.24% increase per year on its export value.[1–3] The statistical data published by FAO has acknowledged Indonesia as the largest producer of the palm oil with a production of 23,672,000 tonnes in 2012, surpassing Malaysia.[4] The productivity of the plantation continuously grows and was 24,431,640 tonnes by the year 2013.[5]

The fruit of oil palm tree has also been known to contain high amount of β-carotene which has important biological function as a vitamin A precursor. In Indonesia, *Elaeis guineensis* and *Elaeis*

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oleifera are the most cultivated oil palm trees. The mesocarp of the oil palm fruit from *Elaeis guineensis* contains 500–700 ppm carotenoids and even it may reach to 4000 ppm in *Elaeis oleifera*. The α- and β-carotene constitute up to 91% of the total carotenoids (including *cis* and *trans* isomers), while the other carotenoids are small amounts of acyclic carotenes (lycopene, phytofluene, phytoene, neurosporene), cyclic carotenes, as well as lutein.[6] Nevertheless, prior to produce the commercial cooking oil, the palm oil has to be processed through stages of bleaching and refining. Hence, the final product of cooking oil in the market has a color of light golden yellow and contains only about 27 ppm of carotenoids.[7]

Due to the plentiful amount of carotenoids, the red palm oil is then used as source of pro-vitamin A. There have been several studies on its effectiveness in elevating vitamin A status of pregnant and lactating women, as well as school-age children.[8–10] In addition, the consumption of palm oil has also been related to reduction of oxidative tissue damages and prevention of some degenerative diseases.[11–13] Among many constituents, palm carotenoids and tocopherols were supposed to give main contribution in those activities.

Recently, the present research group has developed a scaled-up process of the carotenoids extraction from red palm esters in order to produce the concentrate.[14] The carotenes concentrate has a deep yellowish red color and contains less oil fraction. Therefore, it has a potential application for a natural food colorant that has bioactivities such as, pro-vitamin A and antioxidants. The toxicological study has been carried out without any significant effect on vital organs of mice.[15] However, the stability of the palm carotenes upon their application has not been evaluated. The objective of present study was to evaluate the thermal and photo-stability of palm carotenes when it was dissolved in organic solvent and when it was incorporated in a food emulsion. This study will give reference for further product developments as well as proper storages of palm carotenes.

MATERIALS AND METHODS

The carotenes concentrate of palm oil was obtained from The Indonesian Oil Palm Research Institute (North Sumatra, Indonesia). It was prepared by a modified solvolytic micellization method. The concentrate consisted of up to 80% of ester and was stored in a refrigerator to prevent any degradation before it was used.

Thermal and Photo-Stability in an Organic Solvent

In the thermal stability assay, carotenes concentrate was first dissolved in acetone and adjusted to give OD = 1.0 cm^{-1} at its maximum absorption (λ_{max} = 447 nm). The solution was placed on a water bath at 90°C for 11 h. The spectral data was recorded using UV-1700 Spectrophotometer (Shimadzu, Japan) subsequently after 1, 2, 3, 6, and 11 h of heating treatment. The photostability assay of palm carotene was determined by treating the equivalent starting solution under Volpi Lamp at 11,470 lux with intense observations using MultiSpec-1501 Spectrophotometer (Shimadzu, Japan).

Chromatography Analysis

The carotenoid composition in the concentrate was determined by high performance liquid chromatography (HPLC) equipped with photo diode array detector (Shimadzu, Japan) using gradient elution as previously described.[16] The peak area was calculated using the multi-chromatogram approach[17] at 355–510 nm region.

Emulsion Preparation and Storage Conditions

Oil-in-water emulsion (mayonnaise like) was made by mixing 50% (w/w) canola oil, 4% (w/w) soya lecithin (PT Panadia Corp., Indonesia), 1.25% (w/w) xanthan gum (Qingdao ICD Foreign Trade Co., Ltd., China), and the concentrate of palm carotenes. The water was sterilized prior to emulsification, while the lecithin and xanthan gum were subjected to dry sterilization using ultraviolet lamp. No preservative was added to this emulsion system. The amount of palm carotene was varied as follows: 0.000 (control), 0.050, 0.125, 0.250, and 0.500%, resulting in different shades of yellowness. A commercial hand mixer (Zauberstab M100S, Germany) was used to homogenize the components with the following conditions: 7000 rpm for 1 min and then continued mixing at 10,000 rpm for 2 min. Then, the whole series of emulsions with different concentration of palm carotenes were subjected to three different storage conditions: refrigerated at 4°C (dark) for 28 days, illuminated with 2500 lux monochromatic light at 30°C for 8 days, as well as in a dark oven at 60°C for 8 days. The light intensity of illumination was chosen according to average light intensity of most display racks at food stores.

Color Measurement

ColorFlex® EZ No.45/0 (HunterLab, USA) was used to measure emulsion color according to color system of Commission Internationale de l'Eclairage (CIELAB system). It measures L^* for lightness, a^* for redness, and b^* for yellowness. In order to have a valid calculation, the color reading was taken three times and the average value was used. A white tile standard (L^* 92.93, a^* 0.92, b^* 1.48; HunterLab, USA) was used to calibrate the instrument. Change of colors was calculated as ΔE (Eq. 1) from the Hunter L^*, a^*, and b^* values,[18–20] in which higher values indicate a greater color difference;

$$\Delta E = \sqrt{\left(L_0^* - L^*\right)^2 + \left(a_0^* - a^*\right)^2 + \left(b_0^* - b^*\right)^2} \quad (1)$$

The subscript "0" refers to the color reading of emulsion prior to storage.

Statistical Analysis

Data were analyzed statistically by one-way analysis of variance (ANOVA) and Scheffé test using SPSS Statistics program, version 16.0. Statistically significance data was accepted at $p < 0.05$ levels.

RESULTS AND DISCUSSION

Pigment Composition

The result of chromatographic analysis of the carotenes concentrate is shown in Fig. 1. The chromatogram was monitored at 450 nm, recognized as maximum absorption of most carotenes. Two dominant peaks were found at 58.21 (peak 1) and 60.10 min (peak 2) which are identified as α- and β-carotene, respectively. Peak identifications were made by retention times and by UV-vis spectra. Spectral identification was confirmed in *n*-hexane and acetone, as depicted in Fig. 2. The absorption spectrum of α-carotene (β,ε-carotene) in *n*-hexane is characterized with three maxima at 422, 442, and 469 nm, whereas β-carotene (β,β-carotene) exhibits a peak-pattern at 424, 447, and 472 nm.[21,22] Compared to β-carotene, the alpha structural isomer undergoes a hypsochromic shift of 2–5 nm, being similar to that found in geometrical isomerization.[23] The ration of peak area between α- and β-carotene was 0.804, which provisionally represents their composition in the concentrate. This proportion is comparable to those previously reported for palm oil, red palm oil,

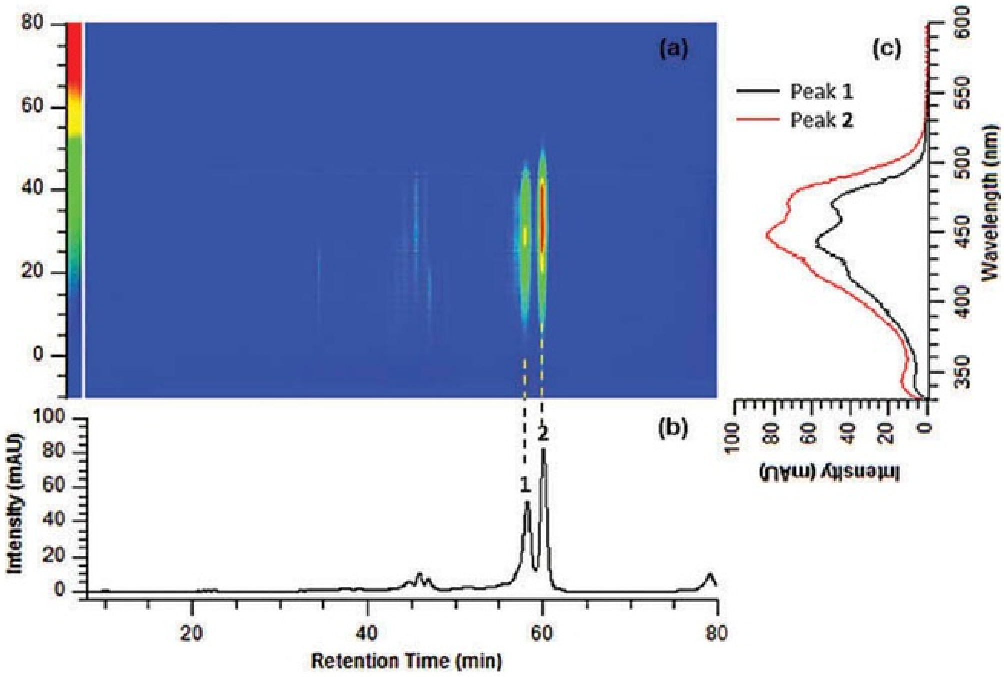

FIGURE 1 HPLC isogram of palm carotenes (a) and the chromatographic profile monitored at 450 nm (b), showing the presence of two major pigments, i.e., α-carotene (peak 1, tR 58.21 min) and β-carotene (peak 2, tR 60.10 min). The photo diode array spectra are portrayed (c) to support the identification.

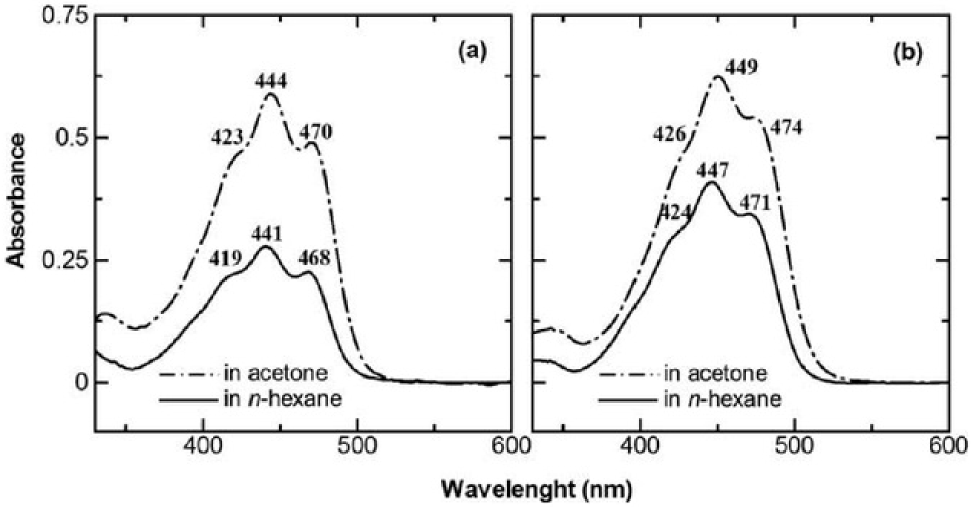

FIGURE 2 UV-Visible absorption spectra of pigment fractions found as peak 1 at tR 58.21 min (a) and peak 2 at tR 60.10 min (b), collected after chromatography, measured in different solvents: acetone and n-hexane.

as well as their olein fraction, having the ratio ranges from 0.627 to 1.327.[24] According to their biological functions, both α- and β-carotene have shown antioxidant and pro-vitamin A activities. One molecule of β-carotene will be enzymatically cleaved into two molecules of vitamin A (retinol), while cleavage of the α-carotene will generate retinol and α-retinol, having only half the activity than that of the β-carotene.[25]

Thermal and Photo Stability in an Organic Solvent

Thermal and photo stabilities of carotenes concentrate were evaluated by monitoring the absorption spectra in UV-visible region. The maximum absorption at 447 nm has decreased to 52.35% after 11 h of incubation in a water bath at 90°C. The decrease was also observed to 56.76% after 60 min of irradiation at 11,470 lux. Figure 3a shows that about one-third of the total decrease has taken place in the first hour of heating and afterward it decreased exponentially within the next 10 h. On the other

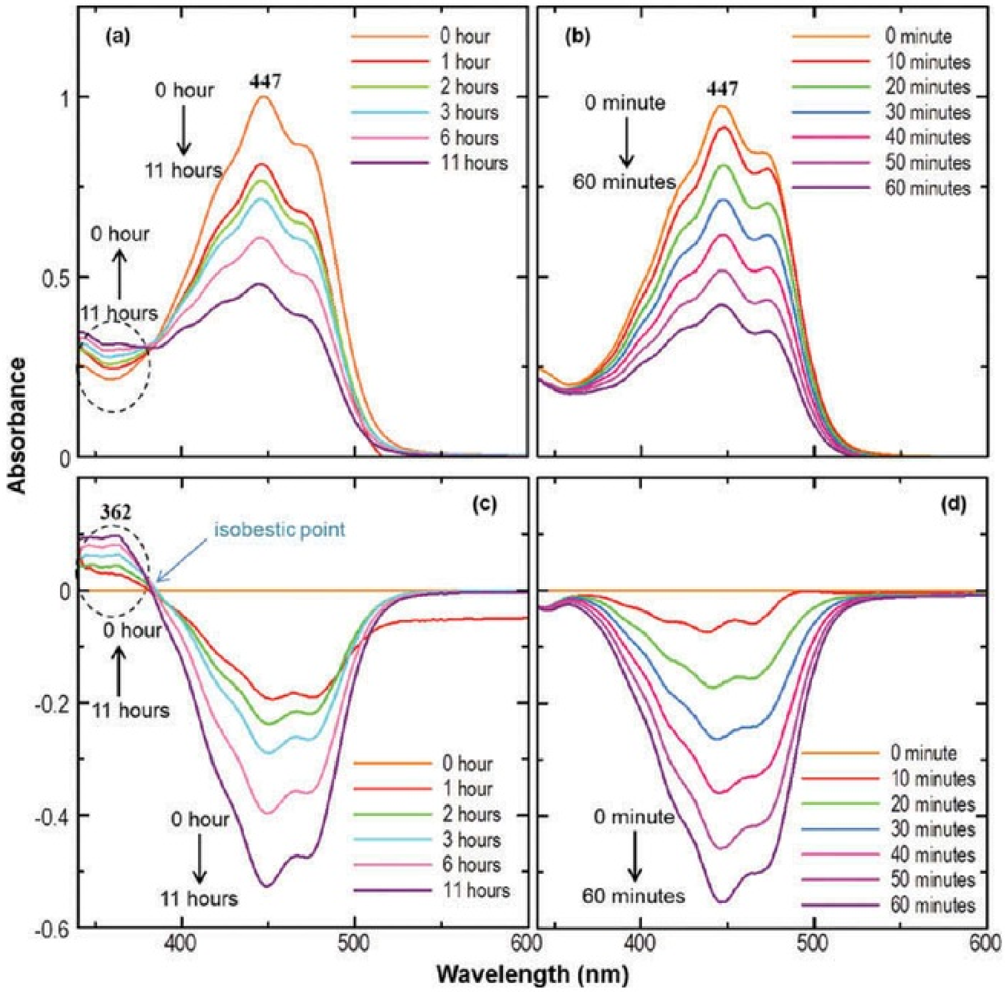

FIGURE 3 Absorption spectra of carotenes concentrate in acetone during 11 h of heating at 900C (a) and 60 min of irradiation at 11470 lux (b), as well as the different absorption spectra for thermal (c) and irradiation (d) treatments.

TABLE 1
Degradation percentage of pigment fractions in palm carotenes upon thermal and irradiation treatment, based on peak area data obtained from HPLC analysis

	Degradation percentage (%)	
Treatment	*Peak 1*	*Peak 2*
Thermal (90°C, 11 h)	48.48	53.39
Irradiation (11,470 lux, 1 h)	67.24	60.89

hand, exposing palm carotene under irradiation has caused the degradation at constant rate (Fig. 3b). It has been known that excessive exposure of light and/or heat leads to geometrical isomerizations of carotenes and even to a chemical breakdown that cause color change.[26] In this regard, Fig. 3c shows that an absorption band emerges at the near-ultraviolet region, which is corresponding to the absorption band of the degradation products of carotenes. However, similar situation is not recorded in Fig. 3d that shows the spectral evolution through exposure to light. The new arising band at around 340 nm in Fig. 3a and 3c can be expected as the *cis* band of α- and β-carotene in the *cis* configuration.[23] Chen and Huang previously identified the degradation and isomerization of all-*trans*-β-carotene in an organic solvent during heating and illumination treatments. Thermo-isomerization leads to the formation of 13-*cis*-β-carotene in largest amount, followed by 9-*cis*- and 15-*cis*-β-carotene, which occurs mostly during the first hour of heating at 70°C.[27] In the case of an excessive illumination, all-*trans*-β-carotene undergoes geometrical isomerization very quickly in the early phase of photo-degradation, then the *cis* isomers break into colorless compounds. This report is similar with the present results. Unfortunately, the evolution of the absorption band of the isomers is beyond the current resolution of instrument. Table 1 provides degradation percentage of each α- (peak 1) and β-carotene (peak 2) based on peak area obtained from HPLC analysis. The thermal stability of β-carotene was supposed to be slightly lower than that of α-carotene, while the photo stability of α-carotene was suspected to be lesser than that of β-carotene. The degradation pathway of α-carotene as it is affected by high temperature and light still needs to be further studied.

Thermal and Photo Stability in Food Emulsion

The use of palm carotene in oil-in-water emulsion gave different shades of yellowness at various concentrations (Fig. 4). The *L**, *a**, and *b** values obtained from color measurement are highly correlated with the carotenoid content as well as its chemical structure.[28] The control emulsion without any colorant gave *L** 88.68, *a** –0.38, and *b** 14.48. When the colorant was added, the lightness parameter (*L** values) was reduced (darker). The chromatic hue *a** (redness) and *b** (yellowness) were greatly varied from *a** 5.827 – *b** 46.04 until *a** 20.403 – *b** 89.48, when 0.050 up to 0.500% carotenes concentrate was incorporated, respectively. It shows the efficacy of this material as a strong colorant. Three different storage conditions were applied based on the possible application of this concentrate, such as ice cream, creamy yogurt, mayonnaise, salad dressings, peanut butter, as well as condiments. Dairy products are usually stored at low temperatures and in the dark for preservation purpose, while the oil-based products are often kept on display racks of the store or kitchen in which there is light exposure at room temperature. Furthermore, dark storage at fairly high temperature (up to 60°C) might be applied inside the container during long distance transportation. A significant statistical shift ($p < 0.05$) of the *L**, *a**, and *b** values over time was observed during storage period in these three different conditions, revealing the existence of color instability. Figure 5 shows the evolution of yellowness (*b** parameter) during certain period of different storage conditions. An adequate linear relation between the *b** parameter and time was

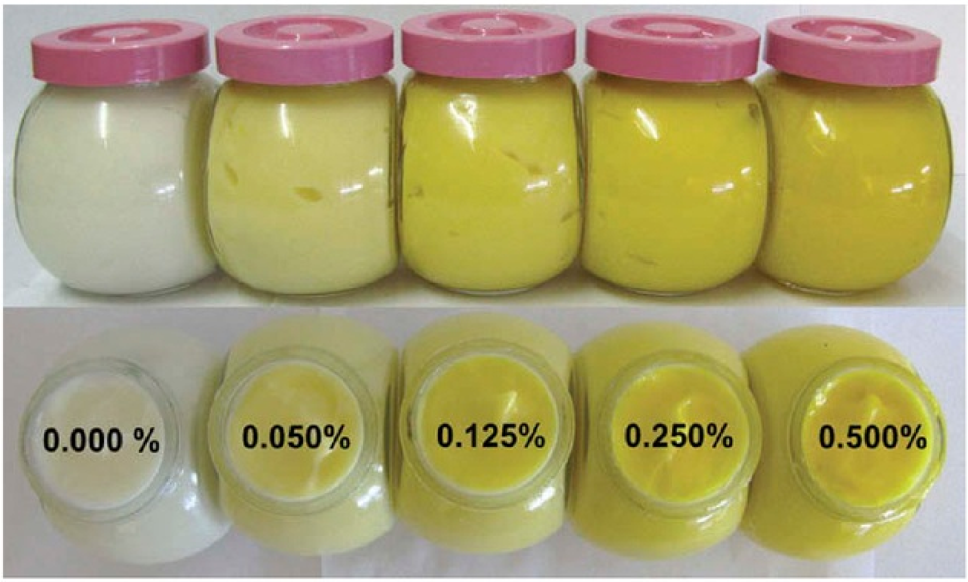

FIGURE 4 Oil-in-water emulsions contain the concentrate of palm carotenes with varied concentrations.

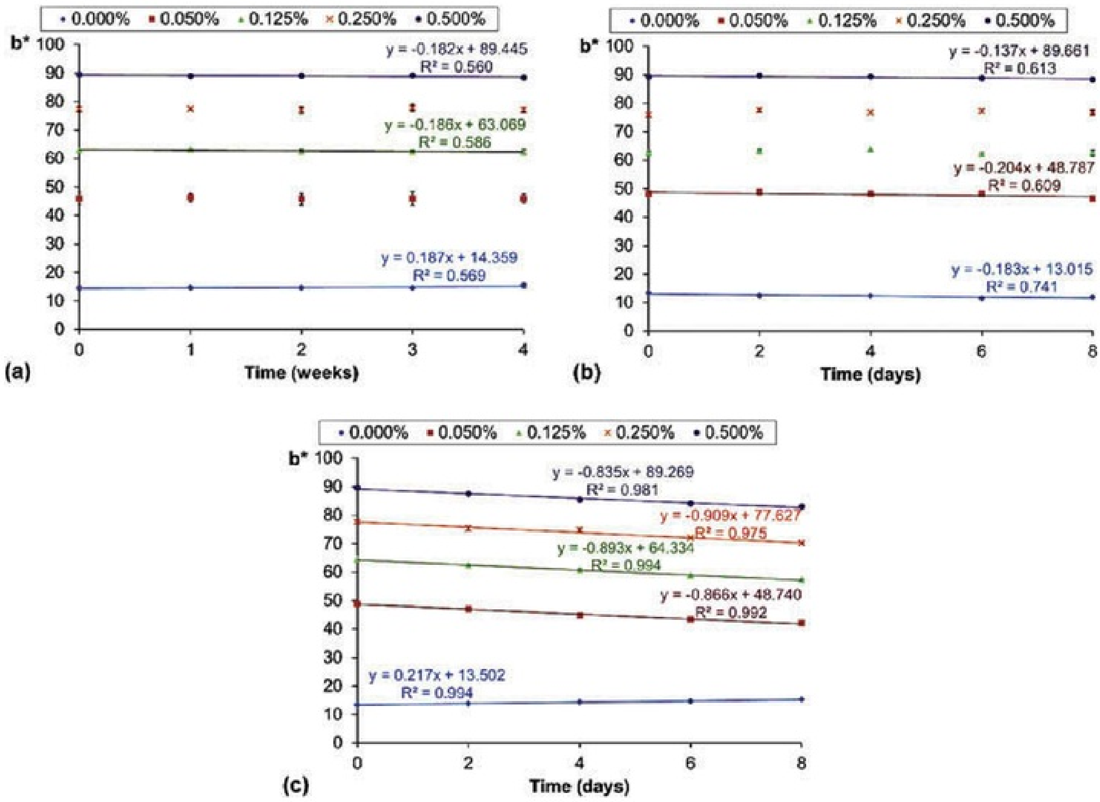

FIGURE 5 Evolution of the yellowness *b** value of oil-in-water emulsion containing palm carotenes during different storage conditions, i.e., dark fridge (T = 40C; 28 days) (a), illumination at 2500 lux (T = 300C; 8 days) (b), and dark oven (T = 600C; 8 days) (c).

TABLE 2
Color differences between the initial condition of food emulsion and those at the end of storage period, when the palm carotenes was applied as natural colorant

Storage condition	*Carotene concentrate (% w/w)*	*ΔE**
T = 4°C; dark fridge; 28 days	0.000	1.11 ± 0.17
	0.050	0.62 ± 0.24
	0.125	0.82 ± 0.17
	0.250	0.40 ± 0.06
	0.500	1.04 ± 0.18
T = 30°C; illumination at 2500 lux; 8 days	0.000	1.64 ± 0.09
	0.050	2.08 ± 0.11
	0.125	1.82 ± 0.27
	0.250	1.99 ± 0.30
	0.500	1.31 ± 0.21
T = 60°C; dark oven; 8 days	0.000	1.90 ± 0.24
	0.050	7.11 ± 0.22
	0.125	7.52 ± 0.21
	0.250	8.10 ± 0.32
	0.500	7.88 ± 0.14

Data were expressed as the mean ± SEM;
**ΔE*: color difference.

found in low temperature and illuminated storage. During illumination, a negative linear relation between yellowness intensity and time was detected in 0.050 and 0.500% application. Opaque properties of oil-in-water emulsion might help to reduce light penetration, and hence, prevent the degradation pigments.[29] On the other hand, high-temperature storage for a long time caused lowered redness and yellowness hues in all concentrations with strong negative linear correlation toward time of observation (Fig. 5). The percentage of degradation is retarded on higher concentration. The reduction of absorption intensity due to degradation of *trans* carotene and formation of *cis* isomer become the reason of this discoloration. Color differences were also determined by calculating ΔE to scale the invisibility of any color change (Table 2). The total color difference, ΔE, which is a combination of the parameters (L^*, a^*, and b^* values), is colorimetric parameters extensively used to characterize the variation of colors in foods during processing. The validated value compromises $\Delta E < 1$ for a normally invisible difference, $1 < \Delta E < 2$ for a very small difference that is only obvious to a trained eye, $2 < \Delta E < 3.5$ for medium difference that is obvious to an untrained eye, and $\Delta E > 3.5$ for an obvious difference.[30] Hence, it can be assumed that at the end of the study, most color emulsions that were stored in a dark fridge as well as under illumination at room temperature did not show a noticeable difference, but those in a dark oven began to give apparent change on the fourth day of storage (data not shown). This prediction was consistent with the characteristic of faster b^* value reduction found during dark oven storage. Overall, there is no statistical significance was found for ΔE among the series of concentration in the same storage condition, suggesting that the concentration of palm carotenes on food emulsion might not influence its color stability.

CONCLUSION

Palm carotenes concentrated from palm oil contained both α- and β-carotene in a nearly equivalent amount. The carotenes concentrate is applicable as a natural yellow colorant, but high-temperature processing and strong illumination should be minimized due to its instability. The investigation of its molecular behavior by means of chromatography and spectroscopy analysis revealed the formation of degradation products during heating (90°C, 11 h) or illumination with high intensity (11,470 lux, 1 h), i.e., the *cis* isomers and even colorless compounds. In an emulsion system, the color of palm carotenes could remain without any obvious changes during 28 days storage in a dark fridge or 8 days with illumination of 2500 lux at room temperature. However, storage at 60°C for 8 days unveiled apparent color fading. Though it was shown that higher concentration of this colorant has lower percentage of degradation, there is no statistical significance was found for its color difference (ΔE), supposing that the application of concentrate at 0.050–0.500% might not influence its color stability. Further study is now in progress in order to learn the kinetics of color change by using varied light intensities and temperatures prior to application in commercial products.

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