Thermal Degradation Kinetics of Encapsulated Palm Carotenes Using Different Combination of Wall Materials

Khoo Mun Hong*, Renny Indrawati¹,²,³, Tatas H. P. Brotsudarmo²,³, and Leenawaty Limantara⁴,⁵,

¹ Department of Cell and Molecular Biology, Faculty of Biotechnology & Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia
² Department of Chemistry, Faculty of Science and Technology, Universitas Ma Chung, Malang 65151, East Java, Indonesia
³ Ma Chung Research Center for Photosynthetic Pigments, Universitas Ma Chung, Malang 65151, East Java, Indonesia
⁴ Center for Urban Studies, Universitas Pembangunan Jaya, South Tangerang 15413, Jakarta, Indonesia

*Corresponding Authors: leenawaty.limantara@upj.ac.id (Tel. +62-21-7455555; Fax +62-21-29861525)

Abstract
Palm oil is known as one of the richest sources of carotenes. Carotenes concentrate could be prepared from crude palm oil and possessing potency to be used as nutraceutical materials. Due to the susceptibility of carotenes against heat, the encapsulation procedure might be applied to provide protections. The present study was aimed to learn the influence of using different combination of wall materials toward the thermal degradation kinetics of palm carotenes. Maltodextrin was used as primary wall material, being combined with various surface-active agents, i.e. Tween-80 (A), Tween-20 (B), and Arabic gum (C). The carotenes concentrate was encapsulated through emulsification steps, and the vivid yellow powder was obtained after freeze drying process. Then, encapsulated palm carotenes were stored in 20 °C, 35 °C, and 50 °C for several days, and the color, carotenes spectrum, as well as water content were monitored periodically. The kinetics of thermal degradation of carotenes were estimated using Arrhenius equation. The results showed that the use of different combination of wall materials exhibited distinct degradation rate. The combination of maltodextrin and Tween-80 gave preferable protection compared to the others, being half-degraded after 51 days in 20°C.

Encapsulated Palm Carotenes: Carotenes concentrate obtained from crude palm oil was encapsulated using maltodextrin in combination with three surface active agents, i.e. Tween-80, Tween-20, and Arabic gum. Thermal degradation kinetics of the encapsulates were calculated using Arrhenius equation, showing the order of stability: Tween-80 > Tween-20 > Arabic gum.

Keywords: palm carotenes, encapsulation, freeze-drying, degradation kinetics

INTRODUCTION
Oil palm (Elaeis guineensis) is one of the world’s major sources for production of vegetable oil. It was estimated that more than 30% of edible oil produced in the world is made from oil palm, followed by soybean, which is 28% [1]. There are some varieties of oil palm, including E. guineensis fo. dura, E. guineensis var. oleifera and E. guineensis fo. tenera, which differ not only in anatomical features but also the oil content in the palm fruits [2, 3]. The crude palm oil has reddish color due to the high concentration of carotenes, typically 500–700 ppm [4].

Carotenes is a group of carotenoid pigments which exhibits yellow to red color and belongs to the secondary metabolite of plants, usually acting as accessory pigments to support the role of energy harvesting by chlorophylls [5]. Furthermore, carotenes also play some important functions in human body, such as vitamin A precursors, enhancer of immune system, and prevention of oxidative stress [6, 7]. Due to its naturalness and health benefits, carotenes group is promising to be more exploited as natural colorants as well as functional ingredients in food products.

Recently, our research group has successfully prepared carotenes concentrate from crude palm oil and evaluate its stability in food emulsion system [8]. The main challenge of involving carotenes in food processing is its susceptibility against heat, light, and oxidation. The encapsulation technique is often used to increase the stability of bioactive compounds as well as tune its solubility. To construct the encapsulating wall of non-polar carotenes, the polysaccarides and surface active agents were needed, such as maltodextrin, Arabic gum, and polysorbate-type nonionic surfactant (Tween) [9]. This study was aimed to learn the influence of using different
combination of wall materials toward the kinetic parameters of carotenones degradation upon thermal treatment. The Arrhenius model was employed to predict the activation energy and degradation rate based on color value and spectroscopic absorption of carotenones.

**EXPERIMENTAL**

**Encapsulation Procedure**

Carotenes concentrate was kindly supplied by Dr. D. Siahaan (Oil Palm Research Institute, Medan, Indonesia). The wall materials used to encapsulate the carotenes concentrate were combination of maltodextrin with different surfactant, i.e. Tween-80 (A), Tween-20 (B), and Arabic gum (C). For (A) and (B), in each separate beakers, 1.05 g of Tween 20 and Tween 80 were weighted. Then, 105 ml of distilled water was added. The solution was heated to 60 °C while stirring, and subsequently 70 g of maltodextrin was added. For (C), a mixture of maltodextrin and Arabic gum was slowly dispersed to distilled water at 60°C. After the polysaccharides was well dispersed, the temperature could be reduced until ambient temperature. An aliquot of carotenes concentrate (2.5 gram) was added to each coating mixture, prior to homogenization with an IKA Turrax T18 (IKA, Germany). The emulsions were frozen overnight prior to lyophilization (Labconco, USA). The freeze-dried samples were ground into small particles (60 mesh) and stored at low temperature until the time of analysis.

**Thermal Storage and Kinetic Analysis**

The samples from each variation were stored in 20 °C, 35 °C and 50 °C using small amber vial, for which the analysis of the samples were carried out within the intervals of 8 days, 2 days, and 15 hours, respectively. At each temperature, five points of storage time were needed to construct an Arrhenius plots. All data were best-fitted by a first-order-kinetic model, $lnC = lnC_0 – k(t)$. The estimation of degradation rate constant ($k$) was taken from the slope of a plot of the natural log of the chromatic value vs. time. Moreover, an Arrhenius model $k = Ae^{Ea/R}$ was determined to obtain the value of activation energy ($Ea$) and frequency factor ($A$), in which $Ea/R$ is the slope and $lnA$ is the intercept of the relationship between natural log k and (1/T) in Kelvins. For a first-order reaction, the half-life was calculated at a determined temperature, of which the difference of chromatic values before and after 50% of degradation was divided by degradation rate. The linear regression with 95% confidence level was used to determine each kinetic parameters.

**Color Measurement**

The color of encapsulated carotenones was measured instrumentally by means of ColorFlex EZ (HunterLab, USA) and the results were expressed in terms of $L^*$ (lightness), $a^*$ (redness), and $b^*$ (yellowness) based on CIELAB system. Color reading was preceded by calibration using standard white tile ($L^* 92.93$, $a^* -0.92$, $b^* 1.48$), and each sample measurement was performed in triplicate.

**Water Content Analysis**

The water content was measured using a moisture tester (Shimadzu, Japan), in which an individual sample (0.3 g) was transferred into the weighing plate. To prevent the absorption of water vapor from air, the stored sample was flowed with an inert gas prior to sealing.

**Spectrophotometry**

The encapsulated carotenones was weighed (0.1 g) and dissolved in distilled water to break the coating material. Then, the carotenones fraction was collected after partition using organic solvent using separating funnel. Acetone and diethyl ether were used for (A) and (B), whereas the combination of acetone, ethanol, and diethyl ether were preferred for (C). The pigments in diethyl ether solutions were measured for its absorption by means of Spectrophotometer UV-1700 (Shimadzu, Japan).

**RESULTS AND DISCUSSION**

The use of surfactant with high hydrophilic-lipophilic balance (HLB) value (>15) was important to increase encapsulation efficiency [10], particularly when the hydrophobic active material was used as the core. The HLB number of Tween 80 was 15 and the HLB number of Tween 20 was 16.7 [11, 12]. Arabic gum, being different, is a polysaccharide complex obtained from acacia trees, possessing HLB value of 8 [13]. However, the previous study reported that Arabic gum was also useful in the encapsulation of both fatty compound as well as natural pigments [14, 15].

The vivid yellow powders were successfully collected after the encapsulation procedure. The variation of surfactant influenced the color of encapsulated product, in which the brownish color of Arabic gum slightly reduced the yellowness and lightness of the powder when it was compared to those which use transparent polysorbate surfactant (Tween-20 and Tween-80). On the other hands, there was no significant difference in the water content of encapsulated powder made of various surfactants. The moisture content of encapsulated palm carotenones after freeze drying and grinding were ranging from 7.5 to 9%, and these were maintained during thermal storage at all temperature (Figure 1). Low moisture content (<10%) is preferred to protect food materials from the possibility of unexpected chemical or biochemical degradation during storage.

The carotenones concentrate obtain from crude palm oil is the rich source of alpha and beta-carotenones with the ratio 0.804, hence it had a typical yellow to orange color and exhibited absorption maxima at 419 to 474 nm [8]. Figure 2 showed the evolution of the absorption spectrum of carotenones during thermal storage at 50°C. The maximum absorption at 446-447 nm revealed the dominancy of beta-carotenones in the present sample. The decrease of absorption signified the degradation of carotenones due to heat. Almost whole carotenones were degraded after being stored for 50 hours. In addition, the rate of degradation was hastened when the combination of maltodextrin and Arabic gum (C) was used, but it was slower at the use of Tween 80 (A).

Furthermore, carotenones degradation was also monitored as color changes using ColorFlex. During thermal storage, the lightness ($L^*$) value was increased, whereas the redness ($a^*$) and yellowness ($b^*$) value were diminished (Figure 3). The evolution of chromatic values followed the first-order kinetic with the increased gradient at higher temperature of storage. The Arrhenius parameters and predicted half-lives of encapsulated palm carotenones in the present study were summarized in Table 1.
Figure 1. The moisture content of encapsulated palm carotenes in maltodextrin-Tween 80 (A), maltodextrin-Tween 20 (B), and maltodextrin-Arabic gum (C) at three levels storage temperature; a) 20 b) 35, and c) 50 °C.

Figure 2. The decrease of absorption spectrum of carotenes in different wall materials during thermal storage at 50 °C, i.e. maltodextrin-Tween 80 (a), maltodextrin-Tween 20 (b), and maltodextrin- Arabic gum (c).

Table 1. Degradation rate constant (k) and calculated half-life for encapsulated palm carotenes using three various wall materials, i.e. maltodextrin-Tween 80 (A), maltodextrin-Tween 20 (B), and maltodextrin-Arabic gum (C) at determined temperature, based on color parameter (a* and b* value).

| Color Parameter | Temperature (°C) | Degradation rate constant (k) | Calculated half-life (days) |
|-----------------|-----------------|-----------------------------|-----------------------------|
|                 |                 | A   | B   | C   | A   | B   | C   |
| a*              | 20              | 0.12| 0.21| 0.3 | 37.83| 26.21| 9.93|
|                 | 35              | 0.82| 1.3 | 1.42| 5.56 | 4.27 | 2.12|
|                 | 50              | 4.66| 6.75| 5.74| 0.98 | 0.82 | 0.52|
| b*              | 20              | 0.08| 0.18| 0.75| 51.79| 27.3 | 2.09|
|                 | 35              | 0.9 | 1.56| 2.91| 4.6  | 3.23 | 0.54|
|                 | 50              | 7.99| 10.77| 9.91| 0.52 | 0.47 | 0.16|
Hence, greater activation energy will prevent the degradation which is needed to start the reaction toward degradation. It has been known that the degradation of carotenes could be initiated by the presence of heat, light irradiation, acid, as well as oxidative substances [16]. The Arrhenius model was constructed based on several assumptions, i.e. the quantitative factor of estimation was only affected by one type of reaction (degradation of palm carotenes), there was no change in others quantitative factor due to other types of reaction (there was no difference in illumination, all samples were kept dark to prevent any color fade due to light), and the changes in the quantitative factor were not caused by the preceding processes.

The activation energy ($E_a$) refers to the minimum energy which is needed to start the reaction toward degradation. Hence, greater activation energy will prevent the degradation and prolong the shelf-life. The activation energy in the use of Tween-80, Tween-20, and Arabic gum were $28.79$ kcal mol$^{-1}$, $25.39$ kcal mol$^{-1}$, and $16.13$ kcal mol$^{-1}$, respectively. This result indicated the advantages of polysorbate surfactant compared to Arabic gum, particularly the use of Tween-80.

Moreover, the degradation rate constant signified the rapidity of this thermal degradation. The degradation rate was strongly influenced by the storage temperature, in which elevated temperature might hasten chemical reaction. The lowest degradation rate constant was at $20 \degree C$ ($0.08$ to $0.75$), followed with $35 \degree C$ ($0.82$ to $2.91$) and $50 \degree C$ ($4.66$ to $10.77$). Additionally, the variation of coating material also gave different degradation rate constant, being comparable to the order of activation energy. The smallest degradation rate constant was found in encapsulated palm carotenes which used maltodextrin and Tween-80. The calculated half-life for (A) was 37 days (based on $a^*$) and 51 days (based on $b^*$), predicted at $20 \degree C$. Due to the yellow color of encapsulated powder, the $b^*$ value (yellowness) was used as a reference.

CONCLUSION

The encapsulation of palm carotenes could be processed by use different coating materials, i.e. maltodextrin, polysorbate surfactants, as well as Arabic gum, with the lyophilization (freeze drying) technology. The Arrhenius model can be employed to determine the kinetic parameters of thermal degradation of encapsulated carotenes. The polysorbate surfactant, particularly Tween-80, gave some more advantageous effects, such as no effect on carotenes color, higher activation energy and lower degradation rate constant during storage, as well as prolonged shelf-life (51 days at $20 \degree C$). This study can be a part of effort to prepare more stable carotenes for further application in the development of functional food or nutraceutical products.

Acknowledgement

The laboratory experiments for this work was carried out by KMH during his internship period at Ma Chung Research Center for Photosynthetic Pigments, under the attentive supervision of Dr. Siti Sarah Othman from Universiti Putra Malaysia. We also acknowledge the contribution of Dr. Donald Siahana from Indonesian Oil Palm Research in the preparation of carotenes concentrate from crude palm oil.

REFERENCES

[1] Aparicio-Ruiz, R., Minguez-Mosquera, I., and, Gandul-Rojas, B., Thermal degradation kinetics of lutein, β-carotene and β-crypotoxanthin in virgin olive oils, J. Food Compos. Anal., 2011, 24(6), 811-820, doi: 10.1016/j.jfca.2011.04.009.
[2] Cadena, T., Prada, F., Perea, A., & Romero, H. M., Lipase activity, mesocarp oil content, and iodine value in oil palm fruits of Elaeis guineensis, Elaeis oleifera, and the interspecific hybrid O× G (E. oleifera × E. guineensis), J. Sci. Food Agric., 2013, 93(3), 674-680, doi: 10.1002/jsfa.5940.
[3] Edem, A. J., Ekwere, I. O., Akpanudo, N. W., Nsi, E. W., & Akpakpan, A. E., Physicochemical Characterization of Oil and Metallic Soaps from Two Varieties of Palm Kernel Oil (Fenera and Dura), Int. J. Modern Chem., 2018, 10(1), 33-46.
[4] Ng, M. H., & Choo, Y. M., Improved method for the qualitative analyses of palm oil carotenes using Uplc., J. Chromatogr. Sci., 2016, 54(4), 633-638, doi: 10.1093/chromsci/bmv241.
[5] Chen, C., Overview of plant pigments. In Pigments in fruits and vegetables, 2015, 1-7, Springer, New York, NY, doi: 10.1007/978-1-4939-2356-4_1.
[6] Seifir, E., Retura, G., & Levenson, S. M., Carotenoids and cell-mediated immune responses, Quality of Foods and Beverages, Recent Developments in Chemistry and Technology, 2012, 4, 335-347.
[7] Kasperek, S., Dobrakowski, M., Kasperek, J., Ostałowska, A., Zalejska-Fiolka, J., & Birkner, E., Beta-carotene reduces oxidative stress,
**Indones. J. Nat. Pigm., Vol. 02, No. 1 (2020), 21-25**

Improves glutathione metabolism and modifies antioxidant defense systems in lead-exposed workers, *Toxicol. Appl. Pharmacol.*, 2014, 280(1), 36-41, doi: 10.1016/j.taap.2014.07.006.

[8] Indrawati, R., Chomiak, A., Indriatmoko, Adhiwibawa, M. A., Siahaan, D., Brotosudarmo, T. H., & Limantara, L., Stability of Palm Carotenes in an Organic Solvent and in a Food Emulsion System, *Int. J. Food Prop.*, 2015, 18(11), 2539-2548, doi: 10.1080/10942912.2014.999374.

[9] Ray, S., Raychaudhuri, U., & Chakraborty, R., An overview of encapsulation of active compounds used in food products by drying technology, *Food Biosci.*, 2016, 13, 76-83, doi: 10.1016/j.fbio.2015.12.009.

[10] Dinarvand, R., Moghadam, S. H., Sheikhi, A., & Atyabi, F., Effect of surfactant HLB and different formulation variables on the properties of poly-D, L-lactide microspheres of naltrexone prepared by double emulsion technique, *J. Microencapsulation*, 2005, 22(2), 139-151, doi: 10.1080/0265204040026392.

[11] Schmidts, T., Schlupp, P., Gross, A., Dobler, D., & Runkel, F., Required HLB determination of some pharmaceutical oils in submicron emulsions, *J. Dispersion Sci. Technol.*, 2012, 33(6), 816-820, doi: 10.1080/01932691.2011.584800.

---

**Abstrak**

Tujuan utama penelitian ini adalah untuk menguji kestabilan termal dari konsentrat karoten yang diperoleh dari minyak sawit dengan perlakuan enkapsulasi menggunakan berbagai kombinasi bahan penyulat, yakni: maltodekstrin/Tween 80, maltodekstrin/Tween 20, dan maltodekstrin/gum Arabik. Sampel dipreparasi menggunakan metode freeze-drying untuk mengurangi efek panas pada sampel selama proses pengeringan. Penguaksaan diuji dalam interval 8 hari, 2 hari, dan 10-15 jam untuk sampel yang disimpan pada suhu 20 °C, 35 °C dan 50 °C. Dengan menggunakan CIELAB dan spektrofotometri, degradasi pigment pada setiap material terkonsentrasi diukur dan umur simpan yang berkaitan dengan kestabilan termal konsentrat karoten yang disimpan pada masing-masing suhu penyimpanan dapat diperkirakan menggunakan persamaan Arrhenius.

**Kata kunci:** beta-karoten, mikroenkapsulasi, pengeringan-beku, termostabilitas