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Formulation and Characteristics of Nanostructured Lipid Carrier (NLC) Red Palm Oil (RPO) Prepared by High-Pressure Homogenization and Application in Orange Juice

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ABSTRACT: A Nanostructured lipid carrier (NLC) is an emulsion system that can encapsulate and improve stability of lipid-based bioactive compounds. This study aimed to develop an optimal RPO-NLC formula with a combination of solid and liquid lipids, lipids and surfactants, and the use of high-pressure homogenizer (HPH). RPO-NLC was made using HPH with a 400 bar and 600 bar pressure. A Completely randomized design (CRD) with three factors was used to determine interactions between factors. The RPO-NLC formula was characterized based on particle size. One of the best formulas was selected with small particle size and high RPO content then characterized based on encapsulation efficiency (EE), loading capacity (LC), storage stability, FTIR, thermal and applied to orange juice including sensory evaluation, encapsulation stability and pH. RPO-NLC yielded particle size 44.9 nm, EE 99.9±0.02%, LC 4.9±0.001%, was stable on storage. β-carotene identified in RPO-NLC lipid matrix based on FTIR analysis and has good thermal stability based on differential scanning calorimeter analysis. Sensory evaluation showed changes in sensory attributes of color, aroma, taste during storage. Encapsulation stability showed significant increase during storage and pH measurement results also increased.

Keywords: *RPO, Nanostructured lipid carriers, High-pressure homogenizer, Orange juice.*

INTRODUCTION

Red palm oil (RPO) is high in micronutrient components consisting of carotenoids with concentration 500 –700 ppm and vitamin E in the form of tocopherols and tocotrienols with concentration 500 – 1000 ppm (Lee et al., 2018). Carotenoids contained in RPO consisting of α- and β-carotene are lipophilic and easily degraded due to exposure to light and high temperature (55 °C) causing oxidation so that RPO loses its functional properties (Demiray and Tulek, 2017; Lee et al., 2018; Mehrad et al., 2018). The degraded RPO have decrease in quality indicated by formation of peroxide compounds and free fatty acids (Budiyanto et al., 2012) causing utilization of RPO to decrease due to reduced β-carotene micronutrient compounds. There are several types of carrier systems that can be used to encapsulate bioactive compounds in foodstuffs including microemulsion, solid lipid nanoparticle (SLN) and nanostructured lipid carrier (NLC).

Nanostructured lipid carrier (NLC) can be used to overcome limitations in utilization of β-carotene as a functional food which can improve encapsulation efficiency, physical stability and loading capacity of the components of bioactive compounds (Rohmah et al., 2022). NLC has an average size about 10 – 500 nm consisting a mixture of solid lipids and liquid lipids so that it can increase loading capacity bioactive compounds and prevent release components bioactive compounds (Duong et al., 2019; A. Sharma and Baldi, 2018; Subramaniam et al., 2020). The combination NLC lipid matrix causes formation an imperfect crystal structure so that it can increase encapsulation bioactive compounds and higher loading capacity (Pezeshki et al., 2019). Several methods that can be used to produce NLC are high-pressure homogenization (HPH), emulsification solvent diffusion, solvent injection, phase inversion, double emulsion method and ultrasonication (A. Sharma and Baldi, 2018). Rohmah et al. (2022) reported NLC made using a mixture of palm stearin and palm olein can increase the bioaccessibility and antioxidant capacity of β-carotene.

High-pressure homogenization (HPH) is a method that uses pressure and heat in emulsion manufacturing process so that it can reduce average size of oil droplets well and increase stability O/W emulsion during storage (Salvi and Pawar, 2019). The type lipid used also plays an important role in physicochemical characteristics NLC. Palm oil has fractionated product consisting stearin as solid lipid fraction and olein as liquid lipid fraction. Red palm olein (RPOL) is red palm oil with olein fraction containing palmitic acid (C16:0) 35.5%, oleic acid (C18:1) 46.2%, linoleic acid (C18:2) 12.0%, linolenic acid (C18:3) 0.3%, lauric acid (C12:0) 0.5%, myristic acid (C14:0) 1.2%, arachidic acid (C20:0) 0.5% (Sulaiman et al., 2022). Sagiri et al. (2015) reported that stearin has good thermal stability using differential scanning calorimeter (DSC) analysis. Nanoemulsion made using high-pressure homogenizer produces β-carotene encapsulation above 98% with good storage stability at 4 ^{0}C (Borba et al., 2019).

NLC has good stability and loading capacity, so it is generally widely applied in pharmaceutical (Trucillo et al., 2022). However, NLC is difficult to apply in beverages this is due to different conditions of each drink compared to NLC when applied in pharmaceutical. Orange juice is a natural source of bioactive compounds in form of vitamin C, carotenoids and flavanones (Klimczak et al., 2007; Stinco et al., 2020) which are widely consumed by society. Orange juice contains several other bioactive substances such as hesperidin and

narirutin (Gattuso et al., 2007), which can prevent emergence degenerative diseases. Zhang et al. (2020) reported that DHA and EPA nanoemulsions applied to apple juice did not cause a significant change in the characteristics of the juice. The results of Gonçalves et al. (2023) applied Curcumin-NLC to a model beverage system was composed by sucrose, citric acid, ascorbic acid and sodium benzoate which did not significantly affect the characteristics and stability of the model beverage during 21 days of storage.

However, research on the application of lipids from fractionated palm oil in NLC is still very limited. The results of study (Rohmah et al., 2022; Rohmah, Raharjo, et al., 2020), palm stearin and palm olein are able to encapsulate β-carotene in NLC in order that to increase the bioaccessibility and antioxidant capacity of β-carotene. Similar fatty acid content of palm stearin and palm olein including optimal formulation result in high encapsulation efficiency of β-carotene in NLC (Rohmah et al., 2022). Meanwhile, the use of red palm oil as a liquid lipid and the application of NLC in beverage products has not been widely reported. The main objective of this study was to determine an optimal RPO-NLC formula with combination of solid and liquid lipids, lipids and surfactants, and HPH pressure used in its application to orange juice. The best formula for RPO-NLC was characterized by particle size, then analyzed further, including encapsulation efficiency (EE), loading capacity (LC), storage stability, Fourier transform-infrared spectroscopy (FTIR), thermal (DSC), stability of RPO-NLC in orange juice, pH and sensory evaluation orange juice with added RPO-NLC.

MATERIALS AND METHODS

Materials

Red palm oil (liquid lipid) was obtained from Palm Oil Research Center (PPKS, Medan), palm stearin (solid lipid) was obtained from PT Smart Tbk (Surabaya, Indonesia), Tween 80 was obtained from Sigma-Aldrich (St. Louis, MO), 96% ethanol was obtained from CV. General Labora (Yogyakarta, Indonesia), standard β-carotene was obtained from Sigma-Aldrich (St. Louis, MO) and commercial orange juice obtained from supermarkets.

Methods

RPO-NLC Preparation

RPO-NLC were prepared using hot high-pressure homogenization (HPH) method (Cornacchia and Roos, 2011; Ng et al., 2015; Rohmah et al., 2019) with several modifications. The lipid phase consisted of palm stearin and red palm oil as solid and liquid lipids, respectively was melted using a hot plate at 60 °C for 10 minutes. Distilled water and tween 80 solutions were heated at same temperature as lipid phase. When distilled water temperature reached 60 °C, tween 80 solution was then dispersed and then heated again at same temperature for 10 minutes using a magnetic stirrer at 600 rpm. The hot lipid phase solution was put into aqueous phase and homogenized at 10.000 rpm for 10 minutes using high-shear homogenizer (IKA T50 basic Ultra-Turrax, Staufen, Germany). NLC has then homogenized again in a hot state using high-pressure homogenizer (GEA PandaPLUS 1000, Germany), with pressure 400 and 600 bar, ten cycles and subsequently cooled at room temperature for 24 hours.

Particle size

Particle size determination was determined by the dynamic light scattering (DLS) technique used by Nanotrac Wave II (Microtrac Inc. USA). The RPO-NLC, which had been diluted ten times with distilled water, was then dispersed at a temperature of 25 °C (Rohmah et al., 2019).

Encapsulation efficiency and loading capacity of β-carotene NLC

The encapsulation efficiency (EE) and loading capacity (LC) of β-carotene loaded in NLC were determined using the method of Akhoond Zardini et al. (2018) with several modifications. 0.5 ml of RPO-NLC and 1 ml of 96% ethanol were dispersed into Eppendorf then centrifuged at 14.000 rpm at 27 °C for 30 minutes (Eppendorf Centrifuge 5424 R, Germany). The obtained supernatant containing free β-carotene was determined at wavelength of 450 nm using UV-vis spectrometer (Shimadzu UV-2100. Tokyo, Japan).

The percentages of EE and LC are calculated using equations (1) and (2):

$$EE\% = \frac{W_{total} - W_{free}}{W_{total}} x100 \quad (1)$$

$$LC\% = \frac{W_{total} - W_{free}}{W_{lipid}} x100 \quad (2)$$

Where W_{total} is the total amount of β-carotene at the time of initial addition, W_{free} is the amount of free β-carotene detected in supernatant after centrifugation of formula, and W_{lipid} is the amount of lipid in NLC preparation. EE and LC analyzes were repeated three times.

Storage Stability RPO-NLC

Stability of RPO-NLC during storage was determined by measuring turbidity value using UV-vis spectrometer (Shimadzu UV-2100. Tokyo, Japan) (Khosh manzar et al., 2020). 10 ml of RPO-NLC sample was placed in closed tube, then stored in incubator at 37 °C for four weeks. Every week RPO-NLC samples were removed and evaluated by measuring changes in turbidity. Distilled water was set as control then measured at wavelength of 600 nm. Measurements were done 3 times.

Fourier transform-infrared spectroscopy (FTIR)

The FTIR spectra of the RPO-NLC were analyzed using the Thermo Scientific Nicolet iS10 FT-IR spectrometer instrument (Waltham, MA, USA). FTIR analysis was performed using attenuated total reflectance (ATR) with ZnSe crystal and a deuterated triglycine sulfate detector. Then sample was placed on the ATR crystal and scanned from 650 – 4000 cm-1 with a resolution of 8 cm-1 and 32 iterations (Rohmah et al., 2019).

Thermal RPO-NLC

Thermal analysis of RPO-NLC formula using method (Rohmah, Choiri, et al., 2020) with several modifications. RPO-NLC was analyzed using a differential scanning calorimeter Shimadzu DSC-60 (Kyoto, Japan). 10 mg of sample was placed in a tightly closed aluminum pan and heated at heating rate of 10 °C/min-1 from 30 °C to 30 °C.

During thermal scan, the container was cleaned with nitrogen and an empty aluminum pan as reference standard.

Encapsulation Stability (ES%) of RPO-NLC in orange juice

Encapsulation stability (ES%) was evaluated for four weeks with storage temperature of 8 °C using method (Babazadeh et al., 2016) with several modifications. Ratio formulation of RPO-NLC and orange juice 1:19 (v/v). 0.5 mL of RPO-NLC which had been applied to orange juice was taken put into microtube, then 1 mL of 96% ethanol was added and centrifuged at 14.000 rpm for 30 minutes with 27 °C temperature. The obtained supernatant was then tested for absorbance at 450 nm using a UV-vis spectrometer (Shimadzu UV-2100. Tokyo, Japan).

The percentage of encapsulation stability (ES) is calculated using the following equation:

$$ES\% = \frac{W_{total} - W_{free}}{W_{encapsulated}} x100 \quad (3)$$

W_{total} is the total amount of β-carotene present when initially added to RPO-NLC orange juice. W_{free} is the amount of free β-carotene detected in supernatant after centrifugation, and $W_{encapsulated}$ is the amount of β-carotene encapsulated in RPO-NLC orange juice.

pH

Measurement pH RPO-NLC applied to orange juice was determined using method (Babazadeh et al., 2016) during storage for four weeks at 8 °C. Prior to measurements, pH meter was calibrated using buffer pH 7. pH measurements were carried out by inserting the electrode directly into sample.

Sensory evaluation

Sensory evaluation of orange juice with added RPO-NLC was performed using discrimination testing (difference pair comparison test) (Lawless and Heymann, 2010) for four weeks of storage at 8 °C with twenty panelists (6 males and 14 females), age ranged 24 to 37. Semitrained students of food technology and agricultural products department (Universitas Gadjah Mada) analyzed samples in good health. Each panelist was presented 10 ml with a control sample along with a test sample is placed in a small plastic container coded with three-digit random numbers and asked to determine the differences between the two samples. Testing includes color, aroma, and taste attributes. To release the residuary flavor and taste, water and crackers were used in between evaluations. Samples with the same parameter attribute are given zero (0), and different parameter attributes are numbered one (1). The samples consisted of two sets and the order of the samples was randomized in each set consisting of orange juice and orange juice samples with the addition of RPO-NLC. The results obtained are then adjusted to the probability different test table.

Data Analysis

All measurements were made on at least two freshly prepared samples, and each sample was measured in triplicate. The results were reported as averages ± standard deviations. The differences among treatments were calculated based on an analysis of variance (ANOVA) and were compared using t-test with Microsoft Office Excel 2010. A confidence level of 99% ($p < 0.01$) was considered statistically significant.

RESULT AND DISCUSSION

Particle size

Particle size is an essential characteristic in an NLC formula because it can determine the release rate of bioactive compounds. The results of the RPO-NLC particle measurements in Table 1 show that the RPO-NLC particle size ranges from 42 to 97 nm. The small RPO-NLC particle size can be caused by influence use of HPH pressure and concentration lipid-surfactant ratio used ($p < 0.01$). Khosa et al. (2018) reported several factors affecting particle size such as processing temperature, pressure used, number of cycles during HPH, lipid-surfactant ratio and type of surfactant used. High surfactant-lipid ratio can result in smaller particle size, while low surfactant concentration increases particle size. Pezeshki et al. (2019) reported the use of surfactant concentrations that are too high can cause micellar accumulation in the emulsion system, which can increase the particle size. Surfactant concentrations that are too high can also cause thickening of the system (Pezeshki et al., 2014).

High-pressure heat homogenization can result in smaller particle sizes due to heat treatment (above the lipid melting point), which reduces the lipid phase viscosity. However, heat treatment that is too high can degrade components bioactive compounds that are sensitive to high temperatures (Mehnert and Mäder, 2012). On the other hand, the type of lipid used also has an important role in diameter particle size. Hu et al. (2005) reported that the use of liquid lipids in NLC formulations can reduce surface tension in forming smaller particle sizes, this is because liquid lipids can reduce NLC viscosity. Hyun Eun et al. (2022) used the high-pressure homogenization method to encapsulate curcumin in NLC resulting small particle size from 115.2 to 141.4 nm compared to the results of previous studies. This is caused by the use of high-energy in emulsification method to generate pressure such as high shear, impact, and cavitation which can reduce size NLC particles.

Table 1. Particle size RPO-NLC

Palm stearin : RPO	Lipid phase : Tween 80	Pressure (400 Bar) Size (nm)	Pressure (600 Bar) Size (nm)
6:4	1:2.5	88.7±1.32	44.9±0.79[a]
6:4	1:3	97.9±3.62	45.8±0.26[a]
7:3	1:2.5	84±5.58	43.4±0.38[a]
7:3	1:3	88.5±1.26	48.3±1.19[a]
8:2	1:2.5	77.9±9.71	42.1±1.45
8:2	1:3	89.1±3.46	44.3±1.14[a]

Note: Data are expressed as mean ± SD (n = 3). Statistical analysis performed using analysis of variance (ANOVA) followed by t-test. [a] statistically different from pressure 400 Bar ($p < 0.01$).

The RPO-NLC formulation was made to investigate tolerance level ratio of solid lipids and liquid lipids in producing small particle sizes. In formulation, ratio of solid lipids and liquid lipids (6:4), (7:3) and (8:2) was still able to produce small particle sizes below 100 nm. Other studies Alcantara et al. (2019) reported formulation ratio of solid lipids and liquid lipids (8:2), (7:3), (6:4) and (5:5) resulting in particle sizes above 100 nm. Determination the best formula for RPO-NLC based on small particle size, high liquid lipid composition (RPO) and surfactant concentration used. The small particle size of NLC plays an important role in controlling rate release of bioactive compounds (Tamjidi et al., 2013) with can increase solubility and stability of colloidal systems (Khosh manzar et al., 2020). Based on results of particle size characterization and high liquid lipid composition (RPO), the best formula was determined namely ratio of palm stearin:RPO = 6:4, lipid phase:Tween 80= 1:2.5, and pressure 600 Bar with particle size 44.9 nm. The high concentration of liquid lipids in NLC can increase loading capacity and affect encapsulation efficiency of bioactive compounds. Solid and liquid lipid formulations (8:2) are capable producing small RPO-NLC particle sizes, but low in loading capacity of bioactive compounds. Alcantara et al. (2019) reported that high ratio of solid to liquid lipid composition (6:4) was able to produce higher encapsulation efficiency and loading capacity than a low solid and liquid lipid composition (8:2).

Encapsulation efficiency (EE%)

Encapsulation efficiency is percentage of the number components bioactive compounds trapped in nanoparticle system. Encapsulation efficiency aims to determine ability of lipids to trap components bioactive compounds. The encapsulation efficiency (EE%) of β-carotene loaded in RPO-NLC according to Table 2 was 99.9±0.02%. Encapsulation efficiency above 90% indicates that bioactive compounds can be trapped properly in NLC (Huang et al., 2017). This is probably caused by the use of liquid lipids and homogenization process using HPH which can break down RPO-NLC particles into smaller ones. Thus causing the crystal structure of NLC particles to be imperfect and results encapsulation for components micronutrient compounds to be maximized(Sirikhet et al., 2021).

Borba et al. (2019) reported that nanoemulsions prepared using HPH generate β-carotene encapsulation efficiency above 98%. In addition, encapsulation efficiency depends on total amount of lipids and solubility of components bioactive compounds in lipids (Singh et al., 2015). Khosa et al. (2018) observed that formation of particles by rigid solid lipids after cooling could increase encapsulation of bioactive compounds in lipid matrix. Moreover nature components of bioactive compounds and lipid phase used can also affect value of NLC encapsulation efficiency, bioactive compounds that are lipophilic have higher encapsulation efficiency value. Increasing oil content can increase encapsulation efficiency, because the encapsulated bioactive components have higher solubility in liquid lipids than solid lipids(Patil et al., 2014).

Loading Capacity (LC%)

Loading capacity describes the amount of bioactive compound loaded based on total lipid used in emulsion system The β-carotene that was successfully loaded into NLC in Table 2 was 4.9±0.001 % based on the total addition of 6% red palm oil. This is probably due to the ability of β-carotene to dissolve in lipid phase, resulting in better loading capacity. Higher loading capacity has the advantage of better encapsulating more bioactive compounds so as to maximize the ability of NLC as a lipid-based delivery system (Tamjidi et al., 2013). High loading capacity value is caused by the imperfect crystal structure in NLC during production process, it can provide a lot of space for loading bioactive compounds. The loading capacity of RPO-NLC can be influenced by the physicochemical properties of liquid lipid, concentration of surfactant and amount of addition components bioactive compounds.

Houacine et al. (2020) reported that the solubility of resveratrol in lipid matrix consisting of trimyristin and several types of liquid lipids can result in varying loading capacities ranging from 2 – 7%. The change in loading capacity value is influenced by the physicochemical properties of bioactive compounds and type of liquid lipid used. Keivani Nahr et al. (2018) reported that loading capacity of cardamom oil in NLC system could increase between 23.38% to 63.31%, along with increase in the concentration of cardamom oil used. However, loading capacity that is too high can degrade surface of nanoparticles, causing leakage of bioactive compounds during storage (Akhoond Zardini et al., 2018; Bashiri et al., 2020).

Tabel 2. Encapsulation efficiency (EE%) and loading Capacity (LC%) β-carotene in RPO-NLC

Palm stearin : RPO	Lipid phase : Tween 80	Pressure (Bar)	Analysis parameters	
			EE%±SD	LC%±SD
6:4	1:2.5	600	99.9±0.02	4.9±0.001

Note: Data are expressed as mean ± SD (n = 3).

Storage Stability RPO-NLC

The stability of RPO-NLC during storage is determined by measuring turbidity. NLC which will be applied to beverage products with aim of enriching nutrients is very important for long-term stability analysis. Figure 1. showed turbidity of RPO-NLC which was accelerated by storage for four weeks in incubator at 37 °C showed slight increase in turbidity 0.31±0.0006 nm to 0.33±0.0006 nm. This is probably caused by water phase of RPO-NLC experiencing evaporation during storage four weeks at 37 °C, therefore lipid phase RPO-NLC experienced little saturation which caused turbidity value to increase slightly. Khosh manzar et al. (2020) also studied value of NLC turbidity after 30 days of storage at room temperature did not show a significant difference, possibly caused by the encapsulated bioactive compounds that underwent evaporation or degradation during storage.

Emulsions with particle size diameter about 45 nm have lower turbidity than emulsions with particle size diameter about 216 nm (Uluata et al., 2016). NLC stored at 4 °C had lower turbidity value during storage than at 37 °C, this is caused by the kinetic energy originating from high temperatures that can accelerate collisions between particles resulting in aggregation and destabilization which can affect the NLC turbidity value (Azevedo et al., 2021). Several factors that can affect turbidity emulsion are particle size and the number of particles in system (Babazadeh et al., 2016).

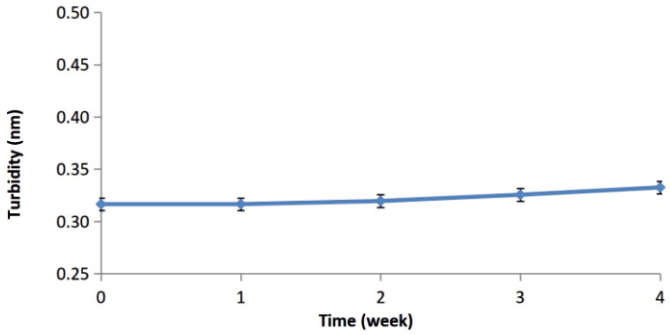

Figure 1. Turbidity RPO-NLC during storage at incubator 37 °C for up to four weeks.

Fourier transform-infrared spectroscopy (FTIR) Analysis

FTIR analysis is an effective method in identifying type of functional group and type of chemical bond present in sample, either in solid or liquid form based on absorption infrared radiation. In Figure 2. shows results of RPO-NLC FTIR spectra observed in wavenumber range 650 – 4000 cm^{-1}. The specific absorption spectrum of RPO-NLC was observed at wavenumber 2924.02 cm^{-1} and 2854.61 cm^{-1} indicates the presence of functional group C-H (Rohmah et al., 2019). The identified C-H group is derived from triacylglycerol in lipid matrix which is used as raw material for production RPO-NLC. Baek et al. (2020) reported that the wavenumber 2923 and 2855 cm^{-1} shows hydrophobic C-H group. Absorption region at wavenumber 1089.59 cm^{-1} shows stretching C-O functional group, according to results of study (Baek et al., 2020; Rostamabadi et al., 2019). Guerrero et al. (2010) reported that stretching C-O bond occurs at C_1 and C_3.

At wavenumber 3356.44 cm^{-1} showed specific peak in form of high intensity OH functional group in presence of confirmed absorption. This is probably caused by the use of surfactants in form tween 80. Tween 80 is non-ionic surfactant which has side chain in form of OH functional group (Rohmah et al., 2019). Rostamabadi et al. (2019) using tween 20 as surfactant confirmed that at wavenumber 3395 cm^{-1} there is vibrational strain originating from OH functional group.

RPO-NLC FTIR spectrum at wavenumber 1638.81 cm^{-1} shows absorption peak C=C, according to research results Pezeshki et al. (2019) on wavenumber 1587–1680 cm^{-1} the absorption peak of C=C group. Then at wavenumber 1462.71 cm^{-1} bending or vibration occurs *methylene* CH_2 from β-carotene (Baek et al., 2020; Neha et al., 2017). Then at wavenumber 947.14 cm^{-1} identified deformation trans-conjugate-alkenes in specific area trans=CH which can be used as identification of β-carotene (Reksamunandar et al., 2017; Rostamabadi et al., 2019). Wavenumber 966.1 cm^{-1} is characteristic peak of β-carotene representing *trans conjugated alkene* CH out of field deformation (Yi et al., 2015).

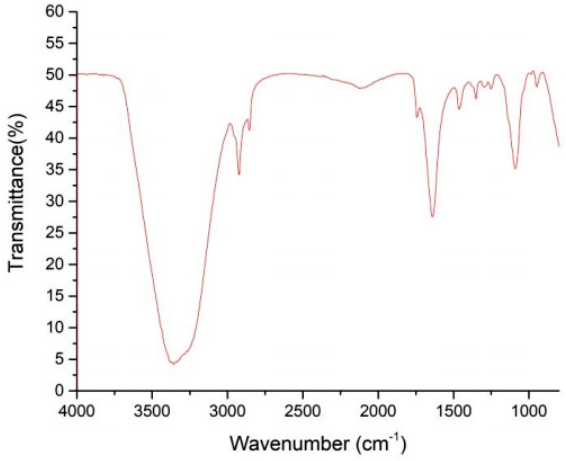

Figure 2. Spectra FTIR RPO-NLC.

Thermal RPO-NLC

Differential scanning calorimetry (DSC) is thermal analysis that measures physical and chemical changes in material response to temperature based on the amount of heat absorbed (endothermic) or released (exothermic). Thermal analysis of RPO-NLC was carried out at temperature of 35 °C to 300 °C with a temperature rate of 10 °C/min^{-1}. Figure 3. shows that the RPO-NLC has two endothermic thermal peaks. At the first peak detected at temperature of 138.45 ^{0}C is probably caused by the presence of components bioactive compounds (β-carotene) which is not completely encapsulated in lipid matrix (*palm stearin*) in small quantities. Chaudhari et al. (2021) there is an endothermic peak at 131 °C based on pure piperine DSC thermogram results but there is no endothermic peak at that temperature on the DSC Piperine-NLC thermogram indicating that piperine is fully encapsulated in NLC lipid matrix with amorphous form and there are no bioactive compounds on the particle surface.

The second peak 168.05 °C probably caused by amorphous of RPO-NLC. β-carotene is encapsulated in dense lipid matrix causes the crystal structure of lipid (palm stearin) become irregular that it becomes amorphous. This result was similar to that of previous research Akhoond Zardini et al. (2018) where an endothermic peak is detected at a temperature of 163.3 °C shows melting point of lycopene which is a carotenoid compound. S. Sharma et al. (2021) producing NLC containing lycopene from oil palm bunches reported that endothermic peak at 150-180 ^{0}C influenced by the amorphous NLC structure. The endothermic peak can also be influenced by type of surfactant used. The use of tween 80 as surfactant can improve stability of RPO-NLC emulsion system. Rohmah et al. (2019) reported that the use of tween 80 as surfactant, can improve stability of β-carotene in NLC and prevent degradation due to high temperature heating.

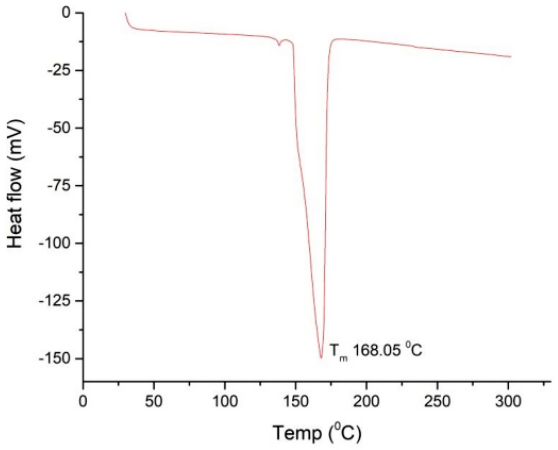

Figure 3. Thermal analysis RPO-NLC.

Encapsulation stability (ES%)

Encapsulation stability was used to identify stability of nanocarrier system in trapping bioactive compounds during storage process (Fathi and Varshosaz, 2013). Figure 4. shows increase in encapsulation stability (ES%) RPO-NLC was applied to orange juice during storage at 8 ^{0}C for up to four weeks. The increase in encapsulation stability from 70.30±0.52 % to 86.87±0.46 % is probably due to the use of palm stearin as solid lipid fraction. Noor et al. (2017) NLC coated with stearic acid and stored at 4 – 8 °C for 30 days has good stability nanoparticles compared to NLC that is not coated with stearic acid, this is probably due to the characteristics of stearic acid at low temperature storage.

Fauzi et al. (2013) reported that palm stearin melts completely at 50 °C, palm stearin also has highest solid fat content (SFC) compared to palm kernel oil and soybean oil at low temperatures. Because high melting point of palm stearin causes RPO-NLC particle structure to be stronger when stored for four weeks at 8 °C. Thus, minimizing components of bioactive compounds that come out in the emulsion system along with longer storage duration. The stability of bioactive compounds encapsulated in NLC can also be affected by type of lipid used. Bashiri et al. (2020) encapsulated cinnamon essential oil combined with almond oil as liquid lipid and cocoa butter as solid lipid showed encapsulation stability which experienced little leakage at 40 days of storage, this is due to the suitability of almond oil in encapsulating cinnamon essential oil component in NLC. While Babazadeh et al. (2016) reported that encapsulation stability (ES%) of Rutin-NLC decreased during 45 days of storage, this is probably due to saturation of bioactive compounds encapsulated in NLC resulting in rutin leakage during storage and decrease in encapsulation stability.

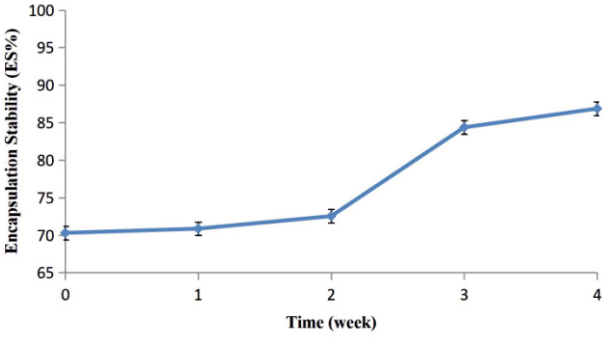

Figure 4. Stability RPO-NLC encapsulation applied to orange juice during storage at 8 ^{0}C for up to four weeks.

pH RPO-NLC in orange juice

Measurement of pH in NLC system aims to determine changes that occur during storage at low temperatures. In Figure 5. results pH measurement of RPO-NLC applied to orange juice with storage temperature at 8 °C there was a slight insignificant increase in pH from 2.81±0.006 to 3.52±0.006 during storage. Changes in pH that occur during storage may be caused by acid-sugar conversion along with length of storage period causing decrease in acidity levels (Osungbade et al., 2021). The changes that occurred were not significant probably because surfactant tween 80 was non-ionic, so it was able to maintain stability of RPO-NLC during storage.

Tween 80 is a type of non-ionic surfactant that can increase stability of emulsion system due to presence of steric resistance from tween 80 (Park et al., 2017). Babazadeh et al. *(2016)* reported that addition of Rutin-NLC in food products with different pH such as milk, orange juice and apple juice did not change significantly during 30 days of storage, this is probably due to the use of tween 80 as a non-ionic surfactant. Bashiri et al. (2020) also reported that different pH conditions in several beverage models did not significantly affect the encapsulation stability (ES%) in NLC.

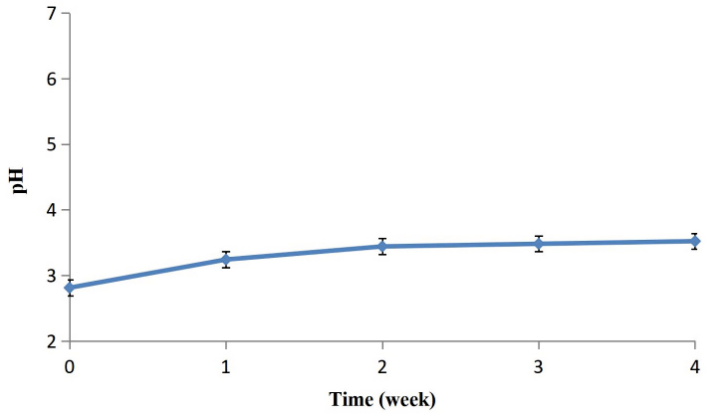

Figure 5. RPO-NLC pH test on application orange juice during storage at 8 °C for up to four weeks.

Sensory evaluation of RPO-NLC orange juice

Sensory evaluation using difference pair comparism test method was carried out to determine difference between control sample (pure orange juice) and RPO-NLC sample that had been applied to orange juice. Based on Table 3. orange juice with addition of RPO-NLC in first week to fourth week there was a very significant change in sensory attributes of color, aroma and taste ($p < 0.01$). The color changes that occur during storage in orange juice with addition of RPO-NLC may be caused by non-enzymatic browning reactions. Non-enzymatic browning reactions result from several reactions such as the maillard reaction, caramelization process, ascorbic acid browning and pigment degradation (Da S. Lima et al., 2009). Chauhan et al. (2014) reported that there was color change in lemon juice mixed with coconut water, the change was caused by a non-enzymatic browning reaction during storage.

The results of sensory evaluation aroma parameters in orange juice that have been added with RPO-NLC have changed during storage compared to orange juice without the addition of RPO-NLC (control) this is probably caused by the use of tween 80 as a surfactant in RPO-NLC so that it can affect aroma of orange juice during storage. Tween 80 is a surfactant with a slightly bitter taste and characteristic rancid odor, consists of a mixture of oleic partial esters of sorbitol and sorbitol anhydride condensed with about 20 moles of ethylene oxide (C_2H_4O) for each mole of sorbitol and mono and dianhydride(Burdock, 2001).

The very significant change in taste RPO-NLC orange juice from first week to fourth week is caused by the use of tween 80 (Polysorbate 80) as a surfactant that has a bitter taste so that it greatly affects taste drinks. Tween 80 is a surfactant that has a slightly bitter taste and characteristic rancid odor (Burdock, 2001). In addition, the use of small amounts of RPO (0.16 g) in RPO-NLC formula 100 ml of orange juice did not affect the sensory attributes of taste, this is probably because red palm olein has a neutral taste. El-Hadad et al. (2010) using red palm olein which was applied to functional biscuits, reported that it did not show significant change in the sensory attributes of taste and aroma compared to biscuits without the addition of red palm olein (control).

Table 3. Sensory evaluation orange juice with added RPO-NLC during four weeks storage at 8 ^{0}C.

Week	Parameter		
	Color	Aroma	Taste
0	14±1.41	15±1.41	15±0.01
1	17±1.41[a]	16.5±0.70[a]	18±0.01[a]
2	19±0.01[a]	15.5±0.70[a]	18±0.01[a]
3	18±0.01[a]	18±0.01[a]	18.5±0.70[a]
4	19±0.01[a]	19±0.01[a]	20±0.01[a]

Note: The mean ± SD value of 2 test sets with different assessments between control and orange juice with the addition of RPO-NLC. Numbers followed by letters indicate very significant difference ($p < 0.01$).

CONCLUSION

NLC was successfully prepared with a mixture of palm stearin and red palm oil (RPO) and Tween 80 surfactant using the HPH method. The best formula RPO-NLC has a particle size 44.9 nm which was selected based on small particle size, high RPO composition and surfactant concentration used. The selected RPO-NLC resulted EE 99.9±0.02%, LC 4.9±0.001%, and remained stable on storage for four weeks in incubator at 37 °C. FTIR analysis showed that β-carotene was encapsulated in RPO-NLC lipid matrix and had good thermal stability based on DSC analysis. There are challenges in selection of orange juice products that will be applied to RPO-NLC because each type orange juice product has different pH which can affect sensory evaluation. Sensory evaluation RPO-NLC applied to orange juice showed changes in sensory attributes of color, aroma, and taste during storage. Encapsulation stability showed a significant increase during storage and pH analysis also increased during storage. RPO-NLC applied to orange juice has great potential for application to beverage products. However, more in-depth studies are needed regarding changes in sensory attributes during storage and interactions that occur between beverage products and NLC.

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