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Original Article

FORMULATION OF RED FRUIT OIL NANOEMULSION USING SUCROSE PALMITATE

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ABSTRACT

Objective: Red fruit (*Pandanus conoideus*) is an endemic plant in the Papua region, Indonesia, and is reported to be very rich in carotenoids (pro-vitamin A), tocopherols (vitamin E), and unsaturated fatty acids. This study aimed to formulate the red fruit oil nanoemulsion in the forms of cream and gel, with sucrose palmitate as the emulsifying agent for topical application to the skin.

Methods: Nanoemulsion was prepared by brute force method using Ultra-Turrax homogenizer as a high-speed mixer. Analyzis of the zeta potential, Polydispersity Index (PDI), particle size, and storage stability of the nanoemulsion cream and gel were also carried out.

Results: Red fruit oil nanoemulsion had pseudoplastic flow properties, a pH of 4.81, a spherical shape, an average particle size of 103.07±1.31 nm, and a PDI of 0.229±0.02. The nanoemulsion cream had plastic flow properties, a pH of 6.45, an average particle size of 391.53±1.31 nm, and a PDI value of 0.701±0.01. The nanoemulsion gel had plastic flow properties, a pH of 5.83, an average particle size of 143.7±3.57 nm, and a PDI value of 0.221±0.03. Antioxidant activities of the cream and gel were determined using 2.2-Diphenyl-1-picrylhydrazyl (DPPH) assay. Inhibition of 50% concentrations (IC_{50}) for cream and gel were 6.14 and 48.85, respectively.

Conclusion: The best red fruit oil nanoemulsion formula contained 10% of surfactant-cosurfactant mix (Smix) composition with a ratio of 9:1. Both the nanoemulsion cream and gel remained physically stable after 12 weeks of storage at three different temperatures, cycling, and centrifugation tests.

Keywords: Nanoemulsion, Red fruit oil, Sucrose palmitate, Cream, Gel

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INTRODUCTION

Nanoemulsion is a transparent, translucent emulsion system. It is a water-oil dispersion stabilized by a film coating of a surfactant or surfactant molecule, having a droplet size of 50-500 nm [1-3]. Nanoemulsion is widely used in cosmetics, pharmaceuticals, and other industries because the amount of surfactant used is relatively low. The product obtained through nanoemulsion is more stable than other forms, has low toxicity and irritant properties, and is widely used in a variety of formulations, such as foams, creams, gels, liquids, and sprays [4, 5].

Nanoemulsion offers several advantages over other dosage forms. These advantages are an increased rate of absorption, reduced variability in absorption, protection from oxidation and hydrolysis in oil-in-water nanoemulsion, delivery of lipophilic drugs after solubilization, aqueous dosage form for water-insoluble drugs, enhanced bioavailability for many drugs, ability to incorporate both lipophilic and hydrophilic drugs, and a delivery system to enhanced efficacy. At the same time, nanoemulsion reduces the total dose and side effects, as it forms non-toxic and nonirritant vehicles for skin and mucous membrane delivery and release control by permeation of drugs through liquid films, whose hydrophilicity or lipophilicity, as well as thickness, can be precisely controlled [6, 7].

Sucrose monoester palmitate is a nonionic surfactant containing sucrose as a hydrophilic group and fatty acids as a lipophilic group developed to manufacture nanoparticles. Sucrose esters are odorless, tasteless, non-toxic, and nonirritant to the skin, so are used in the manufacture of foods, medicines, cosmetics, and other pharmaceutical products [8, 9].

Red fruit oil taken from the flesh of red fruit has various active compounds with medicinal properties [10]. Red fruit oil contains beneficial nutrients or high levels of several active compounds, including beta-carotene and tocopherol, as well as fatty acids such as oleic acid, linoleic acid, linolenic acid, and decanoic acid. The tocopherol content in red fruit oil is high and has biological activity as an antioxidant to prevent damage to cell components causing skin aging [11, 12].

This study aimed to formulate the red fruit oil nanoemulsion in the form of cream and gel with sucrose palmitate as the emulsifying agent for topical application to the skin.

MATERIALS AND METHODS

Materials

Red fruit oil (provided to the reasearchers as a gift from Drs. I Made Budi, M. Si. Papua, Indonesia), sucrose palmitate (Carbosynth, UK), propylene glycol (Brataco, Indonesia), olive oil (Brataco, Indonesia), stearic acid, triethanolamine (TEA), cetyl alcohol, Butylated hydroxytoluene (BHT), benzoic acid, sepigel 305 (CV. Cipta Anugerah Mandiri, Indonesia), glycerin, sodium metabisulfite (Brataco, Indonesia), aquadest (Brataco, Indonesia), DPPH (Sigma Aldrich), methanol (Merck, Germany).

Construction of pseudo-ternary phase diagram

The ternary phase diagram was constructed using red fruit oil, sucrose palmitate, propylene glycol, and aquadest. The sucrose palmitate and propylene glycol were prepared in ratios of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9. The nanoemulsion formula contained 10% and 20% sucrose palmitate and propylene glycol for each ratio and 3% red fruit oil. The pseudo-ternary diagram was created using the CHEMIX School 7.0 program by entering the volume of the experimental results. The program displayed the dots connecting the regions where the nanoemulsion was formed [13].

Preparation of red fruit oil nanoemulsion

The nanoemulsion was prepared by brute force method using an ultra-turrax homogenizer as a high-speed mixer (7). Sucrose palmitate was dissolved in propylene glycol and aqua dest (table 1). The mixture was stirred with a magnetic stirrer and heated at 30 °C. After mixing homogeneously, the red fruit oil was added. After that, the nanoemulsion was homogenized again using a high shear homogenizer Ultra-Turrax mixer at 10,000 rpm for 10 min to obtain a homogenous nanoemulsion with a particle size of less than 200 nm.

Table 1: Formula of red fruit oil nanoemulsion

	Red fruit oil (%)	Smix (%)	Aquadest (%)
Formula 1	3	10	87
Formula 2	5	15	80
Formula 3	7	20	73

Preparation of nanoemulsion cream and gel

Cream preparations were made by mixing the water and oil phases after heating at 70 °C. The oil phase–consisting of, cetyl alcohol, stearic acid, and olive oil-was heated on a heater at a temperature of 70 °C until melted [14]. BHT was added to the dissolved oil phase, and propylene glycol, TEA, and benzoic acid were added to the heated water phase (table 2). The oil and water phases were mixed and homogenized with a homogenizer at 750 rpm to form a cream base. Then, 5% of red fruit oil nanoemulsion was added to the cream base.

Table 2: Formula of nanoemulsion cream

Components	Composition (%w/w)
Red fruit oil nanoemulsion	5
Olive oil	6
Cetyl alcohol	4
Stearic acid	4
Triethanolamine	2
BHT	0.05
Propylene glycol	10
Benzoic acid	0.1
Aquadest	Ad 100

Gel preparations were made by using a homogenizer to make a gel base. Sodium metabisulfite was dissolved in glycerin before adding propylene glycol. The mixture was then mixed with aqua dest and sepigel (table 3). Sepigel was chosen because it is easy to process at both hot and cold temperatures and stable in pH range between 3 and 12 [15]. The mixture was homogenized using a homogenizer at a constant speed of 500 rpm to form a gel base. After developing a gel base, 5% of red fruit oil nanoemulsion was added to the gel base and stirred until homogenous.

Table 3: Formula of nanoemulsion gel

Components	Composition (%)
Red fruit oil nanoemulsion	5
Sepigel	2
Propylene glycol	3
Glycerin	5
Sodium metabisulfite	0.01
Aquadest	Ad 100

Evaluation of red fruit oil nanoemulsion gel and cream

Emulsions should be evaluated for appearance, including phase separation, color, odor, pH, viscosity, and mean size and distribution of dispersed globules [16].

Physical appearance

The color and odor of nanoemulsion gel and cream were observed. For the nanoemulsion cream, phase separation was observed, while syneresis was observed for the gel.

pH measurement

Evaluation was conducted using a semisolid electrode pH meter by dipping the electrode into the sample before recording the results. The test was carried out in triplicate. The pH obtained was expected to match the skin's pH in the range of 4.5-6.5 [13, 17].

Particle size measurement

This experiment was carried out using the dynamic light scattering method and the particle size analyzer. This test was performed to determine the size of the globules formed in the formulations [17].

Transmission electron microscopy (TEM)

TEM analysis was used to examine the red fruit oil nanoemulsion morphology. The nanoemulsion was dissolved in aqua dest (1/100), dropped on a holey film grid, and analyzed using TEM after drying [17].

Rheology properties

The rheology and viscosity of the nanoemulsion gel and cream formulas were determined using a Cole Parmer Viscometer with spindle number 4. The speed was shifted from 2.0, 4.0, 10.0, and 20.0 rpm to 20.0, 10.0, 4.0, 2.0 rpm. It was used to dial in the reading at each revolution. Each system's rheological behavior was assessed by plotting shear stress versus shear rate values. The viscosity values were recorded [18].

Physical stability study of red fruit oil nanoemulsion gel and cream

The cycling test of the nanoemulsion gel and cream was carried out on both preparations. The cycle was repeated six times. Nanoemulsion gel and cream were loaded into a centrifuge tube and were centrifuged at 10,000 rpm for 30 min. The formulas were then observed for any changes in homogeneity. Twelve samples of nanoemulsion gel and cream were stored in three different temperature conditions: room temperature of 27°±2 °C; the high temperature of 40°±2 °C; and low temperature of 4°±2 °C. Each was stored in the condition for 12 w. Physical stability evaluation through visual inspection of physical change, such as color, phase separation, and syneresism, every two weeks [19].

RESULTS AND DISCUSSION

Determination of fatty acid contents in red fruit oil

The level of fatty acids in red fruit oil nanoemulsion was determined using gas chromatography in this study (table 4). The fatty acid has a volatile characteristic, so the method of quantitative analysis by gas chromatography was chosen [20-22]. The highest fatty acid content was oleic acid at 57.467%, and the lowest fatty acid content was myristate fatty acid at 0.068%.

Table 4: Fatty acid contents in red fruit oil

Sample name	Types of fatty acid analysis	Method	Result	Unit
Red fruit oil	Laurate	Gas chromatography	Not detected	%
	Myristate		0.068	
	Palmitate		22.398	
	Stearic		0.866	
	Oleic		57.467	
	Linoleic		2.056	
	Linolenic		-	

Construction of ternary phase diagram

The pseudo ternary phase diagram plotting the three components of oil, surfactant-co surfactant mix, and water was created to calculate the concentration area of the oil-surfactant-water mixture that would form a nanoemulsion [23, 24]. The volumes of water, oil, and surfactant-cosurfactant mixture were entered into the CHEMIX School 7.0 application (fig. 1). The CHEMIX program revealed that the nanoemulsion formation area in water was formed on the oil side. Areas with a multi-phase system are designated outside the area. Surfactants produce nanoemulsions to reduce the interfacial tension between the water and oil phase layers [25]. The nanoemulsion region was marked in a yellow phase diagram. Through visual observation of the formulation, the area identified had good homogeneity, good flowability and did not undergo phase separation.

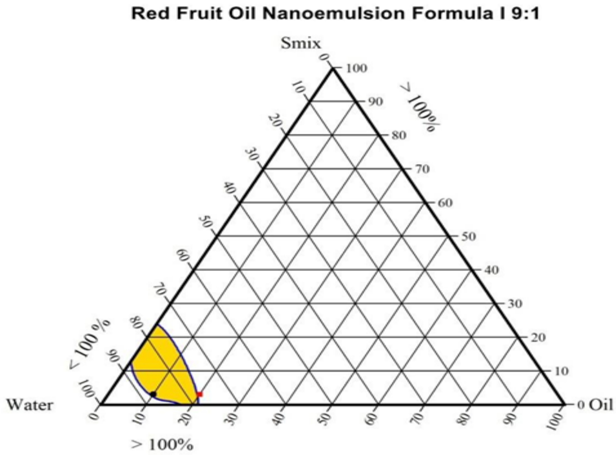

Fig. 1: Ternary diagram of red fruit oil nanoemulsion

Preparation of red fruit oil nanoemulsion

The surfactant-cosurfactant ratio was optimized to create the nanoemulsion formulation. It is important to control the critical formulation and process parameters during formulation using the high shear homogenization method, as they greatly affect nanoparticle size [26]. The percentage of red fruit oil used was 3%. With a Hydrophilic–Lipophilic Balance (HLB) value of 16, sucrose palmitate was used as a surfactant and propylene glycol was used as a co-surfactant. The formula was developed by creating ternary phase diagrams, and had a characteristic of red color, a pH range of 4.5-6.5, did not undergo phase separation, and a particle size of less than 200 nm.

The red fruit oil nanoemulsion was cloudy red, smelled strongly of red fruit oil, and did not undergo phase separation. The nanoemulsion viscosity study at 4 rpm yielded a value of 531.5 cP. The nanoemulsion of red fruit oil had pseudoplastic flow properties (fig. 2). Measurements found that the pH of the nanoemulsion was 4.5-6.5 and thus suitable for the pH of the skin. The obtained nanoemulsion particle size was 103.07±1.31, with a PDI of 0.229±0.02 (table 5). The particle formed was small for penetration of the wall of the film surfactant by the co-surfactant, which later reduced the fluidity and viscosity of nanoemulsion [27]. Using TEM (JEOL 1010, USA), the morphology of the red fruit oil nanoemulsion preparation can be seen microscopically. The result showed that the red fruit oil nanoemulsion preparation has a spherical shape of 200 nm (fig. 3).

Table 5: Particle size of red fruit oil nanoemulsion

Parameter	Red fruit oil nanoemulsion*
Particle size (nm)	103.07±1.31
D90 (nm)	135.43±35.92
Polydispersity index (PDI)	0.229±0.02

*Results are expressed as mean±SD, n=3

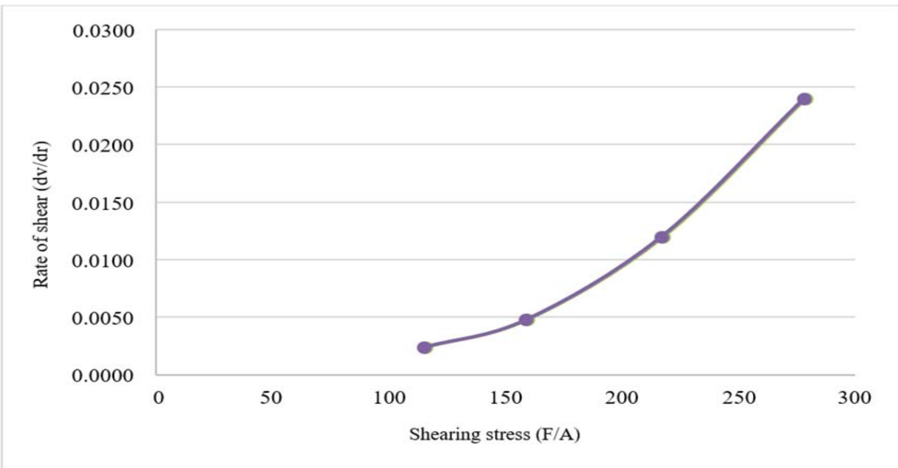

Fig. 2: Rheogram of red fruit oil nanoemulsion

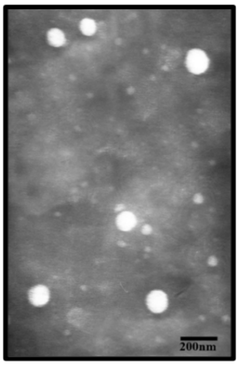

Fig. 3: Morphology of red fruit oil nanoemulsion

Nanoemulsion gel and cream of red fruit oil

Red fruit nanoemulsion concentrations as high as 5% were used in gel and cream formulations. The nanoemulsion formula chosen is consistent with preliminary experiments on the production of ternary phase diagrams, namely the formula with a 9:1 Smix ratio and a total composition of Smix in the formula of 10%.

Table 6: Particle size of red fruit oil nanoemulsion cream and gel

Parameter	Cream*	Gel*
Globul size (nm)	391.53±1.32	143.7±3.57
D90 (nm)	290.67±0.57	275.0±2.64
Polydispersity index (PDI)	0.701±0.01	0.221±0.03

*Results are expressed as mean±SD, n=3

The cream nanoemulsion was a pale orange semi-solid form that smelled mildly of red fruit oil and did not undergo phase separation. The nanoemulsion gel was bright orange in color, odorless, and formed a good gel or did not undergo syneresis (fig. 4). The nanoemulsion cream used the Cole Parmer Viscometer with plastic flow properties. The viscosity value of the gel was 26,018 cP at 4 rpm. The nanoemulsion gel also had plastic flow properties, with a viscosity value of 32,031 cP at 4 rpm (fig. 5). The average particle size of the cream was 391.53±1.32 nm, D90 value of 290.67±0.57 nm, and a PDI value of 0.701±0.01. The gel obtained an average particle size of 143.7±3.57 nm, D90 value of 275.0±2.64 nm, and a PDI value of 0.221±0.03 (table 6). The pH of the cream was 6.45, while the gel had a pH value of 5.83 (table 7).

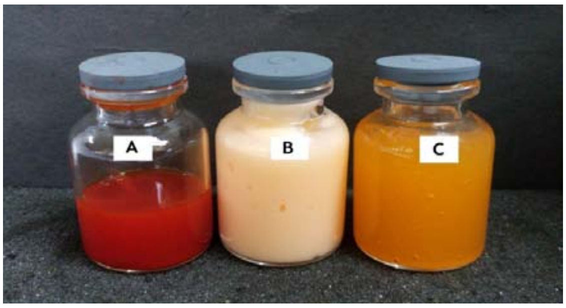

Fig. 4: Physical appearance of (A) red fruit oil nanoemulsion, (B) nanoemulsion cream, and (C) nanoemulsion gel

Centrifugation at 10,000 rpm for 30 min was carried out to test the stability of the cream and gel preparations of red fruit oil nanoemulsion. The cream and gel preparations were stable without undergoing any phase separation or syneresis [28]. The physical stability study was carried out over 12 w at three different temperatures and revealed that the samples of nanoemulsion cream were pale orange with mild red fruit oil odors and no signs of phase separation, while the samples of nanoemulsion gel were bright orange, odorless, and no signs of syneresis. All of the samples maintained a stable pH for 12 w (table 7).

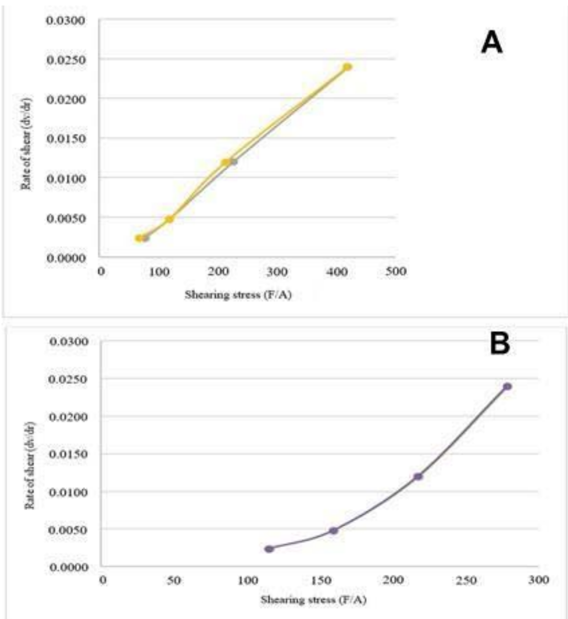

Fig. 5: Rheogram of (A) Nanoemulsion cream and (B) Nanoemulsion gel

Table 7: pH of physical stability test of nanoemulsion cream and gel

Week/Temperature	Nanoemulsion cream*			Nanoemulsion gel*		
	4 °C	27 °C	40 °C	4 °C	27 °C	40 °C
Week 0	6.45±0.06	6.45±0.06	6.45±0.06	5.83±0.09	5.83±0.09	5.83±0.09
Week 2	6.11±0.06	5.87±0.11	5.84±0.02	5.73±0.03	5.84±0.07	5.63±0.07
Week 4	5.97±0.16	5.83±0.12	5.83±0.05	5.71±0.01	5.71±0.13	5.64±0.02
Week 6	6.11±0.02	5.96±0.02	5.86±0.04	5.72±0.02	5.62±0.05	5.64±0.06
Week 8	6.03±0.04	5.94±0.02	5.86±0.03	5.82±0.04	5.80±0.05	5.81±0.13
Week 10	6.05±0.01	5.96±0.04	5.83±0.04	5.79±0.02	5.68±0.04	5.68±0.09
Week 12	5.98±0.01	5.93±0.02	5.93±0.01	5.79±0.02	5.53±0.04	5.63±0.07

*Results are expressed as mean±SD, n=3

Antioxidant determination of nanoemulsion gel and cream of red fruit oil

The DPPH was widely used to assess free radical scavenging activity. The IC_{50} value was calculated by plotting the DPPH radical scavenging activity percentage against the sample concentration. The IC_{50} value is defined as the concentration of the sample required to cause 50% inhibition. The higher the scavenging activity sample, the lower the IC_{50} value [29].

The DPPH suppression method was used to test the antioxidant activity of the nanoemulsion cream and nanoemulsion gel. The nanoemulsion gel had an IC_{50} value of 48.85, while the nanoemulsion cream had an IC_{50} value of 6.14. The antioxidant analysis of the cream and gel using DPPH revealed that the cream had a higher antioxidant profile. The scavenging activity was obtained as the percentage of absorption of the sample minus the absorption of the blank divided to the absorption of the blank [14].

CONCLUSION

The best formula of red fruit oil nanoemulsion was the formula with 10% sucrose palmitate and propylene glycol mixture composition in the ratio of 9:1. It showed good stability and smaller particle size. Both cream and gel nanoemulsions were physically stable over a storage period of 12-weeks at three different temperatures, cycling, and centrifugation tests. The IC_{50} percentage values for cream and gel formulation were 6.14 and 48.85, respectively.

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Nil

AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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