Food Research 8 (3) : 363 - 375 (June 2024)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

RESEARCH PAPER

Characteristic and storage stability of nanostructured lipid carriers containing red palm oil

[1,2]Efendi, Z., [1]Ardhi, A., [1]Santoso, U., [1]Supriyadi, [3]Ulfah, M. and [1,*]Raharjo, S.

[1]*Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Bulaksumur, Yogyakarta, 55281, Indonesia*
[2]*Department of Agricultural Technology, Faculty of Agriculture, Universitas Bengkulu, Jl. WR Supratman, Kandang Limun, Bengkulu, 38371, Indonesia*
[3]*Department of Agricultural Technology, Institute of Agriculture STIPER, Jl. Nangka II, Maguwoharjo, Depok, Sleman, Yogyakarta, 55282, Indonesia*

Article history:
Received: 13 July 2022
Received in revised form: 5 May 2023
Accepted: 3 October 2023
Available Online: 8 June 2024

Keywords:
Nanostructured lipid carriers,
Palm kernel stearin,
Palm stearin,
Red palm oil

DOI:
https://doi.org/10.26656/fr.2017.8(3).375

Abstract

Red palm oil (RPO) is a potential carotenoid source mostly containing β-carotene. RPO instability requires a delivery system such as nanostructured lipid carriers (NLC). This research aimed to develop an NLC delivery system and study the characteristics of NLC-RPO. The melting-emulsification-ultrasonication method was employed to produce the NLC-RPO based on 6% (w/w) lipids, with solid lipid to RPO ratio (SRR) varied 6:4, 7:3, and 8:2. Tween 80 (24% w/w) to lipids ratio of 4:1, and distilled water 70% (w/w). The solid lipids employed in this investigation were palm stearin (PS), and palm kernel stearin (PKS), while the liquid lipid was RPO. The stability of NLC-RPO was evaluated using β-carotene entrapment efficiency (EE), centrifugation, cooling, heating test, color, and pH for 90 days of storage. The NLC-RPO was characterized by particle size, polydispersity index (PDI), zeta potential, and viscosity before and after 90 days. Regression analysis was performed to evaluate the relationships between the storage and stability parameters. The highest encapsulation efficiency of β-carotene in NLC-RPO as a carrier β-carotene from the RPO was achieved when solid lipid to RPO ratio (SRR) of 6:4 and there was no significant difference in the type of solid lipid (PS and PKS) used. Generally, NLC-RPO stored for 90 days at room temperature showed good stability after centrifugation, cooling and heating tests with greenish-yellow color (-a*;+b*), and pH of 6.38-6.54. The particle size (38-87 nm), PDI (0.01-0.54), and zeta potential (-10.17 to -22.67 mV) did not significantly change over 90 days of storage, while the viscosity (8.36-9.11cP) was significantly different. The NLC-RPO with SRR of 6:4 had the highest β-carotene entrapment efficiency and remained stable after 90 days of storage at room temperature.

1. Introduction

Red palm oil (RPO) is known as one of the derivates of palm oil products obtained from the olein fraction of crude palm oil (CPO) that has high added value. Generally, RPO contains bioactive components such as phytosterols, carotenoids, tocopherols, tocotrienols, squalene and coenzyme Q_{10} (Chawla and Saxena, 2013). RPO has a reddish color that is associated with carotenoids. Two mainly categorized carotenoids are carotene and xanthophylls. RPO has a total carotene concentration of 500 ppm, with β -carotene being the most prevalent (Dauqan *et al.*, 2011). β-carotene is well-known as provitamin A and is also recognized as a potent antioxidant (Mayamol *et al.*, 2007). Goh *et al.* (1985) report that RPO is a rich natural source of α-β-carotene (500-1500 ppm) and tocol (700-1000 ppm). Total carotene in RPO is 545 ppm, of which the levels of α-carotene, β-carotene, γ-carotene, δ-carotene, cis-carotene, and lycopene were 37.0, 47.4, 0.5, 0.6, 6.9% and 1.5%, respectively (Choo *et al.*, 1993). The other report indicates a total of 263 ppm of carotene contained in RPO with a melting point of 36.5°C (Hasibuan and Ijah, 2018).

However, the use of β-carotene as a bioactive ingredient has limitations because of its lipophilic characteristic with low solubility and poor stability in water, processing, and storage. Carotenoids can be dissolved in the oil phase of an oil-in-water emulsion to increase their solubility and stability in water (Sari *et al.*,

*Corresponding author.
Email: *sraharjo@ugm.ac.id*

eISSN: 2550-2166 /
Published by Rynnye Lyan Resources

2018). A β-carotene microemulsion can inhibit the photooxidative deterioration of vitamin C in aqueous food (Ariviani *et al.*, 2011a; Ariviani *et al.*, 2011b). Another effort to develop a stable emulsion system containing lipophilic compounds can be carried out through a nano-delivery system.

Nanostructured lipid carriers (NLCs) are a nano-particulate carrier system consisting of solid lipids, liquid lipids, emulsifying agents, and water. NLC is a potential delivery system for food bioactive molecules and it is suitable for application in transparent or opaque beverages (Tamjidi *et al*., 2013). The NLC system has several advantages such as high loading capacity, more controlled release of bioactive components, protection of bioactive components, low crystallinity index and stability in physicochemical characteristics (Varshosaz *et al.*, 2010; Li and Ge, 2012; Azar *et al*., 2020), reduced polymorphic transition, little crystalline index, enhanced encapsulation efficiency and loading capacity, physical stability, improved chemical stability, bioavailability, and the controlled release of encapsulated components (Kanojia *et al*., 2022). From these properties, some disadvantages of microemulsions, nanoemulsions, and solid lipid nanoparticles (SLN) can be corrected by NLC system. The lipid phase has an important influence on the features of NLC, such as loading capacity and physical stability (Tamjidi *et al*., 2014a). The physicochemical stability of NLC is influenced by its constituent components derived from lipids, surfactants, and their formulations. The solubility of bioactive components in liquid lipids must be high because this affects the loading capacity. Liquid lipids and solid lipids used must also be compatible with the bioactive components and miscible at specific concentrations (Tamjidi *et al.*, 2013).

The range of melting points, crystal shape, viscosity, and polarity must be taken into account when choosing the solid lipids to be employed (Qian *et al.*, 2012a). The use of palm stearin as a solid lipid is successfully applied to NLC (Rohmah *et al.*, 2019b; Rohmah *et al*., 2020; Rohmah *et al.*, 2022). However, palm kernel stearin, a derived product of palm kernel processing has not been studied for NLC. Palm kernel stearin has a larger amount of total solid fat content (SFC) than palm stearin at 10-27°C, which causes palm kernel stearin to be harder than palm stearin at room temperature leading to a greater potential of solid lipid as a matrix for trapping bioactive component. Moreover, with the melting point of palm kernel stearin of 32°C (Hasibuan and Siahaan, 2013), it is preferred and recommended for reducing mouthfeel discomfort when consumed because it quickly melts in the mouth.

The components that are widely reported as food-grade ingredients in NLC formulations include medium chain triglycerides (MCT)/caprylic- and capric-triglycerides (Zardini *et al.*, 2017), oleic acid (Tamjidi *et al.*, 2014a; Tamjidi *et al.*, 2014b), palm olein (Rohmah *et al.*, 2019a; Rohmah *et al.*, 2019b), palm oil, rice bran oil, virgin coconut oil (Teeranachaideekul *et al*., 2022) and ricinoleic acid (Hyun *et al*., 2022) as liquid lipid. While stearic acid, glyceryl palmitostearate, glyceryl behenate (Tamjidi *et al.*, 2013), palm stearin (Rohmah *et al.*, 2019a; Rohmah *et al.*, 2019b), and glyceryl monostearate (Teeranachaideekul *et al*., 2022) were reported as solid lipids. The surfactant used, such as Tween 80, one of the polysorbates with a high HLB number of 15, is water soluble, and best used as an oil-in-water emulsifier. A suitable surfactant can reduce interface tension and facilitate droplet dispersion during homogenization (Liu and Wu., 2010). The surfactants play an important role in stabilizing oil-based systems, including reducing surface tension quickly and preventing particle aggregation and recrystallization (McClements and Rao, 2011).

Many different methods have been described in the literature for the production of lipid nanoparticles, especially NLC, including hot/cold homogenization, microemulsion, solvent emulsification–evaporation, emulsification solvent diffusion, phase inversion technique methods, and hot homogenization is the most used method for fabrication of both SLN and NLC (Tamjidi *et al.*, 2013). Several techniques for the production of NLC also are reported including microfluidization method, high shear, homogenization and ultrasonication, and solvent Injection technique (Kanojia *et al*., 2022). The melting-emulsification-ultrasonication has many advantages (such as easy scale-up, lack of organic solvents and short production time) compared to the other methods. In this approach, the component is initially dissolved or dispersed in the melted lipid mixture (5-10°C) above the temperature of the lipid with highest melting point. Then, the lipid melt is dispersed in an aqueous emulsifier solution at the same temperature by high-speed stirring/shearing. The hot emulsion obtained may further be homogenized at the same temperature, by instruments such as high-pressure homogenizer (HPH), high-intensity ultrasonic probe/jet/bath or microfluidizer, to produce a hot nanoemulsion. Subsequently, NLC is produced by cooling the hot nanoemulsion in cold water, room temperature or by a heat exchanger to crystalize lipid droplets and precipitate the lipid nanoparticles (Tamjidi *et al*., 2013).

Moreover, the methods for stable NLC production have been reported, such as palm stearin-palm olein-NLC by melting-emulsification-homogenization-sonication (Rohmah *et al.*, 2019a; Rohmah *et al.*, 2019b), melting-emulsification-sonication (Veider *et al.*,

https://doi.org/10.26656/fr.2017.8(3).375

2022; Tamjidi *et al.*, 2014a, Tamjidi *et al.*, 2017), melting- homogenization-ultrasonication (Zardini *et al.*, 2017; Nahr *et al.*, 2018). All of those methods produce NLC with particle size under 200 nm, with entrapment efficiency reaching >90% (Rohmah *et al.*, 2019b; Rohmah *et al.*, 2022; Hyun *et al.*, 2022), and using 70% amplitude of ultrasonication (Nahr *et al.*, 2018). Based on formulas, NLC can be formed if a solid lipid is more than a liquid lipid. The solid-to-liquid lipid ratios was reported 7:3 (Tamjidi *et al.*, 2014a; Tamjidi *et al.*, 2017), 4:1.35 (Zardini *et al.*, 2017), 5:5 to 9:1 (Rohmah *et al.*, 2019a), yielding optimal entrapment efficiency at 5.5:4.5 ratio, with surfactant:lipid ratio of 4.9:1 and (lipid+surfactant): water ratio of 24:76 (Rohmah *et al.*, 2019b; Rohmah *et al.*, 2022).

The NLC system can improve stability and protect the active ingredients. However, there is no information about the preparation of NLC formulations containing RPO as the liquid lipid, which also contains β-carotene as a bioactive component. Also, an NLC-RPO production based on solid lipid to RPO (SRR) and the use of palm stearin compared to palm kernel stearin as solid lipid is still unknown. In addition, the stability and characteristics of NLC-RPO have not been clearly described. This study aimed to develop an NLC delivery system using the melting-emulsification-ultrasonication method based on SRR, palm stearin, and palm kernel stearin as solid lipids and to study the characteristics and stability of NLC-RPO during storage.

2. Materials and methods

2.1 Materials

The materials used in this study were red palm oil (PT. Nutri Palma Nabati), palm stearin and palm kernel stearin (PT. Wilmar Nabati Indonesia), Tween 80 (Sigma -Aldrich), β-carotene standard (CAS 7235-40-7, Sigma-Aldrich), acetone for HPLC (Merck), pyrogen-free water for HPLC (PT. Khaparmindo Putamas, Indonesia), distilled water purchased from CV. Progo, Yogyakarta, and other chemicals were of analytical reagent grade.

2.2 Preparation nanostructured lipid carriers-red palm oil

NLC-RPO was prepared by melting, emulsification and ultrasonication techniques (Tamjidi *et al.*, 2014a; Tamjidi *et al.*, 2017; Rohmah *et al.*, 2020; Veider *et al.*, 2022) with a slight modification. Briefly, the aqueous and lipid phases were separately prepared. Red palm oil (RPO), palm stearin, and palm kernel stearin were used as the lipid phase. While Tween 80 and distilled water as the aqueous phase. The formulation of NLC-RPO contained total lipid (solid and liquid lipid), Tween 80, and water with the proportions of 6%, 24%, and 70% (w/w), respectively. Tween 80 and distilled water were mixed and solid lipid and RPO (6:4, 7:3, and 8:2) were melted at 65°C and 600 rpm for 15 mins using a magnetic stirrer (AREC, VELP Scientifica, Italy). After that, the aqueous phase was added to the lipid phase for emulsification at 65°C and stirred at 1400 rpm for 15 mins. The emulsion produced was sonicated with an ultrasonic probe (UP200St Hielscher) at 70% amplitude for 20 mins to form the NLC-RPO. The NLC-RPO was cooled down at room temperature for 12 hrs. The NLC-RPO samples were placed in vials with aluminum foil packaging and away from light sources before further analysis.

2.3 Entrapment efficiency of β-carotene

β-carotene in the formulation is derived from RPO. The β-carotene entrapment efficiency (EE) of the NLC-RPO was assessed using a previously described method (Qian *et al.*, 2012b; Fathi *et al.*, 2013; Rohmah *et al.*, 2020) with a slight modification. Briefly, The NLC-RPO dispersion (NLC-RPO: ethanol = 1: 2) was centrifuged at 10.000 rpm for 10 mins at 25°C. The ethanol phase containing free β-carotene was separated and its β-carotene was determined by HPLC. The EE could be calculated according to the following equation :

$$EE\ (\%) = \frac{Wi - Wf}{Wi} \times 100\% \quad (1)$$

where Wi is the mass of initial β-carotene in the NLC-RPO, Wf is the mass of free β-carotene detected in the supernatant after centrifugation of NLC-RPO dispersion. The initial β-carotene was analyzed by Association of Official Analytical Chemists (AOAC) Official Method (AOAC, 2005), and particle morphology of β-carotene loaded NLC-RPO was observed with transmission electron microscopy (TEM).

2.4 Analysis of β-carotene

The β-carotene of NLC-RPO was determined by the AOAC Official Method (AOAC, 2005). Samples were prepared by saponification using 10 mL of absolute ethanol and 2.5 mL of 50% KOH in aquadest (w/v), heated in a water bath at 80°C for 1 hr, and 2.5 mL of glacial acetic acid was added. The solution was subsequently transferred into a 25 mL volumetric flask and the volume was adjusted with a mixture of ethanol: tetrahydrofuran 1:1 (v/v). The solution was passed through a Whatman syringe filter with a pore size of 0.45 µm. The β-carotene was determined using High-Performance Liquid Chromatography (HPLC) (Shimadzu, Japan) by isocratic with a C18 column and UV-Vis detector at 450 nm. A gradient solvent system consisting of acetone (A) and H_2O (B) was employed (Ningrum *et al.*, 2015). Initially, a mixture of 75% A and

https://doi.org/10.26656/fr.2017.8(3).375

25% B was used, and then the mixing was programmed linearly to 95% A within 20 mins and linearly to 100% A at 21 mins. The flow rate was 0.5 mL/min. Quantification of β-carotene was performed using a standard curve of β-carotene solution with concentration range from 0 to 10 ppm Analysis was performed on samples after 0, 30, 60, and 90 days of storage. The β-carotene was analyzed in duplicate.

2.5 Stability of nanostructured lipid carriers-red palm oil based on turbidity

The NLC-RPO was subjected to a centrifugation test at 4500 rpm (Sorvall ST 8R) for 15 mins, a cooling test at 4°C for 12 hrs, or a heating test at 105°C for 15 mins in an oven (Memmert type IN30) according to Rukmini *et al.* (2012). The stability of the NLC-RPO was evaluated based on its turbidity changes. The turbidity was determined using a UV-Vis spectrophotometer (Shimadzu UV-2100) at a wavelength of 600 nm (Qian and McClements, 2011). An NLC-RPO was declared stable if the turbidity was less than 1% and it was analyzed in triplicate. Turbidity was calculated by following the equation of Cho *et al*. (2008):

$$\text{Turbidity (\%)} = 2.303 \times \text{absorbance} \quad (2)$$

2.6 Color of nanostructured lipid carriers-red palm oil

The color of NLC-RPO was measured using Chromameter CR-400 (Konica Minolta). The results were expressed in terms of lightness (L*), red-green (a*), blue-yellow (b*), and ΔE characteristics (Almeida *et al.*, 2018). The different classification of E was measured by the difference between ΔE*(initial) and ΔE*(after 90 days). The color analysis was conducted in triplicate.

2.7 pH of nanostructured lipid carriers-red palm oil

The pH values were measured in triplicate using a digital pH meter (Hanna Instrument HI 2210) at room temperature. The pH meter was calibrated using pH 7.0, 4.0 and 10.0.

2.8 Characteristics of nanostructured lipid carriers-red palm oil

Characterization of NLC-RPO consisted of the average particle size, polydispersity index (PDI), and zeta potential (Hyun *et al*., 2022), which was characterized using Microtrac (Nanotract wave II, USA). Nanoparticle measurement was carried out by dynamic light scattering (DLS)-backscattered laser-amplified scattering reference method utilizing photon correlation spectroscopy at room temperature (25°C), and the sample was diluted with distilled water to the suitable concentration of 1:10 before analysis (Lv *et al*., 2016). The characterization results were presented as average from triplicate data.

2.9 Experimental design and statistical analysis

In this study, a factorial experiment was designed for the source of solid lipid to red palm oil ratio (SRR). Three levels of SRR were fixed at 6:4, 7:3, and 8:2, and analysis was performed on samples after 0, 30, 60, and 90 days of storage at room temperature. There were 24 samples and each sample was replicated three times. The β-carotene levels, stability after the physical test, color, and pH were analyzed by regression and paired slope test between regressions using statistic function: standard error of regression, standard error of slope, t-statistic, and probability (significant 2-tailed) in Microsoft Excel (edition 2019) at $p<0.05$. An SPSS 19.0 program was used for statistical analysis of EE and characteristic parameters. The significance of the differences between the observed mean values ± standard deviation (SD) for characteristics and particle stability was evaluated using one-way ANOVA and Duncan's Multiple Range Tests with $p = 0.05$.

3. Results and discussion

3.1 Entrapment Efficiency of β-carotene of nanostructured lipid carriers-red palm oil

The information in the EE relates to the active substance that was successfully encapsulated by nanoparticles. The finding of an investigation on the EE of NLC-RPO in the lipid matrix is shown in Figure 1. EE value decreased from 96.79% to 96.11% and 96.80% to 96.15% in both palm stearin and palm kernel stearin as solid lipid with increased use of solid lipid to RPO ratio from SRR of 6:4 to 8:2 but EE at SRR of 6:4 and 7:3 showed no significant different ($p<0.05$). However, EE was higher than 90% in all SRR formulations. A similar trend was noticed by other researchers that found EE of β-carotene-NLC of 91.2% (Rohmah *et al.*, 2019b), curcumin-NLC of 94.5% (Hyun *et al*., 2022) and cardamom essential oil-NLC of more 90% (Nahr *et al.*, 2018). EE mainly depends on the solubility of core material in a lipid matrix. However, other factors such as compaction of lipid structure, type and concentration of surfactant, liquid lipid type and content may have significant effects. Rohmah *et al*. (2019a) reported a stable NLC which was prepared using lipid:surfactant ratio of 1:4. The presence of surfactant is capable of reducing the surface tension and preventing lipid particle aggregation (McClements and Rao, 2011). Applying liquid lipids in the mixture led to the limitation of recrystallization and forming an amorphous or less order crystalline state which resulted in imperfection and accommodation of a higher amount for encapsulant

https://doi.org/10.26656/fr.2017.8(3).375

(Fathi *et al*., 2013).

The high EE of β-carotene in the NLC-RPO might be due to its lipophilic characteristic, which led to its higher partitioning into the lipid matrix of NLC compared to the aqueous phase (Ni *et al*., 2015). The EE could be higher in the NLC-RPO due to the ratio of the solid lipid more than the liquid lipid. In addition, when palm stearin and palm kernel stearin were solidified, they formed irregular spaces in the matrix for incorporating bioactive substances. It confirmed the use of palm stearin and palm kernel stearin as solid lipids to incorporate a higher amount of β-carotene. This study found that β-carotene, as its lipophilic nature, had a higher solubility in SRR of 6:4 and 7:3 and no significant difference ($p<0.05$) but a NLC-RPO in SRR of 6:4 had a higher RPO as β-carotene sources.

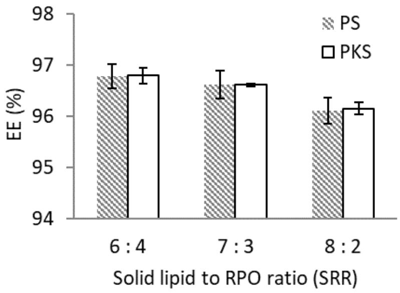


Figure 1. Entrapment Efficiency (EE) of NLC-RPO β-carotene. RPO: red palm oil, PS: palm stearin, PKS: palm kernel stearin.

The microscopic characteristic at NLC-RPO in SRR 6:4 was observed using TEM (Figure 2). The β-carotene as a lipophilic micro-nutrient has successfully incorporated into NLC and smaller particles when used palm kernel stearin (reached 96,0% of EE) than palm stearin as a solid lipid. Spherical particle along with particle size less than 100 nm after preparation and after three months of storage at room temperature. In another report, the particle size of β-NLC had an average of 115.2 nm to 148.4 nm during 90 days (Rohmah *et al*., 2020).

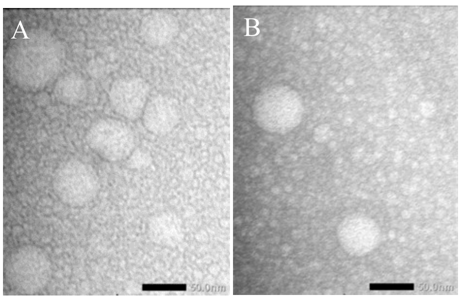


Figure 2. TEM photograph of β-carotene loaded NLC-RPO in SRR 6:4 with (A) palm stearin, (B) palm kernel stearin as solid lipid after preparation (bar scale is 50.0 nm).

3.2 Stability of β-carotene during storage

Figure 3 shows that the initial β-carotene of SRR 6:4 was higher than SRR 7:3 and 8:2. The stability of β-carotene levels with SRR 6:4 > 7:3 > 8:2 occurred in both palm stearin and palm kernel stearin as solid lipid. It has also been proven that SRR and solid lipid sources could affect β-carotene levels. Storage for 90 days may cause degradation of β-carotene, even though, in this case, SRR 6:4-PKS showed a more considerable β-carotene retention of 96.34% (Table 1).

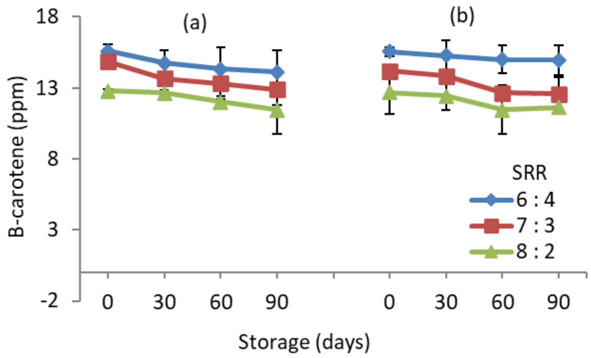


Figure 3. β-Carotene stability of NLC-RPO during storage for 90 days, with palm stearin (a) and with palm kernel stearin (b) as solid lipid. SRR: solid lipip to RPO ratio, RPO: red palm oil, PS: palm stearin, PKS: palm kernel stearin.

Evaluation of the slope value of each trendline showed that the SRR 6:4-PKS had a lower slope of -0.1954 (Table 1), which meant that for every 1% addition of the storage duration variable, there was a decrease of β-carotene of 0.1954 when using palm kernel stearin as the solid lipid. Meanwhile, based on the slope tests on all trendlines, there was a significant difference ($p<0.05$). NLC-RPO using SRR 6:4 with palm kernel stearin as solid lipid had a lower slope of -0.195, indicating that it performed higher entrapment than other SRR. Palm kernel stearin had more solid fat content (SFC) than palm stearin at 10-27°C (Hasibuan dan Siahaan, 2013).

3.3 Physical stability of nanostructured lipid carriers-red palm oil after centrifugation, cooling and heating during storage

In this study, the stability of NLC-RPO was determined based on turbidity value, and it was expressed as stable if the turbidity was below 1% (Cho *et al*., 2008). As seen in Figure 4, the turbidity of all NLC-RPO was raised after 90 days of storage; however, as it was still below 1%, it could be declared stable during storage. The use of palm stearin as the solid lipid showed a high turbidity change after the cooling and heating test (Figure 4a). On the other hand, the use of palm kernel stearin as the solid lipid showed more stability in the cooling and heating test because the turbidity value on day 90 was less than 0.45% (Figure 4b).

The results of the slope test are shown in Table 2.

Table 1. Linear regression of β-carotene content in the NLC-RPO during storage for 90 days.

SRR	β-carotene in NLC-RPO (ppm) Storage (days) 0	30	60	90	β-carotene retention after 90 days (%)	Linear regression
6:4-PS	15.60	14.76	14.34	14.11	90.42	Y = -0.4902x + 15.927 R^2 = 0.9274
7:3-PS	14.85	13.65	13.32	12.88	86.71	Y = -0.6254x + 15.240 R^2 = 0.9096
8:2-PS	12.78	12.64	12.01	11.44	89.54	Y = -0.4631x + 13.374 R^2 = 0.9463
6:4-PKS	15.54	15.06	15.01	14.97	96.34	Y = -0.1954x + 15.685 R^2 = 0.9194
7:3-PKS	14.19	13.83	12.64	12.59	88.72	Y = -0.5993x + 14.811 R^2 = 0.8996
8:2-PKS	12.67	12.43	11.46	11.64	91.87	Y = -0.4063x + 13.065 R^2 = 0.7884
Paired slope test	6:4-PS vs. 7:3-PS *, 6:4-PS vs. 8:2-PS *, 7:3-PS vs. 8:2-PS*, 6:4-PKS vs. 7:3-PKS *, 6:4-PKS vs. 8:2-PKS*, 7:3-PKS vs. 8:2-PKS*, 6:4-PS vs. 6:4-PKS *, 7:3-PS vs.7:3-PKS*, 8:2-PS vs. 8:2-PKS*					

SRR: solid lipid to RPO ratio, RPO: red palm oil, PS: palm stearin as solid lipid, PKS: palm kernel stearin as solid lipid.
* significantly different between slopes at p<0.05.

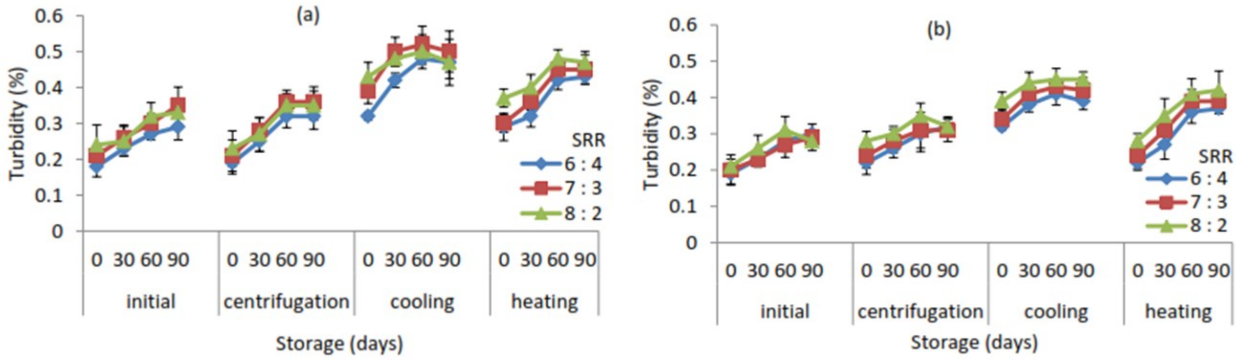


Figure 4. Physical stability of NLC-RPO during storage for 90 days, each with palm stearin (a) and with palm kernel stearin (b) as solid lipid. SRR: solid lipip to RPO ratio, RPO: red palm oil, PS: palm stearin, PKS: palm kernel stearin.

Table 2. The linear regression of NLC-RPO stability during storage for 90 days before (initial) and after centrifugation, cooling and heating test.

SRR	Linear regression of NLC-RPO stability during storage for 90 days: Initial (Before test)	After centrifugation	After cooling	After heating
6:4-PS	Y = 0.378x + 0.1463 R^2 = 0.9624	Y = 0.0465x + 0.1535 R^2 = 0.9051, st*	Y = 0.0522x + 0.2925 R^2 = 0.8192, st*	Y = 0.0518x + 0.234 R^2 = 0.8964, st*
6:4-PKS	Y = 0.038x + 0.155 R^2 = 0.9757	Y = 0.0346x + 0.1877 R^2 = 0.9792, st*	Y = 0.0238x + 0.3163 R^2 = 0.6525, st*	Y = 0.054x + 0.17 R^2 = 0.9287, st*
	st.ns	st*	st*	st*
7:3-PS	Y = 0.043x + 0.1734 R^2 = 0.997	Y = 0.0465x + 0.1535 R^2 = 0.9051, st*	Y = 0.034x + 0.391 R^2 = 0.5655, st*	Y = 0.0532x + 0.2565 R^2 = 0.8949, st*
7:3-PKS	Y = 0.03x + 0.1722 R^2 = 0.9731	Y = 0.0262x + 0.2203 R^2 = 0.8779, st*	Y = 0.0248x + 0.3391 R^2 = 0.6786, st*	Y = 0.0514x + 0.2026 R^2 = 0.9041, st*
	st*	st*	st*	st^{ns}
8:2-PS	Y = 0.0346x+0.2009 R^2 = 0,9136	Y = 0.0428x + 0.1935 R^2 = 0.882, st*	Y = 0.0145x + 0.4361 R^2 = 0.4032, st*	Y = 0.0387x + 0.3309 R^2 = 0.8704, st*
8:2-PKS	Y = 0.0263x+0.1993 R^2 = 0.5988	Y = 0.0166x + 0.2687 R^2 = 0.5641, st *	Y = 0.019x +0.3849 R^2 = 0.7291, st*	Y = 0.0485x + 0.2424 R^2 = 0.9252, st*
	st*	st*	st*	st*

SRR: solid lipid to RPO ratio, RPO: red palm oil, PS: palm stearin as solid lipid, PKS: palm kernel stearin as solid lipid, st: slope comparison test, ns: no significant difference.
* significantly different between slopes at p<0.05.

There was an increase in the slope value after the centrifugation and heating test. Nevertheless, the cooling test generally showed a decrease in the slope value compared to the initial condition of each SRR. An increased slope indicated a decline in the stability of NLC-RPO. Instability systems could be detected by observing the occurrence of creaming, oil phase separation, or sedimentation of the NLC after centrifugation, cooling, and heating treatments (Liu *et al.*, 2014). The initial slope value of NLC-RPO at SRR 6:4 with palm stearin and palm kernel stearin as the solid lipid did not show a significant difference in slope

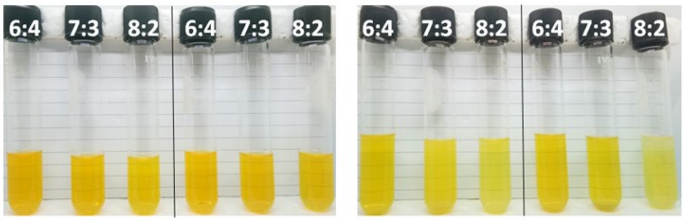
https://doi.org/10.26656/fr.2017.8(3).375

(p<0.05). The slope of NLC-RPO at SRR 6:4 with palm kernel stearin as the solid lipid was lower than the use of palm stearin as the solid lipid after centrifugation, cooling, and heating test at SRR 7:3 and 8:2. Nevertheless, in this case, turbidity was detected below 1% without creaming and phase separation or sedimentation. Therefore, NLC-RPO was considered to be stable for 90 days of storage. The NLC use surfactant:lipid of 4:1 was reported stable at centrifugation, heating and cooling test after 30 days of storage (Rohmah *et al*., 2019a).

The palm kernel used in this study was more solid than palm stearin, which might impact the stability of NLC-RPO during cooling. Table 2 shows that the slope value dropped after cooling more significantly than the slope after centrifugation and heating test (p<0.05). Rukmini *et al.* (2012) reported that the microemulsion became unstable when heated at 70°C or higher, while the instability slightly increased after centrifugation and after 1-2 months. In this investigation, the heating exposure was carried out following the findings of earlier preliminary studies, which showed that NLC-RPO could only be heated at 105°C for a maximum of 15 mins before becoming clouded and entering the separation phase. An increase in NLC-RPO turbidity after heating indicated that heating affected surfactants. According to Flanagan *et al.* (2006), the head-group size of nonionic surfactants, particularly, is affected by changes in temperature, thereby indirectly affecting their ability to solubilize oil.

3.4 Color stability of nanostructured lipid carriers-red palm oil during storage

The color changes of NLC-RPO during 90 days of storage at room temperature can be seen in Figure 5. An increase in the lightness (L*) value indicated a loss of color. The L* of all NLC-RPO increased, however, the value of a*, b* and ΔE* were reduced. During 90-day storage, all a* values were seen in the negative zone, while b* value was in the positive zone, ranging from -1.12 to -4.63 and 38.08 to 27.58 for the NLC-RPO with palm stearin and palm kernel stearin as solid lipid, respectively, which indicated the intensity of greenish-yellow color as in line with Almeida *et al*. (2018). The NLC-RPO with SRR 6:4 of palm kernel stearin showed more negative of a* and positive of b* representing the efficacy of RPO as a stronger colorant. The change in color of NLC-RPO before and after 90 days is shown in Figure 6. The NLC-RPO was observed to be greenish-yellow.



(A) (B)

Figure 6. The color stability and turbidity of NLC-RPO before and after 90 days of storage at room temperature; (A) Initial 0 days and (B) after 90 days, with (a) palm stearin and (b) palm kernel stearin as solid lipid at solid lipid to RPO ratio of 6:4, 7:3 and 8:2.

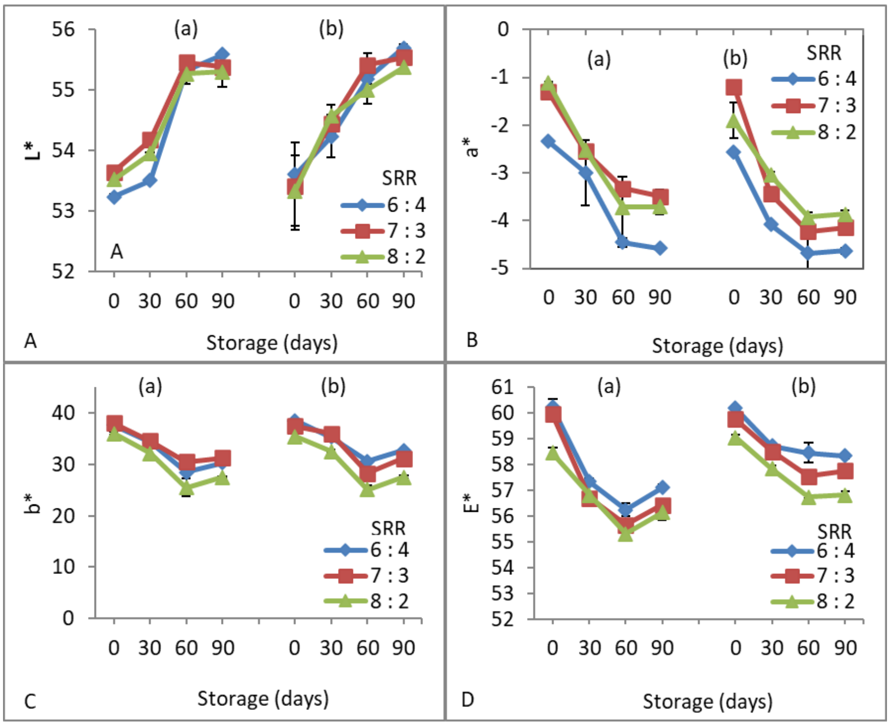


Figure 5. Color stability of NLC-RPO during storage for 90 days, each L* (A), a* (B) , b* (C), ΔE* (D) value, palm stearin (a), dan palm kernel stearin (b) as solid lipid. SRR: solid lipip to RPO ratio, RPO: red palm oil, PS: palm stearin, PKS: palm kernel stearin.

https://doi.org/10.26656/fr.2017.8(3).375

Based on the slope test (Table 3), the use of palm stearin and palm kernel stearin as the solid lipid showed significant differences in the slope trendline values of L*, a*, b* and ΔE*. In general, the slope value of SRR with palm kernel stearin was significantly lower than palm stearin as the solid lipid ($p<0.05$). The value of L* with SRR 8:2 showed no significant difference because the addition of RPO at a low level caused the small effect of carotenoid colorant on the NLC-RPO. The increase in L* indicated that oxidation led to a loss of orange color in the oil during storage, as all oils became more transparent as an effect of degradation (Sikorska *et al.*, 2007; Almeida *et al.*, 2018). This prediction was consistent with the reduction of the b* value found during storage. Overall, the SRR might influence the color stability of NLC-RPO as there was a significant change in L*, a*, b*, and ΔE* among the SRR during storage.

The ΔE parameter was chosen as an indicator of color fading since it allowed the concomitant changes of all color parameters to be taken into account. The stability threshold based on turbidity value was 1% because turbidity >1 showed an indication of cloudiness. The total color difference, ΔE, was a combination of the parameters (L*, a*, and b* values) used to characterize the difference of colors in foods during processing. The validated value compromises ΔE<1 for a normally invisible difference, 1<ΔE<2 for a very small difference that is only obvious to a trained eye, 2<ΔE<3.5 for a medium difference that is obvious to an untrained eye, and ΔE>3.5 for an obvious difference (Habekost, 2013).

The visual threshold allowed an average observer to distinguish the color difference by at least 3.5 (Table 3). Accordingly, the changes in the color of NLC-RPO became apparent after 90 days for SRR 7:3 with palm stearin, and for SRR 6:4 and 8:2 with palm stearin and SRR 8:2 with palm kernel stearin as the solid lipid. In comparison, the NLC-RPO at SRR 6:4 and 7:3 with palm kernel stearin as the solid lipid showed no significant difference in their color after 90 days of storage. Such color changes during storage were mainly caused by the photodegradation of the carotenoids and the presence of oxygen in the headspace is also a contributing factor in the β-carotene degradation (Rodriguez-Amaya and Kimura, 2004).

3.5 pH stability of nanostructured lipid carriers-red palm oil during storage

The change in pH value as one of the parameters of the physical stability of NLC-RPO during storage for 90 days at room temperature is shown in Figure 7. The initial pH values of the NLC-RPO with the formula SRR 6:4, 7:3, and 8:2 in palm stearin and palm kernel stearin as the solid lipids were 6.38, 6.41, 6.46, and 6.44, 6.49, and 6.54, respectively, but it showed not significantly

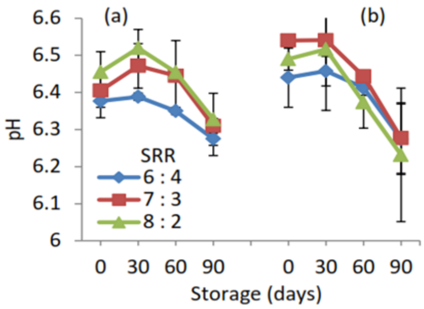


Figure 7. pH stability of NLC-RPO during storage for 90 days each (a) with palm stearin, (b) with palm kernel stearin as solid lipid. SRR: solid lipip to RPO ratio, RPO: red palm oil, PS: palm stearin, PKS: palm kernel stearin.

Table 3. The linear regression of color stability of NLC-RPO during storage for 90 days.

SRR	Linear regression of color parameters during storage for 90 days			
	L*	a*	b*	ΔE*
6:4 -PS	Y = 0.887x + 52.200 R^2 = 0.8905	Y = -0.8210x – 1.5400 R^2 = 0.9917	Y = -2.7653x + 39.630 R^2 = 0.7634	Y = -1.0447x + 60.345 R^2 = 0.6068. ΔE = 3.11
6:4 -PKS	Y = 0.723x + 52.875 R^2 = 0.9871	Y = -0.6837x – 2.2783 R^2 = 0.7905	Y = -2.2160x + 39.872 R^2 = 0.7027	Y = -0.5780x + 60.377 R^2 = 0.7706., ΔE = 1.84
	st*	st*	st*	st*
7:3-PS	Y = 0.650x + 53.045 R^2 = 0.8719	Y = -0.7340x – 0.8300 R^2 = 0.9031	Y = -2.4277x + 39.717 R^2 = 0.8325	Y = -1.1613x + 60.095 R^2 = 0.6264. ΔE = 3.53
7:3-PKS	Y = 0.733x + 52.875 R^2 = 0.9185	Y = -0.9637x – 0.8383 R^2 = 0.7716	Y = -2.6443x + 39.863 R^2 = 0.646	Y = -0.6993x + 60.132 R^2 = 0.8061, ΔE = 1.99
	st*	st*	st*	st*
8:2-PS	Y = 0.666x + 52.850 R^2 = 0.8901	Y = -0.8993x – 0.5183 R^2 = 0.881	Y = -3.3477x + 38.980 R^2 = 0.773	Y = -0.8480x + 58.808 R^2 = 0.6729. ΔE = 2.33
8:2-PKS	Y = 0.655x + 52.940 R^2 = 0.908	Y = -0.6760x – 1.4967 R^2 = 0.8538	Y = -3.1110x + 37.973 R^2 = 0.7357	Y = -0.7793x + 59.563 R^2 = 0.8631, ΔE = 2.23
	st. ns	st*	st*	st*

SRR: solid lipid to RPO ratio, RPO: red palm oil, PS: palm stearin as solid lipid, PKS: palm kernel stearin as solid lipid, st: slope comparison test, ns: no significant difference.
* significantly different between slopes at $p<0.05$.

different ($p<0.05$). The changes in pH could be caused by the characteristics of RPO, palm stearin, palm kernel stearin, and the amount of water in formula NLC-RPO.

The slope tests for each regression pair with the same SRR at different solid lipids showed a negative relationship (Table 4). The decrease in pH of NLC-RPO during 90 days of storage using palm stearin at SRR 6:4, 7:3 and 8:2 was not significantly different ($p<0.05$). The decrease in pH of the NLC-RPO with palm kernel stearin as the solid lipid at SRR 6:4 to 7:3 and 8:2 formulas showed a significantly different slope ($p<0.05$). There was a decrease in pH until 6.23-6.33 after storage for 90 days. The pH of NLC-RPO ranges from 6.23-6.54 indicating a close to pH neutral (pH 7) because the composition in the NLC system was water of 70%. This result was similar to palm stearin-palm olein-NLC had a pH range of 6.32-6.47 (Rohmah *et al*., 2019a).

3.6 Characteristics of particles

3.6.1 Particle size of nanostructured lipid carriers-red palm oil

Characteristics of NLC-RPO particles before and after storage for 90 days were analyzed (Figure 8). The initial particle size before storage of NLC-RPO that used SRR 6:4, 7:3, and 8:2 for both palm stearin and palm kernel stearin as the solid lipid were 45.94, 52.56, 82.25, and 40.70, 38.0, 46.97 nm, respectively as shown in Figure 8(A). After 90-day storage, the particle size did not significantly change in almost all formulas ($p<0.05$). The particle sizes were not significantly reported after storage (Rohmah *et al*., 2019a; Rohmah *et al*., 2020). It could be indicated that no coalescence or phase separation occurred in the NLC-RPO. However, the particle size of NLC-RPO during 90 days of storage was detected below 100 nm. The NLC-RPO storage for 30 days at room temperature also increased to 176-342 nm, which still belonged to the nanoscale and had a large

Table 4. The linear regression of pH stability of NLC-RPO during storage 90 days.

SRR	Linear regression	SRR	Linear regression	SRR	Linear regression
6:4-PS	Y = -0.0343x + 6.4333 R^2 = 0.7575	7:3-PS	Y = -0.0312x + 6.4858 R^2 = 0.323, st.ns	8:2-PS	Y = -0.0448x + 6.5511 R^2 = 0.5166, st*
6:4-PKS	Y = -0.0537x + 6.53 R^2 = 0.6982	7:3-PKS	Y = -0.0887x + 0.6721 R^2 = 0.848 , st*	8:2-PKS	Y = -0.0918x + 6.6325 R^2 = 0.8313, st*
	st*		st*		st*

SRR: solid lipid to RPO ratio, RPO: red palm oil, PS: palm stearin as solid lipid, PKS: palm kernel stearin as solid lipid, st: slope comparison test, ns: no significant difference.

* significantly different between paired slopes in the same row (SRR 6:4, 7:3 and 6:4, 8:2) and in the same column at the same ratio at $p<0.05$.

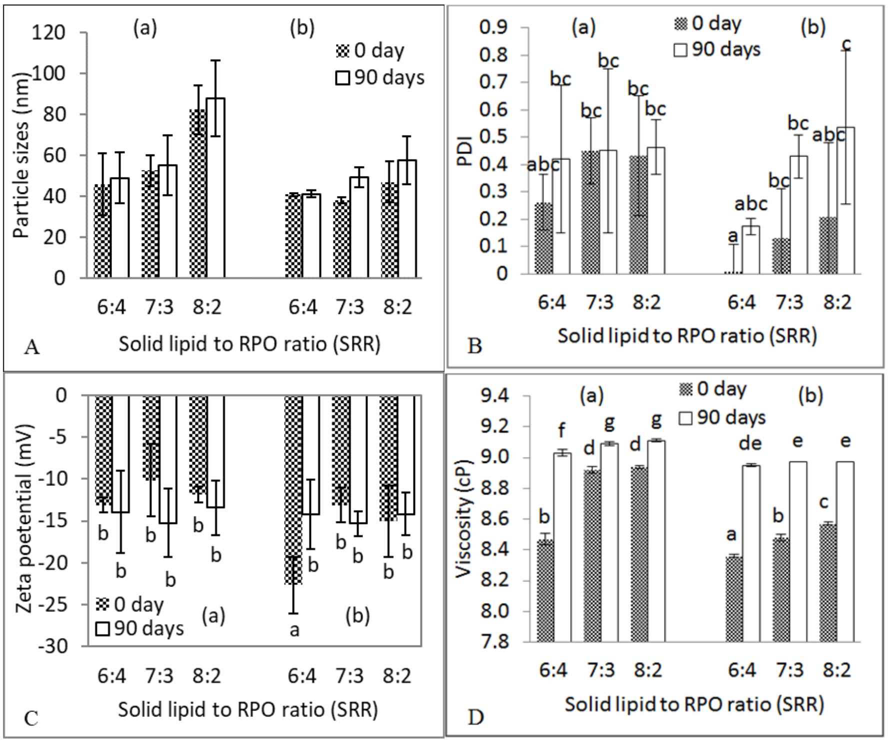


Figure 8. Particle size characteristics of NLC-RPO before and after storage for 90 days, (A) particle size, (B) PDI, (C) Zeta potential, (D) Viscosity, (a) with palm stearin and (b) with palm kernel stearin as solid lipid. RPO: red palm oil, PS: palm stearin, PKS: palm kernel stearin. Different lowercase letters above or under each bar graph indicate statistically significant difference ($p<0.05$).

https://doi.org/10.26656/fr.2017.8(3).375

specific surface area for the bioavailability of bioactive compounds (Rohmah *et al.*, 2019a).

In addition, the surfactant to lipid ratio was fixed at 1:4 to facilitate the lipid phase and NLC-RPO formed. Liquid oil that is added for NLC can be dispersed in a lipid matrix and reduce particle size or produce core-shell type nanocarrier (Zardini *et al*., 2017). Other studies suggested that the droplet size nanoemulsion is controlled by surfactants (Rohmah *et al.*, 2019a; Rohmah *et al.*, 2019b). Tween 80 is a water-soluble and non-ionic surfactant suitable for stabilizing NLCs. Its hydrophile-lipophile balance (HLB) value of 15 can reduce the interface tension and facilitate the dispersion of droplets in the emulsion system (Tadros *et al.*, 2005; Liu and Wu., 2010).

The mean particle size and the particle size distribution (usually as polydispersity index) are the most important characteristics of nanodispersions which govern the physical stability, solubility, biological performance release rate, turbidity and chemical stability (Lakshmi and Kumar, 2010). The usual particle diameter of NLC is in the range of approximately 10-1000 nm. During NLC production, the particle size of final NLC is influenced by a series of factors, depending on the utilized method. The most used method for the production of NLC is the hot homogenization and solvent emulsification–evaporation. The achievable particle size in emulsions depends on the adsorption kinetic of the emulsifier, the interfacial tension between dispersed and continuous phases, viscosities of dispersed and continuous phases, volume fraction of dispersed phase and mechanical energy input to deform and break up the droplets (Gramdorf *et al*., 2008).

3.6.2 Polydispersity index of nanostructured lipid carriers-red palm oil

PDI is a measurement of the distribution and homogeneity of nanoemulsion droplet size to evaluate the stability of a nanoemulsion system. There was no effect of the NLC-RPO storage on the PDI ($p<0.05$), as shown in Figure 8(B). Most of the freshly prepared NLC -RPO were monodispersed-polydispersed. The PDI changed during storage with palm stearin and palm kernel stearin as the solid lipids were 0.26-0.46 and 0.01-0.54, respectively. That means, most NLC-RPO were homogeneous droplet sizes with small particles causing the nanoemulsion system more stable. This proves the formula and melting-emulsification-ultrasonication method used are suitable for producing stable nanoparticles of NLC-RPO.

The PDI values start from 0.01 to 0.5-0.7 for monodispersed particles (Rabima *et al.*, 2018). PDI values smaller than 0.05 are mainly seen with highly monodisperse standards, and bigger than 0.7 indicates that the sample has a vast particle size (Danaei *et al.*, 2018). Thus, the PDI of NLC-RPO before and after 90-day storage was categorized as stable monodisperse with a maximum PDI of 0.54. The NLC became stable at lower PDI because the particle size distribution tended to be narrow, and few particles could aggregate.

3.6.3 Zeta potential of nanostructured lipid carriers-red palm oil

Zeta potential before and after the storage was presented in Figure 8(C). The Zeta potential of NLC-RPO using palm stearin and palm kernel stearin as the solid lipids were -10.17 to -13.1 mV and -13.13 to -22.67 mV, respectively, as initial zeta before storage. After storage for 90 days, their zeta potential was -13.43 to -15.23 mV and -14.2 to 15.33 mV. Zeta potential did not show a significant difference ($p<0.05$) before and after storage for 90 days. The NLC-RPO of SRR 6:4 with palm kernel stearin as solid lipid was observed to have the lowest zeta potential of 22.67 mV due to a large number of homogeneous colloidal in the dispersion system stabilized in the solid lipid matrix. The palm kernel stearin had a high solidity of solid lipid matrix because of more solid fat content at room temperature. Zeta potential in the lycopene-NLC was reported to be -10.23 to -15.87 mV (Zardini *et al.*, 2017), resveratrol-NLC -15 mV (Aisiyah *et al.*, 2019), palm olein-palm stearin-NLC -21 to -33 mV (Rohmah *et al.*, 2019a).

Zeta potential with prominent negativity will have a steric force that causes the particles to repel each other, which makes NLC more stable. This study used a nonionic surfactant and there was no significant zeta potential change during 90 days of storage ($p<0.05$). The negative charge can be mainly attributed to the steric force of Tween 80, although the hydroxyl group of Tween 80 creates a slight negative charge (Zardini *et al.*, 2017). Therefore stabilization nanosuspension by a combination of electrostatic (surfactant) and steric force (Tween 80), a minimum zeta potential of ±20 mV is sufficient for stabilization (Tamjidi *et al*., 2013).

3.6.4 Viscosity of nanostructured lipid carriers-red palm oil

The viscosity of NLC-RPO before and after storage is presented in Figure 8(D). The viscosity of NLC-RPO prepared with palm stearin and palm kernel stearin as the solid lipids were in the range of 8.47-9.11 cP and 8.36-8.97 cP, respectively. The results show a significant increase in the viscosity during storage for 90 days ($p<0.05$). Sari *et al*. (2018) reported an increase in the viscosity of red palm olein nanoemulsion during storage

https://doi.org/10.26656/fr.2017.8(3).375

at room temperature for up to 12 weeks. The viscosity value of the NLC-RPO system depends on the composition of the material used, especially the type of lipid and surfactant used (Rohmah *et al*., 2019a). Lipids that have low density can affect the system's stability allowing creaming, flocculation, and coalescense. In this study, an increasing amount of solid lipid in NLC-RPO causes an increase in turbidity and viscosity.

4. Conclusion

The highest entrapment efficiency of β-carotene in NLC-RPO (96.79% dan 96.80%) was obtained at a solid lipid to liquid lipid ratio (SRR) of 6:4. The NLC-RPO prepared with palm kernel stearin as the solid lipid with SRR of 6:4 had the lowest degradation of β-carotene and ΔE difference. All of the formulated NLC-RPO after storage for 90 days at room temperature remained stable after centrifugation, cooling, and heating tests. The particle size, PDI, and zeta potential of NLC-RPO during storage for 90 days were not significantly different, however, the viscosity during storage was significantly different.

Conflict interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported by a research grant 2020-2021 from the Palm Oil Fund Management Agency (BPDPKS), Republic of Indonesia.

References

Aisiyah, S. Harjanti, R. and Nopianti, V. (2019). Pengaruh Panjang Rantai Karbon Lipid Padat Terhadap Karakteristik Nanostructured Lipid Carrier Resveratrol. *Journal of Pharmaceutical Science and Clinical Research*, 4(2), 69-81. https://doi.org/10.20961/jpscr.v4i2.34408 [In Bahasa Indonesia].

Almeida, D.T., Viana, T.V., Costa, M.M., Silva, C. and Feitosa, S. (2018). Effects of different storage conditions on the oxidative stability of crude and refined palm oil, olein and stearin (*Elaeis guineensis*). *Food Science and Technology*, 39(Suppl. 1), 211-217. https://doi.org/10.1590/fst.43317

Association of Official Analytical Chemists (AOAC). (2005). Vitamin A (retinol) in Food, Liquid Chromatography (AOAC Official Method 2001.13). USA: AOAC.

Ariviani, S., Raharjo, S. and Hastuti, P. (2011a). Potensi Mikroemulsi β-karoten dalam Menghambat Fotooksidatif Vitamin C Sistem Aqueous. *Jurnal Teknologi dan Industri Pangan,* 22 (1), 33-39. [In Bahasa Indonesia].

Ariviani, S., Raharjo, S. and Hastuti, P. (2011b). Aplikasi Mikroemulsi β-karoten untuk Menghambat Kerusakan Fotooksidatif Vitamin C pada Sari Buah Jeruk. *agriTECH*, 31(3), 180-189. https://doi.org/10.22146/agritech.9743 [In Bahasa Indonesia].

Azar, F.A.N., Pezeshki, A., Ghanbarzadeh, B., Hamishehkar, H. and Mohammadi, M. (2020). Nanostructured lipid carriers: Promising delivery systems for encapsulation of food ingredients. *Journal of Agriculture and Food Research,* 2, 100084. https://doi.org/10.1016/j.jafr.2020.100084

Chawla, S. and Saxena, S. (2013). Red Palm Oil - Health Benefits And Their Molecular Executors. *International Journal of Bioassays*, 2(9), 1223-1231.

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), 115-121, https://doi.org/10.1111/j.1750-3841.2008.00688.x

Choo, Y.M., Ma, A.N. and Basiron, Y. (1993). Red palm oil: a potential source of dietary carotene. *Malaysian Oil Science and Technology*, 2, 54-65.

Danaei, M., Dehghankhold, M., Ataei, S., Davarani, F.H., Javanmard, R. and Dokhani, A. (2018). Impact of Particle Size and Polydispersity Index on the Clinical Applications of Lipidic. *Nanocarrier Systems Pharmaceutics*, 10(2), 57. https://doi.org/10.3390/pharmaceutics10020057

Dauqan, E., Sani, H.A., Abdullah, A. and Kasim, Z.M. (2011). Effect of Different Vegetable Oils (Red Palm Olein, Palm Olein, Corn Oil and Coconut Oil) on Lipid Profile in Rat. *Food and Nutrition Sciences*, 2, 253-258. https://doi.org/10.4236/fns.2011.24036

Fathi, M., Varshosaz, J., Mohebbu, M. and Shahidi, F. (2013). Hesperetin-Loaded Solid Lipid Nanoparticles and Nanostructure Lipid Carriers for Food Fortification: Preparation, Characterization, and Modeling. *Food and Bioprocess Technology*, 6, 1464-1475. https://doi.org/10.1007/s11947-012-0845-2

Flanagan, J., Kortegaard, K., Pinder, D.N., Rades, T. and Singh, H. (2006). Solubilisation of soybean oil in microemulsions using various surfactants. *Food Hydrocolloids*, 20(2), 253-260. https://doi.org/10.1016/j.foodhyd.2005.02.017

Goh, S.H., Choo, Y.M. and Ong, S.H. (1985). Minor constituents of palm oil. *Journal of the American Oil Chemists' Society,* 62(2), 237-240. https://doi.org/10.1007/BF02541384

Gramdorf, S., Hermann, S., Hentschel, A., Schrader, K., Müller, R.H., Kumpugdee-Vollra, M. and Kraume,

RESEARCH PAPER

M. (2008). Crystallized miniemulsions: Influence of operating parameters during high-pressure homogenization on size and shape of particles. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 331(1-2), 108-113. https://doi.org/10.1016/j.colsurfa.2008.07.016

Habekost, M. (2013). Which Color Differencing Equation Should be Used? *International Circular of Graphic Education and Research*, 6, 20-33.

Hasibuan, H.A. and Ijah. (2018). Peningkatan Kesukaan Minyak Sawit Merah dengan Penambahan Minyak Nabati atau Flavor dan Stabilitasnya dalam Penggorengan Berulang. *Jurnal Penelitian Kelapa Sawit*, 26(1), 1-9. https://doi.org/10.22302/iopri.jur.jpks.v26i1.57 [In Bahasa Indonesia].

Hasibuan, H.A. and Siahaan, D. (2013). Determination of Iodine Value and Melting Point Based on Solid Fat Content of Palm Oil and Palm Kernel Oil (Comparisons with AOCS Standard Method). *Jurnal Standardisasi*, 15(1), 47-57. https://doi.org/10.1016/j.lwt.2022.113474

Hyun, J.E., Yi, H.Y., Hong, G.P. and Chun, J.Y. (2022). Digestion stability of curcumin-loaded nanostructured lipid carrier. *LWT-Food Science and Technology*, 162, 113474. https://doi.org/10.1016/j.lwt.2022.113474

Kanojia, N., Sharma, N., Gupta, N. and Singh, S. (2022). Applications of Nanostructured Lipid Carriers: Recent Advancements and Patent Review. *Biointerface Research in Applied Chemistry,* 12(1), 638-652. https://doi.org/10.33263/BRIAC121.638652

Lakshmi, P. and Kumar, G.A. (2010). Nanosuspension technology: A review. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2(4), 35-40.

Li, B. and Ge, Z.Q. (2012). Nanostructured Lipid Carriers Improve Skin Permeation and Chemical Stability of Idebenone. *AAPS Pharmaceutical Science and Technology*, 13(1), 276-283. https://doi.org/10.1208/s12249-011-9746-3

Liu, C. and Wu, C. (2010). Optimization of nanostructured lipid carriers for lutein delivery. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 353(2-3), 149-156. https://doi.org/10.1016/j.colsurfa.2009.11.006

Liu, Y., Wang, L., Zhao, Y., He, M., Zhang, X., Niu, M. and Feng, N. (2014). Nanostructured lipid carriers versus microemulsions for delivery of the poorly water-soluble drug luteolin. *International Journal of Pharmaceutics*, 476(1-2). 169-177, https://doi.org/10.1016/j.ijpharm.2014.09.052

Lv, W., Zhao, S., Yu, H., Li, N., Garamus, V.M., Chen, Y. and Zou, A. (2016). *Brucea javanica* oil-loaded nanostructure lipid carriers (BJO NLCs): Preparation, characterization and in vitro evaluation. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 504, 312-319. https://doi.org/10.1016/j.colsurfa.2016.05.068

Mayamol, P.N., Balachandran, Samuel, T., Sundaresan, A. and Arumughan, C. (2007). Process technology for Production of Micronutrient Rich Red palm Olein. *Journal of the American Oil Chemists' Society*, 84(6), 587-596. https://doi.org/10.1007/s11746-007-1078-9

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(4), 285-330. https://doi.org/10.1080/10408398.2011.559558

Nahr, F.K., Chanbarzadeh, B., Hamishehkar, H. and Kafil, H.S. (2018). Food Grade Nanostructured Lipid Carrier for Cardamom Essential Oil: Preparation, Characterization and Antimicrobial Activity. *Journal of Functional Foods,* 40, 1-8. https://doi.org/10.1016/j.jff.2017.09.028

Ni, S., Sun, R., Zhao, G. and Xia, Q. (2015). Quercetin Loaded Nanostructured Lipid Carrier For Food Fortification: Preparation, Characterization And *In Vitro* Study. *Journal of Food Process Engineering*, 38 (1), 93-106. https://doi.org/10.1111/jfpe.12130

Ningrum, A., Minh, N.N. and Schreiner, M. (2015). Carotenoids and Norisoprenoids as Carotenoid Degradation Products in Pandan Leaves (*Pandanus amaryllifolius* Roxb.) *International Journal of Food Properties*, 18(9), 1905-1914. https://doi.org/10.1080/10942912.2014.971186

Qian, C. and McClements, D.J. (2011). Formation of nanoemulsions stabilized by model food-grade emulsifiers using high-pressure homogenization: Factors affecting particle size. *Food Hydrocolloids*, 25(5), 1000-1008. https://doi.org/10.1016/j.foodhyd.2010.09.017

Qian, C., Decker, E.A., Xiao, H. and McClements, D.J. (2012a). Solid Lipid Nanoparticles: Effect of Carrier Oil and Emulsifier Type on Phase Behavior and Physical Stability. *Journal of the American Oil Chemists' Society,* 89(1), 17-28. https://doi.org/10.1007/s11746-011-1882-0

Qian, C., Decker, E.A., Xiao, H. and McClements, D.J. (2012b). Physical and chemical stability of b-carotene -enriched nanoemulsions: Influence of pH, ionic strength, temperature, and emulsifier type. *Food Chemistry*, 132(3), 1221-1229. https://doi.org/10.1016/j.foodchem.2011.11.091

Rabima, R. (2018). Characterization and Antibacterial Activity of Curcumin-Nanostructured Lipid Carrier.

Indonesia Natural Research Pharmaceutical Journal, 3(2), 1-10.

Rodriguez-Amaya, D.B. and Kimura, M. (2004). Harvestplus handbook for carotenoid analysis (HarvestPlus Technical Monograph, No 2. Washington, DC, USA: International Food Policy Research Institute (IFPRI).

Rohmah, M., Choiri, S., Raharjo, S., Hidayat, C. and Martien, R. (2020). Palm stearin and olein binary mixture incorporated into nanostructured lipids carriers: Improvement food functionality for micronutrient delivery. *Journal of Food Processing and Preservation*, 4(10), e14761. https://doi.org/10.1111/jfpp.14761

Rohmah, M., Raharjo, S., Hidayat, C. and Martien, R. (2019b). Application of Response Surface Methodology for the Optimization of ß-Carotene-Loaded Nanostructured Lipid Carrier from Mixtures of Palm Stearin and Palm Olein. *Journal of the American Oil Chemists' Society,* 97(2), 213-223. https://doi.org/10.1002/aocs.12310

Rohmah, M., Raharjo, S., Hidayat, C. and Martien, R. (2019a). Formulation and Stability of Nanostructured Lipid Carrier Prepared from a Mixture of Palm Stearin and Palm Olein. *Jurnal Aplikasi Teknologi Pangan,* 8(1), 23-30, https://doi.org /10.17728/jatp.3722

Rohmah, M., Rahmadi, A. and Raharjo, S. (2022). Bioaccessibility and antioxidant activity of ß-carotene loaded nanostructured lipid carrier (NLC) from binary mixtures of palm stearin and palm olein. *Heliyon*, 8 (2), e08913. https://doi.org/10.1016/j.heliyon.2022.e08913

Rukmini, A., Raharjo, S., Hastuti, P. and Supriyadi, (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.

Sari, F., Sinaga, K.R. and Siahaan, D. (2018). Formulation and Evaluation of Red Palm Olein Nanoemulsion. *Asian Journal of Pharmaceutical and Clinical Research*, 11(9), 237-240. https://doi.org/10.22159/ajpcr.2018.v11i9.26532

Sikorska, E., Caponio, F., Bilancia, M., Summo, C. and Pasqualone, A. (2007). Changes in colour of extra-virgin olive oil during storage. *Polish Journal of Food and Nutrition Sciences*, 57(4), 495-498.

Tadros, T.F. (Ed.) (2005). Applied Surfactant, Principles and Applications. Weinheim, Germany: WILEY-VCH VerlagGmbH and Co. KgaA.

Tamjidi, F., Shahedi, M., Varshosaz, J. and Nasirpour, A. (2017). Stability of astaxanthin-loaded nanostructured lipid carriers in beverage systems. *Journal of the Science of Food and Agriculture,* 98(2), 511-518. https://doi.org/10.1002/jsfa.8488

Tamjidi, F., Shahedi, M., Varshosaz, J. and Nasirpour, A. (2014a). Design and Characterization of Astaxanthin-Loaded Nanostructured Lipid Carriers. *Innovative Food Science and Emerging Technologies,* 26, 366-374. https://doi.org/10.1016/j.ifset.2014.06.012

Tamjidi, F., Shahedi, M., Varshosaz, J. and Nasirpour, A. (2014b). EDTA and α-tocopherol Improve The Chemical Stability Of Astaxanthin Loaded Into Nanostructured Lipid Carriers. *European Journal of Lipid Science and Technology*, 116(8), 968-977. https://doi.org/10.1002/ejlt.201300509

Tamjidi, F., Shahedi, M., Varshosaz, J. and Nasirpour, A. (2013). Nanostructured lipid carriers (NLC): A potential delivery system for bioactive food molecules. *Innovative Food Science and Emerging Technologies*, 19, 29-43. https://doi.org/10.1016/j.ifset.2013.03.002

Teeranachaideekul, V., Boribalnukul, P., Morakul, B. and Junyaprasert, V.P. (2022). Influence of Vegetable Oils on In Vitro Performance of Lutein-Loaded Lipid Carriers for Skin Delivery: Nanostructured Lipid Carriers vs. Nanoemulsions. *Pharmaceutics,* 14, 2160. https://doi.org/10.3390/pharmaceutics14102160

Varshosaz, J., Eskandari, S. and Tabakhian, M. (2010). Production and optimization of valproic acid nanostructured lipid carriers by the Taguchi design. *Pharmaceutical Development and Technology,* 15(1), 89-96. https://doi.org/10.3109/10837450903013568

Veider, F., Akkus-Dagdeviren, Z.B., Knoll, P. and Bernkop-Schnürch, A. (2022). Design of nanostructured lipid carriers and solid lipid nanoparticles for enhanced cellular uptake. *International Journal of Pharmaceutics*, 624, 122014. https://doi.org/10.1016/j.ijpharm.2022.122014

Zardini, A.A., Mohebbi, M., Farhoosh, R. and Bolurian, S. (2017). Production and Characterization of Nanostructured Lipid Carrier and Solid Lipid Nanoparticles Containing Lycopene for Food Fortification. *Journal of Food Science and Technology,* 55(1), 287-298. https://doi.org/10.1007/s13197-017-2937-5

RESEARCH PAPER