Engineering Chemistry
ISSN: 2813-6535, Vol. 6, pp 35-43
doi:10.4028/p-Cpp44V

Submitted: 2023-07-03
Revised: 2023-08-08
Accepted: 2023-08-09
Online: 2024-03-05

Factors Affecting Stability of Particle Size in Tocotrienol Emulsion Prepared by Spontaneous Emulsification

Nabilah Kamaliah Mustaffa[a]* and Harrison Lik Nang Lau[b]

Engineering and Processing Division, Malaysian Palm Oil Board, 6, Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia.

[a]*nabilah@mpob.gov.my, [b]harrison@mpob.gov.my

Keywords: Tocotrienols, particle size, emulsification

Abstract. Incorporation of vitamins into food, feed, and personal care is realised through microencapsulation of their nanoparticle in powder form. Vitamin E consisting of α-, β-, γ-, and δ-tocopherols and α-, β-, γ-, and δ-tocotrienols, is a powerful antioxidant that has been used in food applications and supplements. However, due to its fat-soluble characteristic, the application of tocotrienol in food and beverages is still limited. Therefore, microencapsulation of tocotrienol via emulsification could help maximise the dissolution of tocotrienols during processing and prolong the product's shelf lives. In this study, factors affecting the particle size, including surfactant concentration, stirring time and speed, and mixing temperature for the preparation of tocotrienol emulsion, were investigated. Emulsions were prepared using 12 wt% surfactant concentration, 800 rpm stirring for 30 minutes. Emulsions with 190 nm particle size were found stable after 30 days storage at ambient temperature.

Introduction

Vitamin E is an essential nutrient for the body which exist in eight form i.e α-, β-, γ- and δ-tocopherol and α-, β-, γ-, and δ-tocotrienol. It is essential as the body cannot manufacture vitamin E; thus, it must be supplemented via food [1]. The difference between tocotrienols and tocopherols lies in their side chains in which tocotrienols have three double bonds at carbons 3, 7, and 11 whereas tocopherols possess saturated phytol side chains [2]. As part of the vitamin E family, tocotrienols are absorbed in the same way as fat from food in the gut. Tocotrienols are biologically available and are deposited in lipid-rich organs, including the liver, brain, spleen, lung, kidney, and heart. Tocotrienols, as powerful antioxidants, protect the body from attack by free radicals and DNA damage. The health benefits of tocotrienols include cancer and tumour suppression, carotid atherosclerosis reversal, cholesterol levels maintenance, anti-aging, and skin protection. The safe dosage of various tocotrienols for human consumption is estimated to be less than 1,000 mg/day. The supplement is best taken with meals to increase absorption in the gut [3].

Nanoemulsion formation requires energy input to be in the equilibrium state because of the unstable behavior. It can be produced using a variety of methods that can be classified as a high-energy approach or low-energy approach. High energy technologies such as homogenizer, microfluidizer, or sonicator are used to form small droplets from two immiscible oil and water phase. While, the low energy relies on the spontaneous formation of oil droplets, which is achieved by varying emulsifying variables such as surfactant and oil concentration and temperature [4].

Microencapsulation can improve the retention time of nutrients in food and allow controlled release over a specific period [5]. Therefore, microencapsulation in the food industry will help maximise the retention time of the bioactive components during processing and storage of the formulated products [6]. Besides that, vitamin E nanoparticles can be produced by ultra-high-pressure homogenisation and stabilised by microencapsulation with starch. Vitamin E which was specifically designed for beverage application has alleviated the turbidity and physical stability problems [7]. On the other hand, the preparation of palm-based tocols nanoemulsion using

ultrasonication to produce small average droplet size (<100 nm) and narrow droplet size distribution with low polydispersity index (PDI) value has been studied [8].

One of the technologies to produce palm tocotrienols has been commercialized by a few local companies [9]. Some applications using these tocotrienols have also been studied [10 - 13]. Products of palm tocotrienol manufactured by local companies that can be found in the market are SuperVitamin TRF suspension oil, SD Bioganic TRF products, and Carotino TRF oil suspension products etc. These products differ from each other in their concentrations and specifications. The price of each product varies depending on the concentration of tocotrienols as well as the type of emulsifier used in the formulation.

The demand for natural vitamin E has increased in recent years. The end-user applications of vitamin E include animal feed, nutritional/dietary supplements, food and beverages and cosmetics. Vitamin E consumption is projected at 10.8 thousand metric tons by 2024 from an estimated 8.5 thousand metric tons in 2018 and the product market will grow at a compound annual growth rate (CAGR) of 6.22% by 2026 [14].

Several functional food and beverage applications tocotrienols have been commercialised in various parts of the world. Biscuits, cereal, chocolate bars, mayonnaise, energy drink, carbonated drink and juices are examples of products containing vitamin E. The demand for ready-to-eat processed foods and beverages with high nutritional quality is steadily increasing due to consumers' awareness, growing urbanisation, and increased employment. However, functional food products are not homogeneously distributed over all food market segments. Product preferences may vary between markets. Another important product category within the functional food segment is non-alcoholic beverages fortified with vitamins or other functional ingredients. Although nutritional products are available in the functional food segment, the market is still small and fragmented in most European countries [15]. In general, functional food can improve consumers' health and wellness besides reducing healthcare costs by providing a convenient form of healthy ingredients [16].

Materials and Method

Materials

Palm tocotrienols (T50) were purchased from Sime Darby Jomalina Sdn Bhd and SOP Nutraceuticals Sdn Bhd. Specification of the tocotrienols concentrates is shown in Table 1. A non-ionic surfactant, Tween 80, carrier oils such as medium-chain triglycerides (MCT) and long-chain triglycerides (LCT) were purchased from Sigma-Aldrich Co. (St. Louis, MO). Other chemicals for gas chromatography (GC) analysis were purchased from local chemical companies.

Table 1. Vitamin E Content of Tocotrienol Concentrate (T50).

Parameter	Specifications
Tocols content (HPLC)	50% min
Tocols composition:	
d-Alpha Tocopherol	10.0% min
d-Alpha Tocotrienol	8.0% min
d-Beta Tocotrienol	0.75% min
d-Gamma Tocotrienol	15.05% min
d-Delta Tocotrienol	3.0% min

Method

Preparation of Tocotrienols Emulsions

The tocotrienols emulsion was prepared by adding the oil phase into an aqueous phase. The aqueous phase was prepared by dissolving the carrier oil in water at ambient temperature under mechanical stirring. The emulsions were prepared with four different surfactant (Tween 80 and Tween 20) concentrations (6 wt%, 8 wt%, 10 wt%, and 12 wt%). Emulsification was performed by adding the oil phase to the aqueous phase (pH 3.0) at 40°C. The oil phase was dispersed into the aqueous phase solution and mixed for 30 min using a mechanical stirrer with 800 rpm stirring speed under nitrogen blanketing. A sample of the emulsion was taken every 5 min starting from 0 min to 30 min. The particle size distribution was determined using Zetasizer analyser and vitamin E concentration was determined using GC. Fig.1 shows the process flow for the preparation of tocotrienols emulsion.

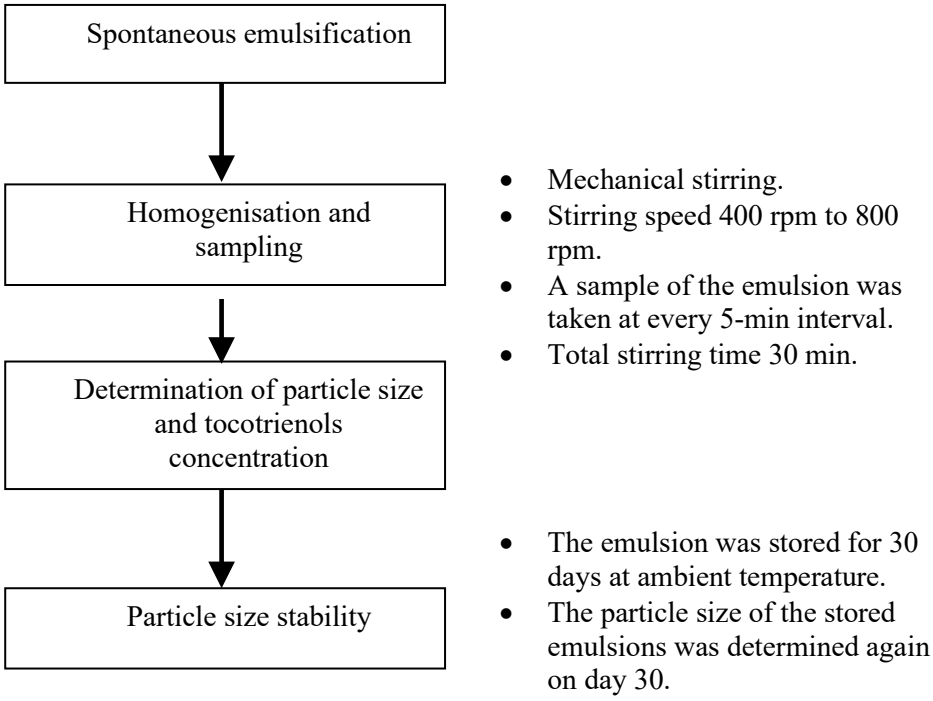

Fig. 1. Process flow for the preparation on tocotrienols emulsion.

Results and Discussions

Effect of temperature on the particle size

The effect of temperature on the emulsion's particle size was investigated by preparing two sets of emulsions with fixed oil composition, surfactant type, and stirring speed. In order to test the viability of adopting low-energy methods, a temperature range of 30°C to 60°C was chosen. The smallest droplet diameters were observed at oil phase's lowest temperature i.e., at 30°C. Fig. 2 shows a moderate increase in mean particle size with increasing temperature for both types of surfactants. When oil phase was heated from 30°C to 60°C, the mean particle size increased from 107 nm to 116 nm for the surfactant to oil ratio of 2:1 and 142 nm to 158 nm for the surfactant to oil ratio of 1:1. This scenario indicated that homogenisation temperature did not significantly affect the particle size for the range of temperatures studied which allows the formation of emulsion at room temperature using simple stirring rather than high-energy methods. The increase in particle size with increasing temperature may have occurred due to several reasons, such as the viscosity of the oil phase, oil solubility, oil-water interfacial tensions, and others [17]. The emulsion formed at 30°C's droplet size remained physically stable over a month of storage at ambient temperature. However, it was anticipated to increase when exposed to higher temperatures.

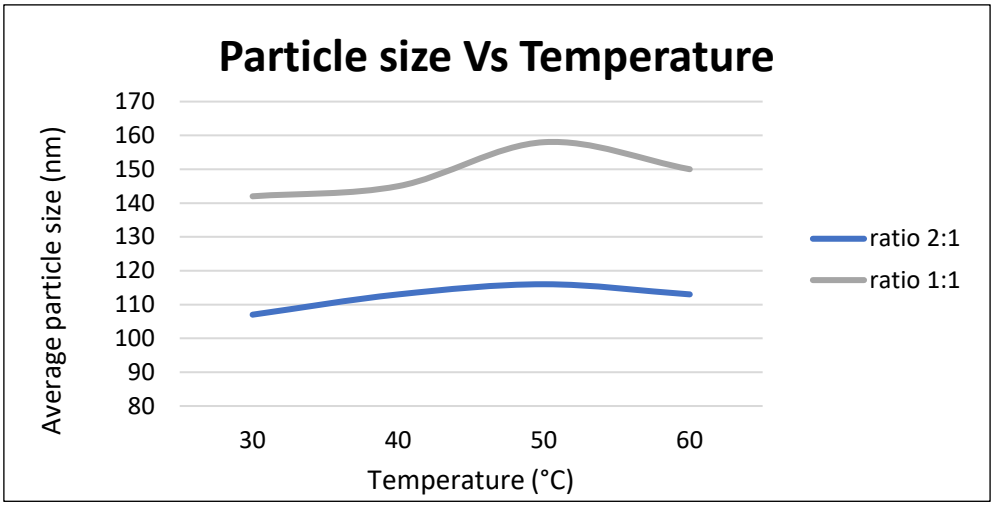

Fig. 2. Average particle size of emulsions prepared with a different surfactant-to-oil ratio at different oil phase temperatures.

Effect of surfactant concentration and time on particle size

Surfactant concentration is another important variable in nanoemulsion preparation due to the increase in the oil-water interface area which requires more emulsifiers to completely cover the interface [18]. The effect of the surfactant's concentration was investigated by preparing the emulsion with four different surfactants at various concentrations (6 wt%, 8 wt%, 10 wt%, and 12 wt%). Results showed that the smallest emulsion's particle size was achieved at day 1 (180 nm) at 12 wt% surfactant concentration after 30 min stirring time at 800 rpm (Fig. 3). The reduction in particle size with increased stirring time and surfactant concentration is due to the increased shearing force when higher speed is applied and greater amount of surfactant molecules diffusing from the oil phase to the aqueous phase, leading to the formation of finer oil particle.

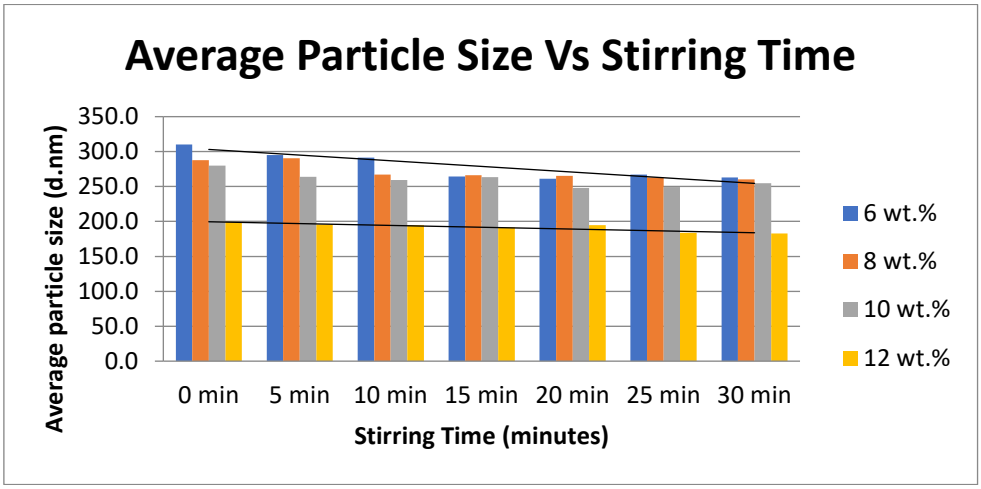

Fig. 3. Average emulsion's particle size prepared with different surfactant concentrations after stirring for 0 min to 30 min.

Fig. 4 shows the size distribution for emulsion with 6 wt% and 12 wt% surfactant concentrations. The particle size distributions for 6 wt% surfactant at 0 and 30 min stirring showed narrower distribution compared to the emulsion with 12 wt% surfactant concentration (at 0 and 30 min). These results suggested that tocotrienols can be formed through spontaneous emulsification even at low surfactant concentration. Nevertheless, in order to prepare a stable emulsion with particle size less than 100 nm the surfactant's concentration must be optimised. Emulsion for commercial applications must remain stable for a certain period.

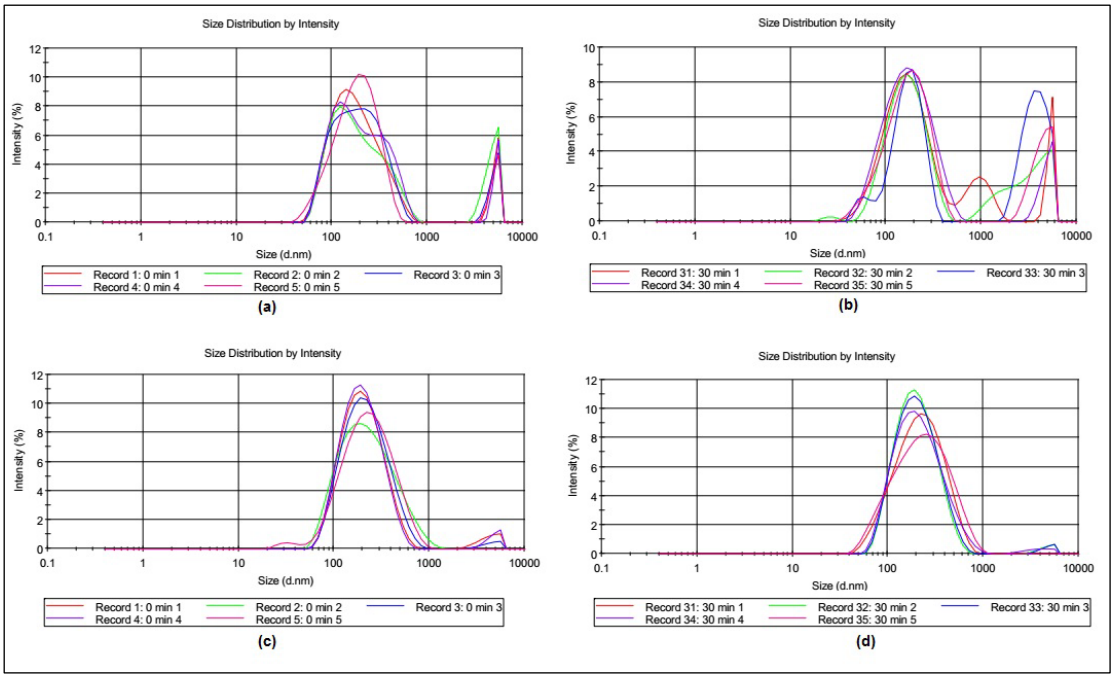

Fig. 4. Particle size distribution (a) 6 wt%, 0 min (b) 6wt%, 30 min (c) 12wt%, 0 min and (d) 12wt%, 30min.

Fig. 5 shows the phase stability of emulsion prepared with four different surfactant concentrations (6 wt%, 8 wt%, 10 wt%, and 12 wt%). Emulsion with low surfactant concentration separated into two phases after 2 days storage at ambient temperature, while emulsion with high surfactant concentrations (8 wt% and 12 wt%) remained single phased for at least 10 days storage. Emulsion with 12 wt% surfactant concentration is stable after 30 days storage at ambient temperature, with the particle size maintained at 180 nm (Fig. 6).

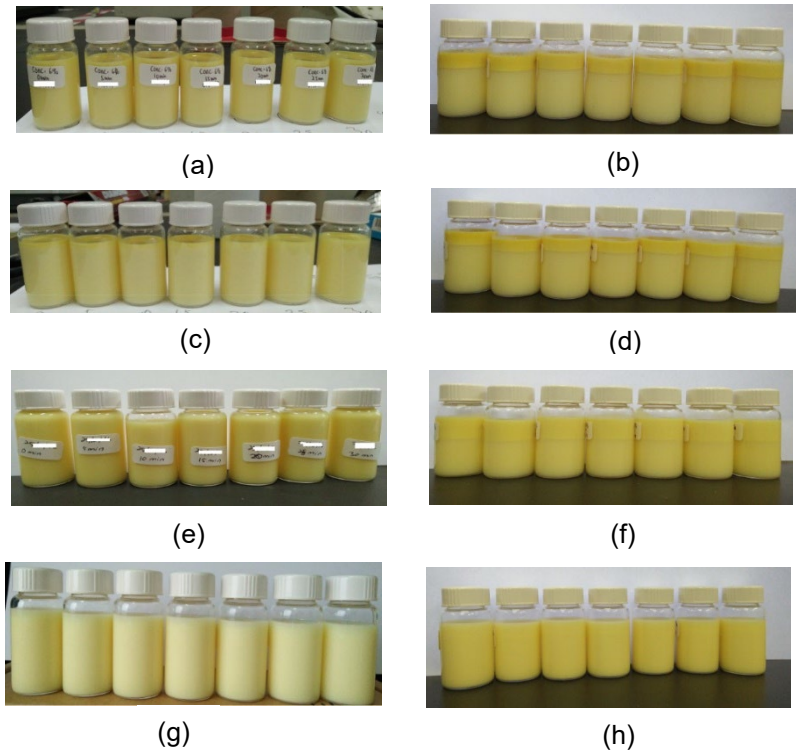

Fig. 5. Storage observation of the emulsion with various surfactant concentrations (a) 6 wt%, day 1, (b) 6 wt%, day 2, (c) 8 wt%, day 1, (d) 8 wt%, day 2, (e) 10 wt%, day 1, (f) 10 wt%, day 10, (g) 12 wt%, day 1 and (h) 12 wt%, day 30.

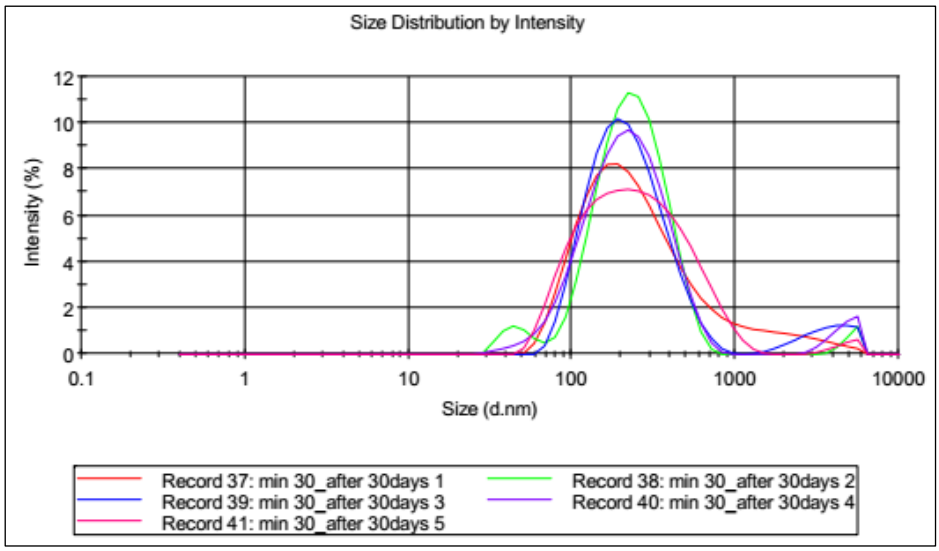

Fig. 6. Particle size distribution emulsions prepared with 12 wt% surfactant concentration after 30 days of storage at ambient temperature.

Effect of two different carrier oils on particle size

The smallest particle size (182 nm) of the emulsion was obtained with MCT as a carrier oil and 12 wt% surfactant concentration after 30 min stirring (Fig. 7). Particle size of the emulsion prepared using LCT was 240 nm with 12 wt% surfactant concentration after 30 min stirring. It was postulated that viscosity and interfacial tension of the carrier oils influenced the emulsion particle size as MCT and LCT have much lower viscosities than vitamin E. MCT has lower viscosity of 0.028 (Pas) compared to LCT (0.052 (Pas)) [19]. Viscosity of the oil phase might be expected to influence the diffusivity of the non-ionic surfactant (Tween 80) molecule from the organic phase to the aqueous phase [20]. Smaller droplet formation is facilitated by the adsorption of surfactant molecules to the oil-water interface, which results in a reduction in the interfacial tension. The development of finer oil droplets at the boundary has been attributed to a higher amount of surfactant molecules diffusing from the organic phase to the aqueous phase. When the surfactant concentration was increased, the droplet size was found to decrease too. Therefore, the lower the viscosity of the oil phase with sufficient surfactant concentration, the smaller the particle size produced, as the surfactant molecules can diffuse at a higher rate.

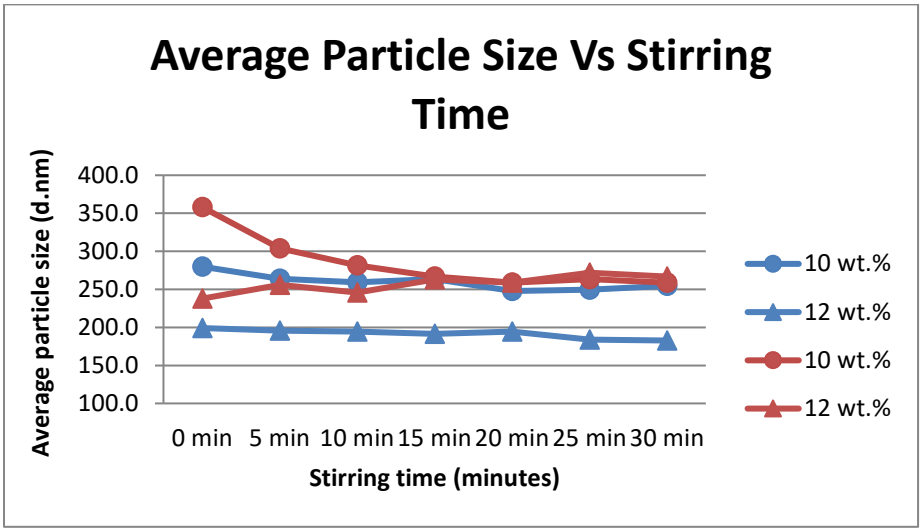

Fig. 7. Average particle size of emulsions prepared with different surfactant concentrations after 0 min to 30 min stirring.

Note: ■ *emulsion with MCT as carrier oil*
■ *emulsion with LCT as carrier oil*

The particle size distribution for emulsion prepared with 12 wt% is the narrowest for both MCT and LCT carrier oils (Fig. 8). However, the graph distributions for both 10 wt% and 12 wt% surfactant concentrations with MCT as carrier oil were better than emulsions prepared using LCT as carrier oil. The narrowest peak distribution suggested that the particles formed in the emulsions were consistent. Phase stability for both emulsions is shown in Fig. 9. Both emulsions formulated with MCT and LCT as carrier oils consisted of particles with less than 500 nm. However, emulsion using LCT as carrier oil at 10 wt% surfactant concentrations separated into two phases in the same day, while emulsion prepared using LCT as carrier oil at 12 wt% concentration separated into two phases on the second day. Therefore, MCT is more suitable as a carrier oil to produce tocotrienol emulsion as it is more stable. The emulsion's phase stability depended on the surfactants, surfactant composition and particle size distribution [20].

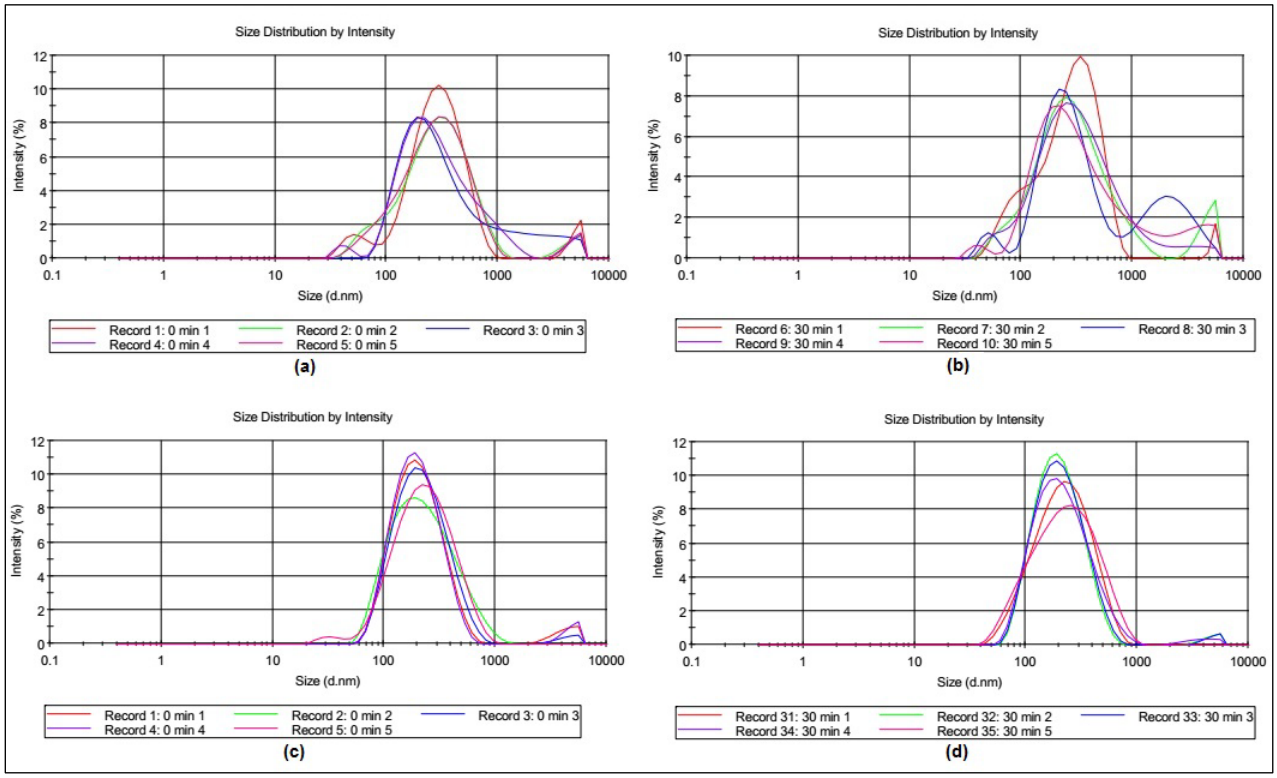

Fig. 8. Particle size distribution of (a) LCT, 12 wt%, 0 min (b) LCT 12 wt%, 30 min (c) MCT, 12 wt%, 0 min and (d) MCT, 12 wt%, 30min

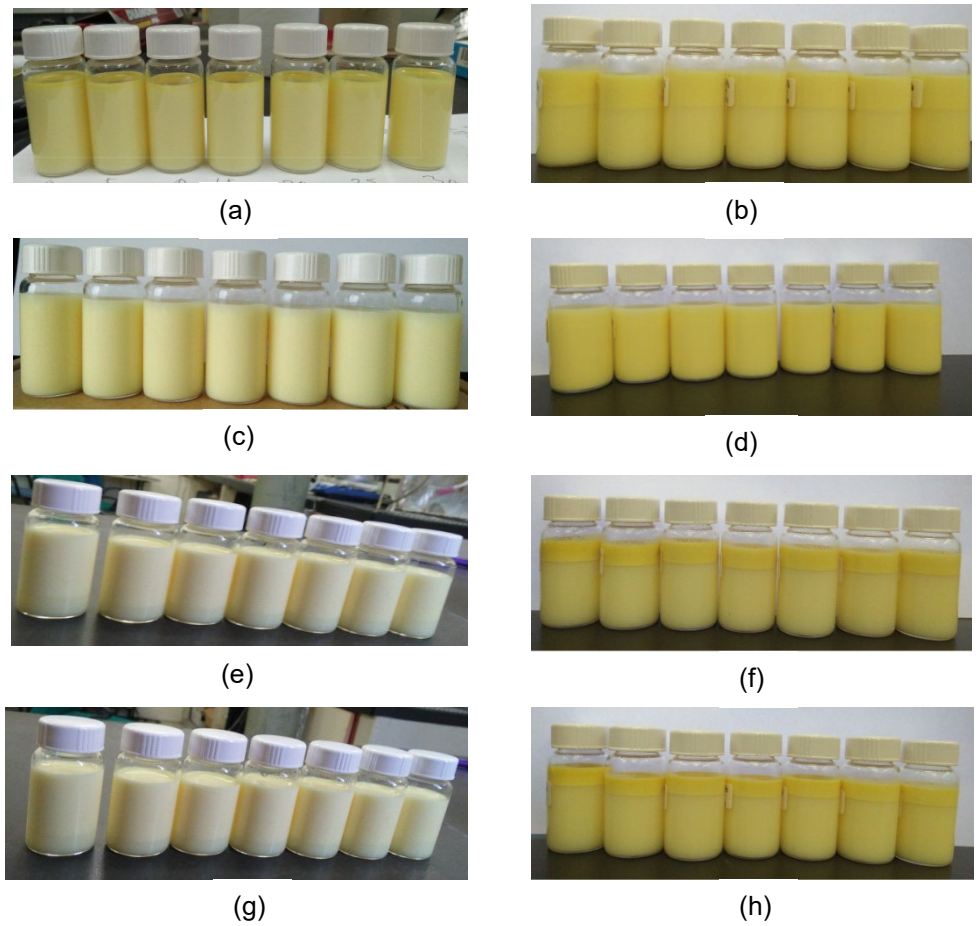

Fig. 9. Storage observation of emulsion (a) MCT, 10 wt%, day 1, (b) MCT, 10 wt%, day 10, (c) MCT, 12 wt%, day 1, (d) MCT, 12 wt%, day 30, (e) LCT, 10wt%, day 1, (f) LCT, 10 wt%, day 1, (g) LCT, 12 wt%, day 1 and (h) LCT, 12 wt%, day 2.

Conclusion

In conclusion, four tocotrienol emulsions can be produced via spontaneous emulsification at low surfactant concentrations (< 10 wt%). Emulsion was found stable after 30 days storage at ambient temperature using 12 wt% surfactant concentrations at highest stirring speed for 30 min and at ambient mixing temperature. Particle size of the emulsion maintained at 180 nm. Intermediate palm tocotrienol products for food and beverages application can be produced via spontaneous emulsification for application and incorporation of vitamin E into these products.

References

[1] K.S Chandan, K Savita and R Sashwati. Tocotrienols: Vitamin E beyond tocopherols. Life Sciences,78 (2006) 2088–2098.

[2] J.Y Fu, H.L Che, D.M.Y Tan and K.M Teng. Bioavailability of tocotrienols: evidence in human studies. Nutrition & Metabolism,11 (2014) 5.

[3] What is tocotrienol on https://www.verywellhealth.com/tocotrienol-5093146#toc-dosage-and-preparation

[4] N. Dasgupta and S. Ranjan. Fabrication of Nanoemulsion: A Brief Review in: An Introduction to Food Grade Nanoemulsions. Springer, (2018) 49-62.

[5] N. Wilson and N.P Shah. Microencapsulation of vitamins. ASEAN Food Journal 14 (1), (2007) 1-14.

[6] H. Korhonen. Technology options for new nutritional concepts. International Journal of Dairy Technology 55 (2), (2002) 79-88.

[7] C.C Chen and G. Wagner. Vitamin E nanoparticle for beverage applications. Chemical Engineering Research and Design 82(11), (2004) 1432-1437.

[8] P.S Goh, M.H Ng, Y.M Choo, B.A Nasrulhaq and C.H Chuah. Production of tocols nanoemulsion by ultrasonication. Journal of Oil Palm Research 28(1), (2016) 121-130.

[9] Y.M Choo, L.L.N Harrison, C.W Puah, M.H Ng, S.C Bong, A.N Ma and Y. Basiron. Production of phytonutrients (carotenes, vitamin E, sterols, squalene, co-enzyme q and phospholipids) from palm methyl esters. In: MPOB Transfer of Technology, 2002 MPOB Information Series No. 151.

[10] P.T Voon, W.T Lai, M.S Miskandar, W.H Lim, O.M Lai and C.P Tan. Nutramee – A lipophilic antioxidant noodle. In: MPOB Transfer of Technology, 2014, MPOB Information Series No. 563.

[11] K. Nesaretnam, K.R Selvaduray, W.H Lim and A.R Ghazali. A transdermal tocotrienol breast cream. In: MPOB Transfer of Technology, 2007, MPOB Information Series No. 378.

[12] I. Zahariah, A. Salmiah, I. Rosnah, A. Zafarizal and T.L Ooi. palm-based skin care and colour cosmetic products. In: MPOB Transfer of Technology, 2005, MPOB Information Series No. 273-274.

[13] H.N Sook, M.W Pei, B. Mahiran and I. Zahariah. Characterization of structural stability of palm oil esters-based nano cosmeceuticals loaded with tocotrienol. Journal of Nanobiotechnology 11(27), (2013)1477-3155.

[14] Natural source vitamin E (tocopherols and tocotrienols) – A global market overview on https://www.researchandmarkets.com/reports/4749730/natural-source-vitamin-e tocopherols-and#src-pos-1

[15] I. Siro´, E. Ka´Polna, B. Ka´Polna and A. Lugasi. Functional food. Product development, marketing and consumer acceptance - A review. Appetite 51 (2008) 456-467.

[16] M.C Kristi and F. Coni. Position of the academy of nutrition and dietetics: Functional foods. Journal of the academy of nutrition and dietetics 113, (2013)1096-1103.

[17] A.H Saberi, Y. Fang and D.J Mcclements. Fabrication of vitamin E – enriched nanoemulsions: Factors affecting particle size using spontaneous emulsification. Journal of Colloid and Interface Science 391, (2013) 95-102.

[18] S.M Jafari, Y He and B Bhandari. Production of sub-micron emulsions by ultrasound and microfluidization techniques. J. Food Engineering 82, (2007) 478-488.

[19] Y. Yang and D.J McClements. Encapsulation of vitamin E in edible emulsions fabricated using a natural surfactant. Food Hydrocolloids 30, (2013) 712-720.

[20] A.H Saberi, Y. Fang and D.J Mcclements. Stabilization of vitamin E-enriched mini-emulsions: Influence of organic and aqueous phase compositions. Colloids and Surfaces A: Physochemical and Engineering Aspects 449, (2014) 65-73.