Physical Characterization and Sunscreen Effectivity of Avocado Oil in Nanoemulsion Using Isopropyl Myristate Variations

by TURNITIN ID

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ABSTRACT

Avocado oil contains unsaturated fatty acids which can prevent erythema due to excessive UV-B exposure. Nanoemulsion using isopropyl myristate (IPM) will increase the efficacy of sunscreen absorbtion into the skin. The purpose of this study was to determine the physical characteristics and effectivity of avocado oil nanoemulsion (AVN) with IPM variations as a sunscreen. AVN was made with 5% oil and IPM variation: 1% (FI), 3% (FII), and 5% (FIII). The AVN were tested for physical characteristics such as organoleptic, pH, viscosity, rheology, particle size and polydispersity index (PI). The products were also tested for sunscreen effectivity by in vitro and Minimum Erythemal Dose (MED) method. The data obtained were analyzed statistically. The results showed that the AVN was pale yellow and clear with transmittance percentage were 96%. The rheogram showed that the products were newtonian. The pH values range were from 6.62 to 6.66; viscosity 1.65-1.84 dPa.s; particle size < 17 nm, zeta potential was in range of -30,54±1,72 to -37,85±3,11 and PI < 0.5 for all formula. In vitro SPF values were 16.34 ± 5.50 (FI), 16.70 ± 5.20 (FII) and 17.80 ± 3.20 (FIII) (p >0.05), and categorized as ultra protection. MED value were 12.28 ± 1.34 (FI); 12.51 ± 1.68 (FII); and 13.22 ± 1.84 (FIII) (p< 0.05) and categorized as maximum protection. Isopropyl myristate did not affect physical characteristics yet increased MED value as the parameter of sunscreen product.

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Keywords: Avocado oil, enhancer, erythema, nanoemulsion

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INTRODUCTION

Chemical sunscreen absorbs sun rays and converts it into heat energy. This sunscreen can work after being absorbed into the skin, so it has the potential to irritate (Minerva, 2019). Sunscreen works by absorbing or dispersing sunlight so that radiation from sunlight does not directly hit the skin (Riascos, 2012). The effectivity of sunscreen is expressed as SPF value of more than 10, thus describing its ability to prevent erythema due to sun exposure (Araújo et al., 2016). FDA has prohibited sunscreen that contains Para Amino Benzoic Acid (PABA), avobenzone and its derivate due to its side effect(Pirotta, 2020). PABA can be absorbed into the skin that it may cause several hormone disorder and breast cancer (Barel et al., 2009). Active compound such as Z-3, octyl-methoxycinnamate will increase luteinizing hormone. Due to this side effect, it is necessary to develop natural sunscreen.

Avocado oil is one of the natural ingredients that can potentially be used as a sunscreen preparation and can absorb UV-B rays (Flores et al., 2019). Avocado oil has bioactive components that benefit the skin such as palmitic acid and linoleic acid, which can increase skin moisture and prevent skin erythema (Li et al., 2019). Components of avocado oil are also used to heal inflammation and skin erythema through skin barrier repair mechanisms (De Oliveira et al., 2013) (De Oliveira et al., 2013). Previous study had been shown that avocado oil has sunscreen activity with SPF value of 6 to 16 (Fares et al., 2023).

Nanoemulsion system is one of a promising delivery system for cosmetics. This system has good stability and skin penetration ability for topical use, especially for active substances from essential or plant oils (Mohite et al., 2019). Nanoemulsion system can reduce the globule size and increase the efficacy of oil in cosmetic preparations (Hashim et al., 2019). Preparations that are applied topically, such as sunscreen, must be absorbed quickly by the skin to provide a good therapeutic effect (Argenta et al., 2014). Adding enhancers such as isopropyl myristate (IPM) will increase the absorption of active substances (Eichner et al., 2017).

Isopropyl myristate (IPM) has low surface tension and viscosity, suitable for good nanoemulsion characteristics. IPM is advantageous in producing nanoemulsions due its low molecular weight and matches the characteristics of nanoemulsions, which have a globule size of less than 1000 nm (Souto et al., 2022). The use of IPM in the nanoemulsion formula can prevent intergranular agglomeration during storage to stabilize the nanoemulsion. IPM combined with Tween 80 surfactant can increase the penetration of the active substances, thereby increasing product efficacy (Sondari & Tursiloadi, 2018). Based on the background above, it is necessary to characterize and study the effectivity of avocado oil in nanoemulsion using IPM as an enhancer.

MATERIALS AND METHOD

Materials and Tools

The tools used in this research were a magnetic stirrer (Scilogex®), UV-Vis spectrophotometer (Shimadzu®), particle size analyzer (SZ-100®), cone and plate viscometer (Rheosys Merlin VR II®), micropipette (Scilogex®), pH meter (Electro Lab®), vortex, nonirritant bandage (Hypafic®) and sterile gauze (Onemed®).

The main ingredient used in this research was avocado oil (cosmetic grade) obtained from PT. Daarjeling, Bandung and with a certificate of analysis. The carrier materials used were tween 80, PEG 400, isopropyl myristate and benzyl alcohol with cosmetic grade and methanol with analytical grade obtained from CV. Multi Kimia Raya, Semarang. The comparison used is Parasol SPF 25++ Spray Sunscreen.

The test animals used in the research were 3 rabbits. Inclusion criteria were male, 3 months old, with weight at 1,5-2 kg, in good health condition, with strain of New Zealand White. This test had obtained ethical clearance from the Faculty of Medicine and Health Sciences, Muhammadyah University of Yogyakarta with number: 014/EC-KEPK FKIK UMY/III/2023.

Methods

Avocado oil nanoemulsion formula with variations of isopropyl myristate

The avocado oil nanoemulsion formula can be seen in Table 1.

Table 1. Avocado oil nanoemulsion formula (Shabrina & Khansa, 2022)

Components	Concentration (%)		
	F1	F2	F3
Avocado oil	5	5	5
Isopropyl Myristate	1	3	5
Tween 80	40	40	40
PEG 400	30	30	30

Benzyl alcohol	1	1	1
Purified Water		up to 100	

Purified water was heated to a temperature of 30°C. Benzyl alcohol was dissolved into purified water at 30 °C and then stirred using a magnetic stirrer at speed of 700 rpm until homogeneous. The first mixing stage was carried out by adding Tween 80 and PEG 400 to a solution of benzyl alcohol and purified water until a homogeneous mixture was produced. Avocado oil and isopropyl myristate were mixed in the second stage until a clear and transparent nanoemulsion was formed. The second stage of mixing was carried out at a speed of 1000 rpm for 30 minutes.

Evaluation of physical properties of avocado oil nanoemulsion (Shabrina et al., 2022)

Organoleptic examination of avocado oil nanoemulsion was carried out by observing visually of the color, odor and phase separation. Nanoemulsion pH testing was carried out using 10 mL of sample which was tested with a pH meter that had been calibrated in a buffer solution of pH 4 and pH 7. Viscosity and rheology tests were carried out using 5 mL of sample inserted into a cone and plate type viscometer at a speed of 12 rpm with 6 rotations (the duration of each round was 30 seconds). Sample clarity was tested using 3 mL of sample placed in a cuvette and the transmittance percentage was read using a spectrophotometer. Particle size, polydispersity index (PI) and zeta potential tests were carried out using a Particle Size Analyzer (PSA) and zeta sizer with 5 mL samples. All tests were replicated three times.

Effectivity Test of avocado oil nanoemulsion (Costa et al., 2015; Heckman et al., 2013)

Determination of SPF begins by calculating the Correction Factor (CF) value with a comparison using Parasol SPF 25++. FI, FII and FIII and Parasol SPF 25++ were each taken as much as 0.5 ml, then put into a 10 mL flask, then methanol PA was added to the mark, then homogenized using a vortex. The absorbance was measured with a UV spectrophotometer at a wavelength of 290-320 nm and the absorbance values were recorded at each 5 nm interval. The results obtained were calculated using the Mansur formula to determine the CF value.

Determination of the sunscreen effectivity was carried out using the Minimum Erythema Dose (MED). Test rabbits had their hair removed 24 hours before treatment using a shaver. The back skin of animals that have been shaved were marked into 5 points for treatment: FI, FII, FIII, comparison (Parasol SPF 25++) and no treatment. Each test point was marked with a size of 4x4 cm. Test animals were exposed to 311 nm UV light. The number of red spots (erythema) that appeared in each group of test animals were observed. The duration of UV light exposure until appearance of the erythema was calculated as Minimum Erythema Dose (MED). The sunscreen effectivity was calculated by comparing the MED of unprotected skin with skin protected by the preparation and the comparison.

Data Analysis

Organoleptic and rheogram data were described descriptively. Data on pH, viscosity, transmittance percentage and particle size, polydispersity index and zeta potential for each formula were analyzed statistically using one way ANOVA. In vitro SPF and MED value data were analyzed using an independent t-test to compare the level of sunscreen effectivity. The SPF value data for each formula was analyzed using one way Anova.

RESULT AND DISCUSSION

The physical appearance of avocado oil nanoemulsion can be seen in Figure 1.

ISSN: 2088 4559; e-ISSN: 2477 0256

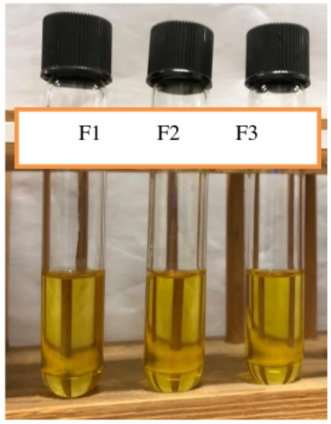


Figure 1. Physical appearance of avocado oil microemulsion

The physical characteristics of avocado oil nanoemulsion can be seen in table 2. The results showed that all avocado oil nanoemulsion formula had a pH value suitable for topical preparations at 4.5-8.0 (Badan Standarisasi Nasional, 1996). The pH of avocado oil before formulation was 6.70 ± 1.50. Additional ingredients such as surfactants, cosurfactants and enhancers had a pH range of 6.0-7.5. The system experienced an increase in pH after avocado oil was incorporated in nanoemulsion yet did not differ significantly. The preparation will cause a skin irritation if the pH is below 4,2 (BPOM, 2014). The pH results of the preparation met the SNI standards for sunscreen preparations. The results showed that increasing the IPM concentration did not affect the pH of the preparation ($p > 0.05$). The results of the viscosity values can be seen in table 2.

Table 2. Physical characteristics of avocado oil nanoemulsion with variations in IPM

Formula	Physical characteristics					
	pH	Viscosity (d.Pas)	Transmittance Percentage (%)	Particle Size (nm)	Polydispersity Index	Zeta Potential (Mv)
FI	6,66±0,03	1,75 ± 0,87	96,50 ± 1,51	15,50 ± 2,13	0,476 ± 0,215	-32,33±2,30
FII	6,65±0,04	1,84 ± 0,83	96,70 ± 1,53	14,75 ± 1,41	0,331 ± 0,227	-30,54±1,72
FIII	6,62±0,03	1,65 ± 0,84	96,90 ± 1,28	13,25 ± 1,35	0,213 ± 0,234	-37,85±3,11

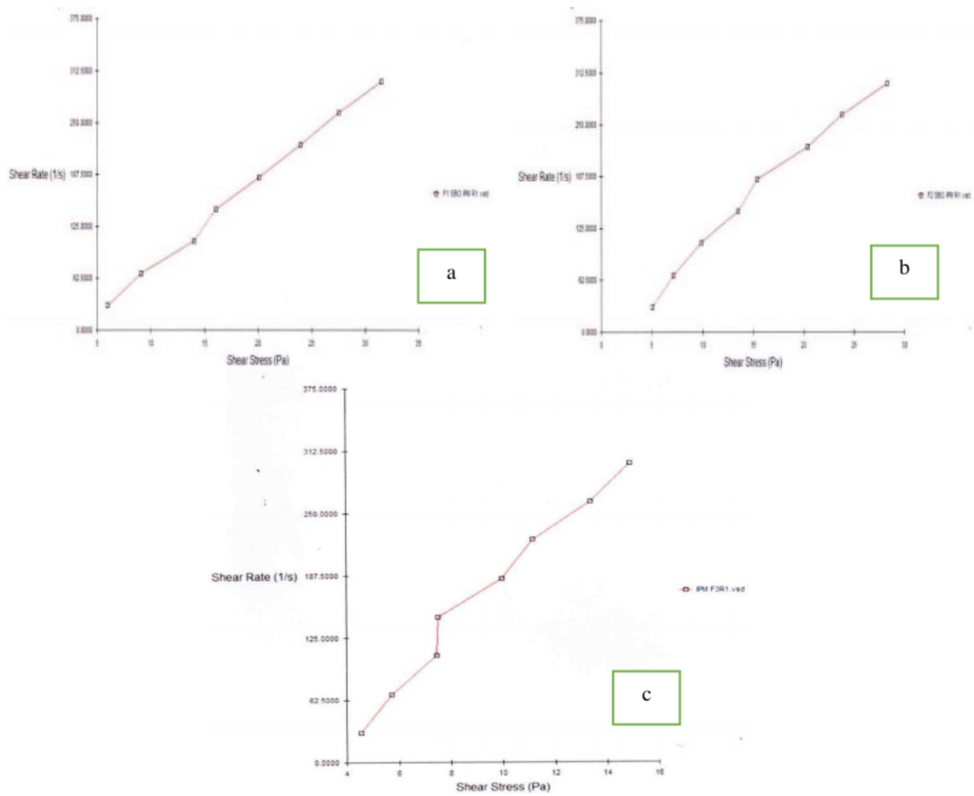
Data displayed were 3 replications with standard deviation

A low viscosity will increase the spreadability and compliance of the user to apply on the skin. IPM combined with Tween 80 as surfactant will increase in the viscosity of the preparation (Goyani et al., 2018). This situation can increase the stability of nanoemulsions because they can form stable globules and oil globules cannot be locked between surfactants and cosurfactants (Thomas et al., 2014). IPM has interfacial tension and low viscosity which is very advantageous in making nanoemulsions because it is easier to produce small globule sizes compared to other mineral oils (Muthi et al., 2016). IPM can integrate with the lipid bilayer and bind with hydrophilic ester groups so that the structure of the stratum corneum bilayer membrane becomes more tenuous.

IPM belongs to the fatty acid ester group which meets safety requirements and is widely used as an enhancer (Abdullah et al., 2022). The results of the rheology of avocado oil nanoemulsion can be seen in Figure 2. All avocado oil nanoemulsion formula were Newtonian. This rheology is defined as a fluid with a linearly proportional shear stress whose velocity gradient is in a direction perpendicular to the shear stress (Marques et al., 2018). These results showed that the droplets formed were smaller than emulsion so that the preparation resembles solution or liquid that easily flows (Hasrawati et al., 2016).

Medium chain fatty acids such as IPM have been used in mixed micelle nanoemulsion formulations for good absorption of active substances and as permeation enhancers (Zhao et al., 2016). Isopropyl myristate when combined with Tween as surfactant will increase the viscosity of the preparation (Sun et al., 2012; Zhao et al., 2016).

 ISSN: 2088 4559; e-ISSN: 2477 0256



Gambar 2. The result of avocado oil nanoemulsion (a) FI, (b) FII, (c) FIII

Nanoemulsion had a clear and transparent visual appearance with a high transmittance percentage. Making nanoemulsions with smaller dispersed droplet sizes can reduce globule agglomerations in nanoemulsion preparations so that transparency can be formed and the percent transmittance value (%T) can be determined (Lv et al., 2018). The ideal percent transmittance requirement for nanoemulsion is 90-100% (Gurpreet & Singh, 2018). Based on table 2, the transmittance percentage meets the requirements for an ideal nanoemulsion. The statistical test results show that there is no difference in the percentage of transmittance in each formula. Variations in IPM concentration did not affect the clarity and transparency of the avocado oil nanoemulsion preparation.

A good nanoemulsion has a particle size of 10-100 nm (Chen et al., 2017). The results in table 2 show that all avocado oil nanoemulsion formulas have a particle size of less than 100 nm. This happens due to the influence of surfactant-cosurfactant and IPM. The mehcanism of Tween 80 and PEG 400 combined with IPM is that the surfactant will adsorp the oil and water interface to support the formation of small particle sizes (Cho, 2016; Dalibera et al., 2021). The smaller the globule size will give a slow aggregation of the particles so that creaming in the nanoemulsion can be prevented (Mariadi et al., 2019). The small particle size can extend the shelf life of nanoemulsion preparations, besides that the preparations are not easily damaged and are more easily absorbed by the body (Dwipayana et al., 2022). The globule size results showed that there was a decrease in each formula but it was not significant. IPM is a triglyceride oil that is capable of producing low globule sizes in nanoemulsion systems (De Azevedo Ribeiro et al., 2015).

The PI value < 1 indicates that the nanoemulsion is monodisperse (Eid et al., 2013). The PI value has a correlation with particle size and zeta potential (Gaber et al., 2023). The results of this PI value are in line with previous research that a low PI value indicates a monodispersion system (Caya et al., 2020). Based on table 2, it is known that variations in IPM concentration did not affect the PI value.

Based on the table 2, the zeta potential showed that all formula had negative potential. The good zeta potential was close to 30 mv or -30 mv(Shakeel et al., 2021). The statistical analysis showed that there were no significant different of the zeta potential between each formula. The zeta potential of avocado oil nanoemulsion was still fulfill the requirement of nanoemulsion characteristics (Rachman et al., 2023). IPM at 3-5% in formula will show negative zeta potential from -22.5 until -34.6 mV (Abdullah et al., 2022). The zeta potential result that close to zero (0) will intend the short term stability of nanoemulsion and increase the particle aggregation during the storage (Maha & Sinaga, 2018).

The results of the effectiveness of avocado oil nanoemulsion sunscreen using in vitro and MED methods

The result of avocado oil nanoemulsion effectivity with in vitro measure and MED methods.

Table 3. Results of SPF values using the MED and in vitro methods

Formula	MED (minutes)	SPF based on MED	Category based on MED Data	SPF in vitro	Kategori SPF in vitro	P Value of In vitro SPF and MED
Unprotected Skin	13,5 ± 7,41	-	-	-	-	-
FI	220,5 ± 6,34^{c,d}	16,34 ± 5,50^{c,d}		12,28 ± 1,34		
FII	225,4 ±5,41^{c,d}	16,70 ± 5,20^{c,d}	Ulta Protection	12,51 ± 1,68	Maximum Protection	0,032
FIII	240,2 ±3,45^{a,b,d}	17,80 ± 3,20^{a,b,d}		13,22 ± 1,84		
Parasol®	328,2 ± 4,50^{a,b,c}	24,31 ± 4,30^{a,b,c}		6,68 ± 3,41^{a,b,c}		

Notes
n = 3 ± standard deviation
a: Significantly different with F1
b: Significantly different with F2
c: Significantly different with F3
d: Significantly different with Parasol®

The sunscreen effectivity is based on their chemical structure, which includes an aromatic molecule conjugated with a carbonyl group. This structure allows high-energy UV radiation to be absorbed, resulting in an excited state. As the molecule returns to the ground state, it releases the lower energy associated with longer wavelengths (Aguilera et al., 2023). The particular wavelength range that a sunscreen absorbs varies. Topical application of sunscreen must be absorbed into the skin layers so that the preparation can be effective both as a photoprotection effect, prevention of sunburn and nourishing the skin (Reza et al., 2023). One of the method to increase sunscreen penetration into the skin is nanoemulsion system. The sunscreen in nanoemulsion system has a rapid transdermal absrobtion compared to sunscreen in cream or emulgel (Chavda et al., 2023).

The results of sunscreen effectivity can be seen in table 3. The correction factor was determined from a sunscreen product with a known SPF value. This correction factor functions as a tolerance limit for the use of the spectrophotometer and solvent so that accurate results are obtained. The correction factor result determined from the parasol cooling mist sun SPF 25+ PA++ product was 6.68.

The results of the independent t-test by comparing the SPF values from MED and in vitro tests showed that there were significant differences in all formulas. Factors that can influence the determination of SPF values in vitro include the absence of appropriate methods so that sunscreen products can be evaluated

(Zarkogianni & Nikolaidis, 2016). The combination and concentration of sunscreen, the use of different solvents when the sunscreen is dissolved, the type of emulsion, as well as the interaction of other additional components such as emulsifiers used in a formulation, the addition of other active substances can affect the difference in determining the SPF value of sunscreen in vitro and MED (Heckman et al., 2013; Kausar et al., 2017).

The results of the oneway ANOVA test on SPF values based on MED show that there was a significant difference between FI and FII compared to FIII. This shows that variations in IPM concentration can increase the MED duration so that the SPF value obtained is higher. The presence of IPM can increase product absorption into the skin, where sunscreen from natural ingredients can provide effectiveness when the preparation can be absorbed into the skin (Barradas & de Holanda e Silva, 2021). High levels of isopropryl myristate are able to disturb the rigid lipid structure and reduce the tension of the stratum corneum thereby increasing penetration and the active substance easily entering the skin layers (Jiang et al., 2017). Apart from that, isopropyl myristate in the nanoemulsion formulation also acts as a co-surfactant and provides good stability to the resulting nanoemulsion formula (Dalibera et al., 2021).

The use of IPM together with surfactants and cosurfactants, namely tween 80 and PEG 400, is able to absorb the active substance components of the avocado oil nanoemulsion because the smaller droplet size of the nanoemulsion can increase the permeation ability of the active substance into the membrane (Iliopoulos et al., 2022; Pakki et al., 2019). Tween and PEG 400 play an important role in the ability of nanoemulsions to release active ingredients by influencing the surface layer of the nanoemulsion (Akhtar et al., 2011; Nastiti et al., 2017).

CONCLUSION

Avocado oil nanoemulsion has a clear appearance and meets the criteria for a nanoemulsion delivery system. Isopropyl myristate does not affect the physical characteristics of the preparation and at a concentration of 5% shows the highest sunscreen effectiveness.

ACKNOWLEDGEMENT

The author would like to thank the Ministry of Education, Culture, Research and Technology, Directorate General of Higher Education, Research and Technology (Kemendikbud Ristekdikti) for the Research Grant under the Penelitian Dosen Pemula scheme.

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