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Characterization and chemical stability evaluation of β-carotene microemulsions prepared by spontaneous emulsification method using VCO and palm oil as oil phase

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Abstract

β-carotene is one of the major carotenoids in the human diet which shows pro-vitamin A activity, and is associated with prevention of cardiovascular diseases, cancer, and immune system enhancer. However, the poor water-solubility, high melting point, and chemical instability of carotenoids is currently a challenge to their application in the food sector. In this research, the characteristics of β-carotene microemulsions (0.025%, 0.05% wt) prepared with ternary food grade surfactants (Span 80, Span 40, Tween 80) and VCO (virgin coconut oil) or palm oil as oil phase, using spontaneous emulsification method were evaluated. Chemical stability of β-carotene loaded microemulsions during storage was also examined. The result showed that β-carotene microemulsions prepared using either VCO or palm oil had viscosity, specific gravity, refractive index and pH values which were not significantly different. The mean particle diameters (z-average) of the β-carotene microemulsions ranged from 20 – 23 nm and the size distributions were monomodal with a narrow particle size range from 10 – 50 nm. The β-carotene microemulsions showed significantly different zeta potential, i.e.: -14.4 ± 0.8mV (VCO, 0.025 %wt), -10.6 ± 0.3 mV (VCO, 0.05 %wt), -24.6 ± 1.0 mV (palm oil, 0.025 %wt) and -16.6 ± 0.9 mV (palm oil, 0.05 %wt). The β-carotene degradation during storage was slower in microemulsions with palm oil as an oil phase than that of VCO as an oil phase. These results have important consequences for the design and utilization of microemulsions as delivery systems to encapsulate and stabilize β-carotene for food or pharmaceutical applications.

Introduction

β-carotene is the most commonly detected carotenoid in human tissue and plasma (Aherne *et al*., 2010), provides the highest provitamin A activity (Castenmiller and West, 1998; Tan and Nakajima, 2005; Wang *et al*., 2012; Qian *et al*., 2012b) and also exhibits anti-inflammatory (Chou *et al*., 2010) as well as anti-cancer activity (Cui *et al*., 2007). Due to their potential health benefits, there is a growing interest in using β-carotene and other carotenoids as functional ingredients in food products. However, the application of carotenoids in food formulations is currently limited because of their poor water-solubility, high melting point, and chemical instability (Qian *et al*., 2012a; Qian *et al*., 2012b). Piorkowski and McClements (2014) revealed that one of the major factors limiting the incorporation of carotenoids into many food and beverage products is their high susceptibility to chemical degradation. Boon *et al*. (2010) explained that the conjugated polyene chain which is characteristic of carotenoids makes these compounds susceptible to degradation. Heat, light, singlet oxygen, acid, iron and iodine, and free radical promote this degradation (Dutta *et al*., 2005; Boon *et al*., 2010).

Emulsion-based delivery systems, such as conventional emulsions, nanoemulsions as well as microemulsions are a particularly convenient means of encapsulating, protecting, and delivering poorly water soluble nutraceuticals like carotenoids for both functional food and pharmaceutical application thereby increasing its solubility, stability, bioaccessibility and bioactivity (Flanagan and Singh, 2006; Chakraborty *et al*., 2009; McClements and Li, 2010; Huang *et al*., 2010). Previous studies had investigated the formation, characteristic and stability of β-carotene emulsions or β-carotene nanoemulsions (Tan and Nakajima, 2005; Yuan *et al*., 2008; Mao *et al*., 2009; Silva, *et al*., 2011; de

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Paz *et al.*, 2013; Yi *et al.,* 2014). The emulsions or nanoemulsions were prepared using high-energy methods such as high-pressure homogenization, high shear homogenization, emulsification–evaporation technique, ultrasound, or microfluidization.

Microemulsions have several advantages over conventional emulsions or nanoemulsions, such as microemulsions can be prepared without involving high energy called spontaneous emulsification method (Flanagan and Singh, 2006; Anton and Vandamme, 2011), generally easier to prepare than nanoemulsions and conventional emulsions (Rao and McClements, 2011a), have transparent appearances, smaller droplets sizes, and thermodynamically stable (Huang *et al.*, 2010; Rao and McClements, 2011b ; Ziani *et al*., 2012). The authors had conducted microemulsion researches, such as Ariviani *et al.* (2011a), Ariviani *et al.* (2011b) and Rukmini *et al.* (2012). In the previous studies, microemulsions were prepared without involving high energies, i.e., by spontaneous emulsification method.

This research aimed to characterize β-carotene microemulsions prepared by spontaneous emulsification method using VCO or palm oil as oil phase, and to evaluate chemical stability of the β-carotene loaded microemulsions during storage at ambient temperature, 15ºC and 4ºC. The different storage temperatures were chosen to reproduce eventual commercial conditions. Thus, the results are expected to provide information for an optimal handling to maintain the stability of β-carotene during storage.

Materials and Methods

Materials

Materials: β-carotene (Type I, C9750), sorbitan monooleate (Span 80, HLB 4.3) and sorbitan monopalmitate (Span 40, HLB 6.7) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA), polyoxyethylene sorbitan monooleate (Tween 80, HLB 15), sodium dihydrogen phosphate monohydrate and disodium hydrogen phosphate dihydrate were purchased from Merck Millipore Co. (Darmstadt, Germany), virgin coconut oil (VCO) and palm oil were purchased from a local supermarket and used without further purification. Distilled water was used to prepare all solutions and microemulsions.

Preparation of β–carotene microemulsions

β-carotene microemulsions were prepared with spontaneous emulsification method as described previously (Ariviani, 2009), use ternary food grade nonionic surfactants mixture consisting of Span 80, Span 40 and Tween 80 with ratio 10 : 5: 85 (w/w). 10 µM phosphate buffer pH 7 was used as aqueous phase (Hur *et al*., 2009; Hur *et al*., 2011; Rao and McClements, 2012), whereas VCO or palm oil was used as the oil phase. Virgin coconut oil is known as triglyceride which is rich in medium chain fatty acids (Dayrit *et al*., 2007) and medium chain triglycerides shown several health benefits, e.g.: anti-diabetic (Nagao and Yaganita, 2010), prevention of obesity (Tsuji *et al*., 2001; St-Onge and Jones, 2002), and anti-inflammation of colon (Kono *et al*., 2010). Palm oil was most widely oil consumed in the world since 2005 (Badrun, 2010; Abdullah and Wahid, 2012). The proportion of surfactant, oil, and the aqueous phase were 16%, 4%, and 80% wt respectively. Briefly, β-carotene (0.025 or 0.05 %wt) was added in mixed surfactant-oil, heated and stirred using heating magnetic stirrer (AREC, VELP Scientifica, Italy) at 70ºC and 800 rpm. After 10 minutes, the aqueous phase was added dropwise while stirring and heating up to 20 minutes. The microemulsions were maintained at ambient temperature for 24 h to reach equilibrium before further investigation.

Characterization of β-carotene microemulsions

In addition to the mean particle size and size distribution as well as rheological properties, determination of physical properties such as pH and specific gravity are also required for the characterization of microemulsion (Kumar *et al*., 2014). Roohinejad *et al.* (2015) have performed characterization of β-carotene microemulsion by measurement of the viscosity, pH, refractive index, mean particle size and zeta potential. In the present study, characterization of β-carotene microemulsions was performed by measuring the viscosity, specific gravity, pH, refractive index, the mean particle diameter and size distribution as well as zeta potential. The viscosities were determined using Brookfield viscometer (Model LVT, Brookfield Co., Mass., USA) with spindle 61 at 25ºC and 60 rpm. Specific gravity measurements were taken using Specific gravity bottle (Pycnometer 25 ml, BRAND, Germany) at 20ºC. The refractive indices were measured by Abbe refractometer (ATAGO, NAR-1T 1217-LO) at ambient temperature (27±2ºC). The pH values were determined using pH meter (CyberScan pH 510, Eutech Instrument, Singapore) at ambient temperature (27±2ºC), calibrated using standard buffer solution (pH 7 and 4). The mean particle diameters (z-average) and size distributions, as well as the zeta potentials of β-carotene microemulsions were determined using zetasizer nano ZS (Malvern Instrument Ltd., Worcestershire, UK). Samples

Table 1. Characteristic of β-carotene microemulsions with different oil phase and β-carotene loaded

Oil phase and β-carotene loaded	Viscosity (mPa s)	Specific gravity	Refractive Index	pH
VCO, 0.025 %wt	5.90 ± 0.18[a]	1.011 ± 0.0004[a]	1.366 ± 0.001[a]	6.70 ± 0.05[a]
VCO, 0.05 %wt	6.05 ± 0.06[a]	1.010 ± 0.0003[a]	1.366 ± 0.000[a]	6.63 ± 0.05[a]
Palm Oil, 0.025 %wt	6.08 ± 0.13[a]	1.010 ± 0.0001[a]	1.366 ± 0.001[a]	6.70 ± 0.03[a]
Palm Oil, 0.05 %wt	5.98 ± 0.10[a]	1.011 ± 0.0003[a]	1.366 ± 0.001[a]	6.67 ± 0.07[a]

Different superscript in the same column indicated significant differences ($p < 0.05$)

were diluted using 10 µM phosphate buffer solution (pH 7) prior to analysis to avoid multiple-scattering effects during measurement. The mean particle diameters and the size distributions of the β-carotene microemulsions were measured by DSL (dynamic light scattering) at a wavelength of 633 nm and temperature of 25ºC (Salvia-Trujillo *et al*., 2013a). Zeta potentials of particles were measured by the PLS (phase-analysis light scattering) use dip Zeta cells (Salvia-Trujillo *et al*., 2013a).

Chemical stability evaluation of β-carotene loaded microemulsions

The β-carotene microemulsion samples prepared freshly were transferred into screw-capped glass vials immediately after preparation. The samples were divided into four groups which were stored in the dark at (1) ambient temperature (27 ± 2ºC), (2) ambient temperature with heating treatment (oven, 105ºC for 5 h) prior to storage, (3) 4ºC and (4) 15ºC. The stability of β-carotene loaded microemulsion against chemical degradation was monitored by measuring the β-carotene concentration over storage time. The results are expressed as the β-carotene retention which are defined as 100 x (Ct/C0), where C0 is the initial β-carotene concentration, Ct is the beta-carotene concentration at storage time t.

Determination of β-carotene concentration

β-carotene concentration was determined by measuring the absorbance of prepared β-carotene microemulsions at 461 nm (wavelength of maximum absorption) using UV-vis spectrophotometer (UV-1650 PC, Shimadzu, Japan). The absorbance determined with this method is proportional to the amount of β-carotene dispersed in solution (de Paz *et al*., 2013). Empty microemulsions (Microemulsions without β-carotene) were used as blanks.

Statistical analysis

All measurement were performed at least three replication using freshly prepared samples and were reported as means and standard deviations. The viscosity, specific gravity, pH, refractive index, mean particle diameter and zeta potential values were analyzed using the program IBM SPSS Statistics 22 (SPSS Inc., Chicago, USA) by analysis of variance (ANOVA). Significant differences of mean ($p<0.05$) were determined by Duncan's multiple range test (DMRT).

Results and Discussion

Characteristics of β-carotene microemulsions

The characteristics of β-carotene microemulsions included the viscosity, specific gravity, refractive index, and pH were presented in Table 1. The type of an oil phase and levels of β-carotene loaded had no effect on the viscosity, specific gravity, refractive index or pH values of the β-carotene microemulsions. β-carotene microemulsions prepared using either VCO (VCO microemulsions) or palm oil (palm oil microemulsions) as the oil phase had specific gravity range from 1.010 – 1.011. It was close to the specific gravity of several beverages, such as fruit drinks (1.010 – 1.030), fruit juice drinks (1.030 – 1.040), Guava nectar (1.020), sport drink (1.030) and soft drinks (1.020) (Charrondiere *et al.*, 2012). The specific gravity of orange juice and orange soft drink from different brands were 1.044 – 1.046 and 1.003 – 1.049 respectively (Vieira *et al.*, 2007). Specific gravity is also required for conversion from volume to weight and vice versa. Thus, it will simplify the application of β-carotene microemulsions as functional food ingredient. The β-carotene microemulsions prepared in this study showed refractive index ranged between 1.365 and 1.366. Sahoo *et al.* (2014) reported that the microemulsion refractive index which ranged between 1.34 and 1.40 signifies that the microemulsions were clear and transparent. The pH of emulsions had a significant impact on the stability of carotene, with most rapid degradation occurring in emulsions at pH 4 and below (Boon *et al*., 2009). Xu *et al*. (2013) reported that pH had an effect on β-carotene emulsions degradation, the degradation rate of β-carotene was much greater at pH 4 compared to pH 7.0. Qian *et al.* (2012a) studied the physical and chemical stability of β-carotene-enriched nanoemulsions. They showed that β-carotene degradation was faster at pH 3 than pH 4–8. Carotene (lycopene) degradation of WPI microemulsions was lower at pH 6.01 and 7.01 than

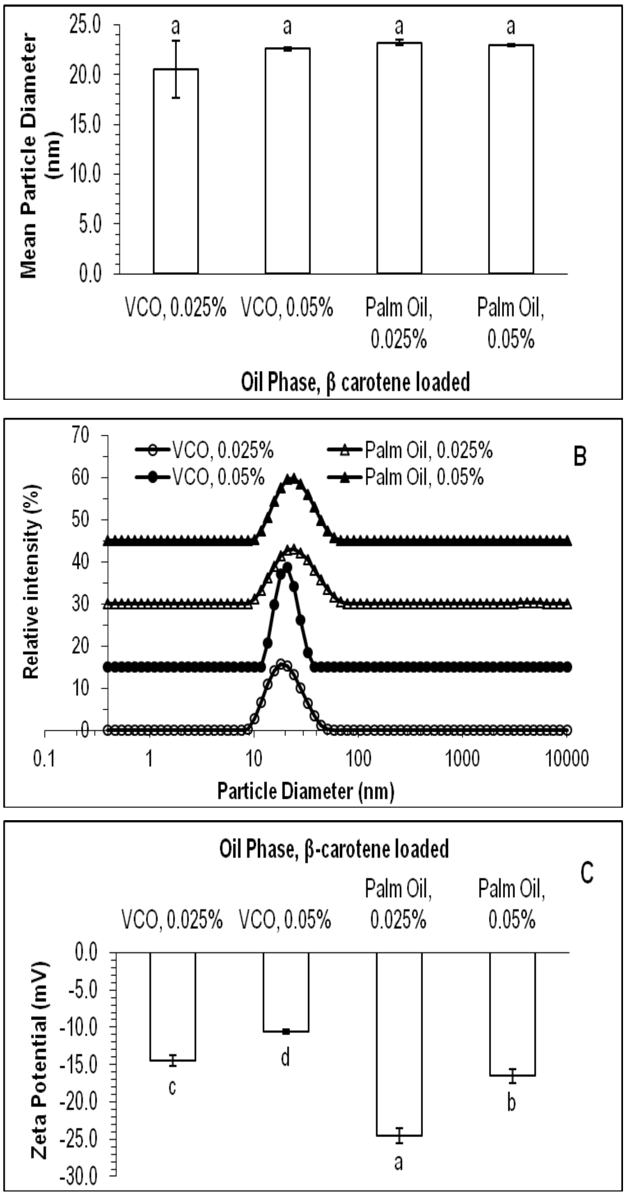

Figure 1. Mean particle diameter (A), particle size distribution (B) and zeta potential (C) of β-carotene microemulsions with different oil phase and β-carotene loaded. Different letter indicated significant differences ($p<0.05$).

pH 5.01 and below. The carotene degradation rate at pH 6.01 was not significantly different with those at pH 7.01, i.e. 34.7 ± 0.9 and 33.2 ± 1.4 mg/100g respectively (Shi *et al*., 2015). In the present study, β-carotene microemulsions showed pH values ranged from 6.63 – 6.70. Viscosity of microemulsion needs to be defined for the physical characterization. A lower viscosity at ambient temperature is useful for microemulsion applications on liquid food products such as beverages (Cho *et al*., 2008). All β-carotene microemulsions prepared in this study showed very low viscosities, ranged between 5.90 – 6.08 mPa s. Fanun (2010) stated that one of the unique properties of microemulsion is the very low viscosities.

The fundamental differences between

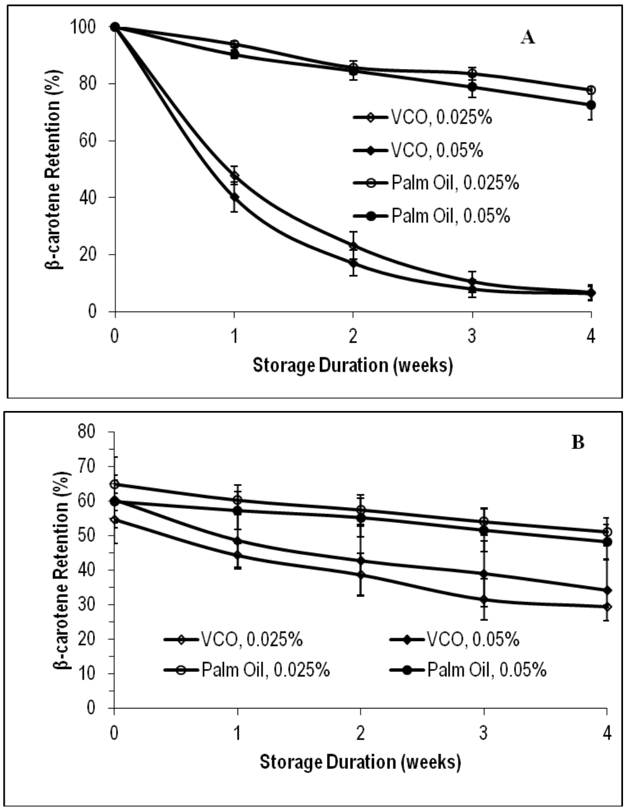

Figure 2. Chemical stability of β-carotene loaded microemulsions during ambient temperature storage. Without (A) and with (B) heating treatment (oven, 105ºC for 5 h) prior to storage.

conventional emulsions, microemulsions and nanoemulsions are their particles size, stability and appearance. Conventional emulsions have a larger particle size, i.e. 100-100000 nm, whereas microemulsions and nanoemulsions have fine particle size, i.e. 5 – 50 nm for microemulsions and 20-100 nm for nanoemulsions (McClements, 2010). Conventional emulsions and nanoemulsions are kinetically stable, while the microemulsions are thermodynamically stable (Mason *et al*., 2006; McClements, 2010; Anton and Vandamme, 2011). Conventional emulsions tend to appear either turbid or opaque, microemulsions tend to appear transparent or translucent, while the nanoemulsions tend to appear either transparent or only slightly turbid (McClements and Rao, 2011). Therefore, β-carotene microemulsions characterizations were also carried out by determining the mean particle diameters and size distributions, as well as the zeta potential. The results were presented in Figures 1.

All of the β-carotene microemulsions tested had very small mean particle diameter (z-average) and were not significantly different between samples, i.e. 20±2.83 nm and 22.60±0.16 nm for VCO microemulsions loaded β-carotene 0.025 and 0.05 %wt, 23±0.29 nm and 22.92±0.12 nm for palm oil microemulsions loaded β-carotene 0.025 and 0.05

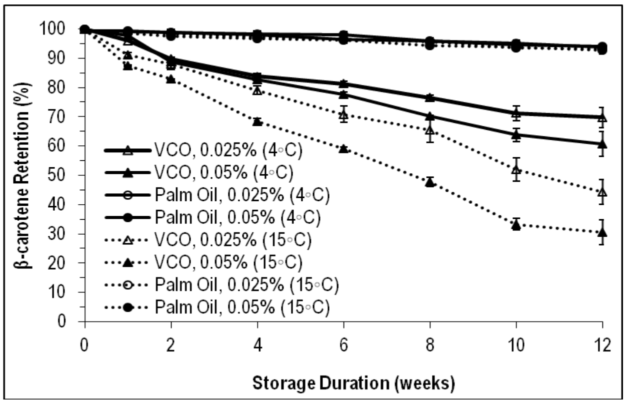

Figure 3. Chemical stability of β-carotene loaded microemulsions during storage at 15ºC (dotted line) and 4ºC (full line).

%wt respectively (Figure 1A). Figure 1B showed that all β-carotene microemulsions which were prepared by spontaneous emulsification method using different oil phases and β-carotene levels have monomodal particle size distribution, with a narrow size range from 10 – 50 nm. These results indicated that all β-carotene microemulsions had relatively homogeneous particle size. It was also supported by the low values of polydispersity index (PdI), i.e. 0.139 ± 0.040 (VCO, 0.025 %wt), 0.170 ± 0.044 (VCO, 0.05 %wt), 0.189 ± 0.014 (Palm oil, 0.025 %wt) and 0.143 ± 0011 (Palm oil, 0.05 %wt). The PdI measures the spread of the particle size distribution and, PdI values range from 0 to 1, if the value is close to 1 indicate a heterogeneous distribution of particle size (Salvia-Trujillo *et al*., 2013b). The small PdI value indicates a narrow particle size distribution (Yuan *et al*., 2008). Generally, it can be concluded that the type of the oil phase did not have an effect on the mean particle diameters and the size distributions of β-carotene microemulsions. Similar result was also reported by Qian *et al.* (2012b). In their study, β-carotene nanoemulsions were prepared with different oil phases, namely MCT (medium chain triglycerides), corn oil or orange oil. The β-carotene nanoemulsions had similar mean particle size and monomodal particle size distributions. Levels of β-carotene loaded microemulsions also had no effect on the mean particle diameter and its size distribution, this result was in line with the study of Chu *et al.* (2007).

The results of zeta potential analysis in Figure 1C showed that the β-carotene microemulsions particles had a negative electrical charge (-), even though all surfactant used in this study were nonionic surfactant. Several previous studies such as Mao *et al.* (2009), Qian *et al.* (2012b), Salvia-Trujillo *et al*. (2013a), and Salvia-Trujillo *et al*. (2013b) which used a nonionic surfactant Tween 20 to stabilize β-carotene nanoemulsions also showed negative value of the zeta potential. It has been possible due to preferential adsorption of hydroxyl ions (OH-) from the aqueous phase or the presence of anionic impurities (such as free fatty acids) in the surfactant or oil used to prepare the emulsion (McClements, 2005). Type of the oil phase influences the zeta potentials of β-carotene microemulsions. VCO which is rich in medium chain fatty acids showed lower zeta potential than palm oil that known has predominant long chain fatty acids. This result was in line with the study of Salvia-Trujillo *et al*. (2013a) which showed that the nanoemulsions with MCT (medium chain triglycerides) as oil phase have significantly lower zeta potential than that of LCT (long chain triglycerides) as oil phase. Levels of β-carotene loaded microemulsions also had an effect on zeta potential of prepared microemulsions. Higher β-carotene level provided lower zeta potential value.

Chemical stability of β-carotene loaded microemulsions

Since the chemical stability of β-carotene in emulsions-based delivery system was affected by storage temperatures (Qian *et al*., 2012a; Liang *et al*., 2013), four different storage conditions were carried out to evaluate chemical stability of β-carotene loaded microemulsions. Three different storage temperatures, i.e. ambient temperature, 15ºC, and 4ºC were chosen to reproduce eventual commercial conditions. Chemical stabilities of β-carotene during ambient temperature storage were presented in Figure 2. The β-carotene microemulsion samples with and without heating pretreatment (105ºC, 5h) incubated at ambient temperature for 4 weeks in dark conditions. Heating treatment was intended to accelerate the degradation (accelerated stability test). Either with or without heating pretreatment, the VCO microemulsions showed β-carotene degradation rates were greater than that of palm oil microemulsions. It was possible due to the differences in β-carotene solubility in the oil phases. β-carotene has very limited solubility in both oil and water, at concentrations above the saturation levels, β-carotene will form crystals. The presence of crystalline material in the emulsion-based delivery systems often promotes instability during storage (McClements, 2012). This was confirmed by the β-carotene degradation rate of the VCO microemulsions with heating pretreatment (Figure 2B) which were significantly lower compared to those without heating pretreatment (Figure 2A). The heating pretreatment (oven 105ºC for 5 h) could dissolve crystalline β-carotene in the oil phase, thus the deterioration rate of β-carotene loaded microemulsions become slower. The second possibility was due to the presence of an endogenous antioxidant tocopherol in palm oil. Gunstone *et al*. (2007) stated that palm oil contains 650 ppm tocopherol consisting of 260 ppm α-tocopherol, 320 ppm γ-tocopherol, and 70 ppm δ-tocopherol. The research conducted by Xu *et al.* (2013) showed that both transition metals and free radicals induce β-carotene degradation, nevertheless free radicals were found to be the predominant mechanism of β-carotene degradation. The study also proved that the presence of α-tocopherol significantly impacts on the β-carotene degradation. The β-carotene degradation rate was slower in the presence of α-tocopherol rather than the presence of EDTA.

Figure 3 exhibited that the β-carotene degradation rate of palm oil microemulsions which were stored at 4ºC have no significantly different with those stored at 15ºC, but it was significantly lower compared with those stored at ambient temperature (Figure 2A). Qian *et al*. (2012a) reported that the degradation rate of β-carotene in the nanoemulsions stored at 5ºC were not different with those stored at 20ºC. In all storage conditions (i.e.: 4ºC, 15ºC and ambient temperature), the VCO microemulsions showed greater β-carotene degradation compared with the palm oil ones. It could be concluded that in order to minimize the β-carotene degradation, palm oil microemulsions could be stored at 15ºC, whereas the VCO microemulsions should be stored at temperature not more than 4ºC.

Conclusion

The present study has shown that β-carotene microemulsions prepared by spontaneous emulsification method using either VCO or palm oil as oil phase, had the viscosity, specific gravity, pH, refractive index, and mean particle diameter which were not significantly different. The mean particle diameter (z-average) of the β-carotene microemulsions ranged from 20 – 23 nm and the particle size distribution were monomodal with narrow size ranged from 10-50 nm. The type of oil phase and levels of β-carotene loaded microemulsions had effect on the zeta potential of prepared microemulsions. The VCO microemulsions had significantly lower zeta potential than that of palm oil microemulsions. The higher β-carotene level provides lower zeta potential value. β-carotene loaded in palm oil microemulsions were more stable toward chemical degradation during storage rather than those loaded in VCO microemulsions. In order to minimize β-carotene degradation, the VCO microemulsions must be stored at temperature not more than 4ºC, whereas the palm oil microemulsions could be stored at 15ºC.

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References

Abdullah, R. and Wahid, M. B. 2012. World palm oil supply, demand, price and prospects: Focus on Malaysian and Indonesian palm oil industries. Downloaded from *http://mpoc.org.my/upload/WorldPalmOil_SupplyDemandPriceProspects_MalaysianIndonesianIndustry_FullReport.pdf* on 20/7/2012.

Aherne, S. A., Daly, T., Jiwan, M. A., O'Sullivan, L. and O'Brien, N. M. 2010. Bioavailability of β-carotene isomers from raw and cooked carrots using an in vitro digestion model coupled with a human intestinal Caco-2 cell model. Food Research International 43: 1449–1454.

Anton, N. and Vandamme, T. F. 2011. Nano-emulsions and micro-emulsions: Clarifications of the critical differences. Pharmaceutical Research 28: 978–985.

Ariviani S. 2009. Formulation of β-carotene microemulsion to inhibit vitamin C photodegradation in aqueous system. Yogyakarta, Indonesia: Gadjah Mada University, Msc thesis.

Ariviani, S., Raharjo, S. and Hastuti, P. 2011a. The potential inhibition of β-carotene microemulsion on vitamin C photo-oxidation in the aqueous system. Jurnal Teknologi dan Industri Pangan 22(1): 33–40.

Ariviani, S., Raharjo, S. and Hastuti, P. 2011b. Application of β-carotene microemulsion to inhibit vitamin C photodegradation in orange juice. Agritech 31(3): 180–189.

Badrun, M. 2010. The facts track: Palm oil is more efficiently. In Badrun M., and Supriono, A. The Thirty Years' Development Tracks of Palm Oil, p. 7 – 16. Indonesia: Directorate General of Horticulture, Ministry of Agriculture, The Republic of Indonesia in cooperation with Indonesian Palm Oil.

Boon, C. S., McClements, D. J., Weiss, J. and Decker, E. A. 2010. Factors influencing the chemical stability of carotenoids in foods. Critical Reviews in Food Science and Nutrition 50: 515–532.

Boon, C. S., McClements, D. J., Weiss, J. and Decker, E. A. 2009. Role of iron and hydroperoxides in the degradation of lycopene in oil-in-water emulsions. Journal of Agricultural and Food Chemistry 57: 2993–2998.

Castenmiller, J. J. M. and West, C. E. 1998. Bioavailability and bioconversion of carotenoids. Annual Review of Nutrition 18: 19–38.

Chakraborty, S., Shukla, D., Mishra, B. and Singh, S. 2009. Lipid - an emerging platform for oral delivery of drugs with poor bioavailability. European Journal of Pharmaceutics and Biopharmaceutics 73(1): 1–15.

Charrondiere, U. R., Haytowitz, D. and Stadlmayr, B.

2012. FAO/INFOODS Density Database Version 2.0 (2012). the Food and Agriculture Organization of the United Nations (FAO). Downloaded from *http://www.fao.org/docrep/017/ap815e/ap815e.pdf.* on 5/9/2014.

Cho, Y. H., Kim, S., Bae, E. K., Mok, C. K. and Park, J. 2008. Formulation of a cosurfactant-free o/w microemulsion using nonionic surfactant mixtures. Journal of Food Science 73(3): E115–E121.

Chou, P.-Y., Huang, G.-J., Cheng, H.-C., Wu, C.-H., Chien, Y.-C., Chen, J.-S., Huang, M.-H., Hsu, K.-J. and Sheu, M.-J. 2010. Analgesic and anti-inflammatory activities of an ethanol extract of *Dunaliella salina* Teod. (Chlorophyceae). Journal of Food Biochemistry 34(6): 1288–1302.

Chu, B-S., Ichikawa, S., Kanafusa, S. and Nakajima, S. 2007. Preparation and characterization of β-carotene nanodispersions prepared by solvent displacement technique. Journal of Agricultural and Food Chemistry 55: 6754–6760.

Cui, Y., Lu, Z., Bai, L., Shi, Z., Zhao, W. and Zhao, B. 2007. β-Carotene induces apoptosis and up-regulates peroxisome proliferator-activated receptor γ expression and reactive oxygen species production in MCF-7 cancer cells. European Journal of Cancer 43: 2590–2601.

Dayrit, F. M., Buenafe, O. E. M., Chainani, E. T., de Vera, I. M. S., Dimzon, I. K. D., Gonzales, E. G. and Santos, J. E. R. 2007. Standards for essential composition and quality factors of commercial virgin coconut oil and its differentiation from RBD coconut oil and copra oil. Philippine Journal of Science 136(2): 119–129.

de Paz, E., Martín, A., Mateos, E. and Cocero, M. J. 2013. Production of water-soluble β-carotene micellar formulations by novel emulsion techniques. Chemical Engineering and Processing 74: 90–96.

Dutta, D., Chaudhuri, U. R. and Chakraborty, R. 2005. Structure, health benefits, antioxidant property and processing and storage of carotenoids. African Journal of Biotechnology 4(13): 1510–1520.

Fanun, M. 2010. Formulation and characterization of microemulsions based on mixed nonionic surfactants and peppermint oil. Journal of Colloid and Interface Science 343: 496–503.

Flanagan, J. and Singh, H. 2006. Microemulsions: A potential delivery system for bioactive in food. Critical Reviews in Food Science and Nutrition 4: 221–237.

Gunstone, F. D., Harwood, J. L. and Dijkstra, A. J. 2007. The lipid handbook with CD-ROM. 3rd ed. New York: CRC Press.

Huang, Q., Yu, H. and Ru, Q. 2010. Bioavailability and delivery of nutraceuticals using nanotechnology. Journal of Food Science 75(1): R51–R57.

Hur, S. J., Decker, E. A. and McClements, D. J. 2009. Influence of initial emulsifier type on microstructural changes occurring in emulsified lipids during *in vitro* digestion. Food Chemistry 114: 253–262.

Hur, S. J., Joo, S. T., Lim, B. O., Decker, E. A. and McClements, D. J. 2011. Impact of salt and lipid type on in vitro digestion of emulsified lipid. Food Chemistry 126: 1559–1564.

Kono, H., Fuji, H., Ishi, K., Hosomura, N. and Ogiku, M. 2010. Dietary medium-chain triglycerides prevent chemically induced experimental colitis in rats. Translational Research 155: 131–141.

Kumar, A., Kushwaha, V. and Sharma, P. K. 2014. Pharmaceutical microemulsion: Formulation, characterization and drug deliveries across skin. International Journal of Drug Development & Research 6(1): 1–21.

Liang, R., Shoemaker, C. F., Yang, X., Zhong, F. and Huang, Q. 2013. Stability and bioaccessibility of β-carotene in nanoemulsions stabilized by modified starches. Journal of Agricultural and Food Chemistry 61: 1249−1257.

Mao, L., Xu, D., Yang, J., Yuan, F., Gao, Y. and Zhao, J. 2009. Effects of small and large molecule emulsifiers on the characteristics of β-carotene nanoemulsions prepared by high pressure homogenization. Food Technology and Biotechnology 47(3): 336–342.

Mason, T. G., Wilking, J. N., Meleson, K., Chang, C. B. and Graves, S. M. 2006. Nanoemulsions: Formation, structure, and physical properties. Journal of Physics: Condensed Matter 18: R635–R666.

McClements, D. J. 2005. Food emulsions. Principles, practices and techniques. Boca Raton, FL: CRC Press.

McClements, D. J. 2010. Emulsion design to improve the delivery of functional lipophilic components. Annual Review of Food Science and Technology 1: 241–269.

McClements, D. J. 2012. Crystals and crystallization in oil-in-water emulsions: Implications for emulsion-based delivery systems. Advances in Colloid and Interface Science 174: 1–30.

McClements, D. J. and Li, Y. 2010. Structured emulsion-based delivery systems: Controlling the digestion and release of lipophilic food components. Advances in Colloid and Interface Science 159(2): 213–228.

McClements, D. J. and Rao, J. 2011. Food-grade nanoemulsions: Formulation, fabrication, properties, performance, biological fate, and potential toxicity. Critical Reviews in Food Science and Nutrition 51: 285–330.

Nagao, K. and Yaganita, T. 2010. Medium-chain fatty acids: Functional lipids for the prevention and treatment of the metabolic syndrome. Pharmacological Research 61: 208–212.

Piorkowski, D. T. and McClements, D. J. 2014. Beverage emulsions: Recent developments in formulation, production, and applications. Food Hydrocolloids 42: 5–41.

Qian, C., Decker, E. D., Xiao, H. and McClements, D. J. 2012a. Physical and chemical stability of beta carotene-enriched nanoemulsions: Influence of pH, ionic strength, temperature and emulsifier type. Food Chemistry 132: 1221– 1229.

Qian, C., Decker, E.A., Xiao, H. and McClements, D. J. 2012b. Nanoemulsion delivery systems: Influence of carrier oil on b-carotene bioaccessibility. Food Chemistry 135: 1440–1447.

Rao, J. and McClements, D. J. 2011a. Food-grade microemulsions, nanoemulsions and emulsions:

Fabrication from sucrose monopalmitate & lemon oil. Food Hydrocolloids 25: 1413–1423.

Rao, J. and McClements, D. J. 2011b. Formation of flavor oil microemulsions, nanoemulsions and emulsions: influence of composition and preparation method. Journal of Agricultural and Food Chemistry 59: 5026–5035.

Rao, J. and McClements, D. J. 2012. Food grade microemulsions and nanoemulsions: Role of oil phase composition on formation and stability. Food Hydrocolloid 29: 326–334.

Roohinejad, S., Oey, I., Wen, J., Lee, S. J., Everett, D. W. and Burritt, D. J. 2015. Formulation of oil-in-water β-carotene microemulsions: Effect of oil type and fatty acid chain length. Food Chemistry 174: 270–278.

Rukmini, A., Raharjo, S., Hastuti, P. and Supriyadi, S. 2012. Formulation and stability of water-in-virgin coconut oil microemulsion using ternary food grade nonionic surfactants. International Food Research Journal 19(1): 259–264.

Sahoo, S., Pani, N. R. and Sahoo, S. K. 2014. Microemulsion based topical hydrogel of sertaconazole: Formulation, characterization and evaluation. Colloids and Surfaces B: Biointerfaces 120: 193–199.

Salvia-Trujillo, L., Qian, C., Martin-Belloso, O. and McClements, D. J. 2013a. Modulating β-carotene bioaccessibility by controlling oil composition and concentration in edible nanoemulsions. Food Chemistry 139: 878–884.

Salvia-Trujillo, L., Rojas-Graü, A. M., Soliva-Fortuny, R. and Martín-Belloso, O. 2013b. Effect of processing parameters on physicochemical characteristics of microfluidized lemongrass essential oil-alginate nanoemulsions. Food Hydrocolloids 30: 401–407.

Shi, J., Xue, S. J., Wang, B., Wang, W., Ye, X. and Quek, S. Y. 2015. Optimization of formulation and influence of environmental stresses on stability of lycopene-microemulsion. LWT - Food Science and Technology 60: 999–1008.

Silva, H. D., Cerqueira, M. A., Souza, B. S. W., Ribeiro, C., Avide, M. C., Quintas, M. A. C., Coimbra, J. S. R., Carneiro-da-Cunha, M. G. and Vicente, A. A. 2011. Nanoemulsions of b-carotene using a high-energy emulsification–evaporation technique. Journal of Food Engineering 102: 130–135.

St-Onge, M. and Jones, P. J. H. 2002. Physiological effects of medium-chain triglycerides: Potential agents in the prevention of obesity. Journal of Nutrition 132: 329–332.

Tan, C. P. and Nakajima, M. 2005. β-Carotene nanodispersions: Preparation, characterization and stability evaluation. Food Chemistry 92: 661–671.

Tsuji, H., Kasai, M., Takeuchi, H., Okazaki, M. and Kondo, K. 2001. Dietary medium-chain triacylglycerols suppress accumulation of body fat in a double-blind, controlled trial in healthy men and women. Journal of Nutrition 131: 2853–2859.

Vieira, S. M., Theodoro, K. H. and Gloria, M. B. A. 2007. Profile and levels of bioactive amines in orange juice and orange soft drink. Food Chemistry 100: 895–903.

Wang, P., Liu, H. J., Mei, X. Y., Nakajima, M. and Yin, L. J. 2012. Preliminary study into the factors modulating b-carotene micelle formation in dispersions using an in vitro digestion model. Food Hydrocolloids 26: 427–433.

Xu, D., Wang, X., Jiang, J., Yuan, F., Decker, E. A. and Gao, Y. 2013. Influence of pH, EDTA, a-tocopherol, and WPI oxidation on the degradation of b-carotene in WPI-stabilized oil-in-water emulsions. LWT - Food Science and Technology 54: 236–241.

Yi, J., Li, Y., Zhong, F. and Yokoyama, W. 2014. The physicochemical stability and in vitro bioaccessibility of beta-carotene in oil-in-water sodium caseinate emulsions. Food Hydrocolloids 35: 19–27.

Yuan, Y., Gao, Y., Mao, L. and Zhao, J. 2008. Characterization and stability evaluation of beta carotene nanoemulsions prepared by high pressure homogenization under various emulsifying conditions. Food Research International 41: 61-68

Ziani, K., Fang, Y. and McClements, D. J. 2012. Fabrication and stability of colloidal delivery systems for flavor oils: Effect of composition and storage conditions. Food Research International 46: 209–216.