

The Effect Of Surfactant And Co-Surfactant Ratio On The Characteristics And Stability Of A Microemulsion Containing Avocado And Olive Oil

Devi Ratnasari^{1*}, Ahsanal Kasasiah², Vesara Ardhe Gatera³

^{1,2,3}Universitas Singaperbangsa Karawang, Indonesia

Abstract

Avocado and olive oil are known to be effective against the causes of degenerative diseases. Furthermore, avocado oil reduces blood sugar levels, normalizes blood cholesterol levels, and improves Liver metabolism. Meanwhile, olive oil is a low molecular weight compound that easily penetrates cells which leads to an involvement in the body's biochemical reactions and a significant reduction in cholesterol levels. These two active ingredients are non-polar and insoluble in water. Therefore, there is a need for a good delivery system, such as a microemulsion dosage formulation. Microemulsion can be used to deliver compounds with low solubility and permeability. Furthermore, it is an emulsion preparation process with sizes ranging from 50-200 nm that contains surfactants and co-surfactants as constituents. The microemulsion formulation that contains a mixture of avocado and olive oil aims to determine the appropriate ratio of surfactants and co-surfactants, therefore forming a stable microemulsion system.

Keywords: Microemulsion, surfactant, co-surfactant, avocado oil, lemon essential oil

Introduction

Avocado oil is widely used as an antioxidant because it contains monounsaturated fatty acids with its highest content being oleic acid¹. Furthermore, the oil contains polyphenols, proanthocyanidins, tocopherols, and carotenoids². Cold extraction from the avocado plant (*Persea gratissima*)³ is a process used to obtain avocado oil. The oil content in avocados is 15-30 grams per 100 grams of fruit⁴ and it is known to increase High-Density lipoprotein (HDL) which provides benefits for the health of the cardiovascular system⁵. Furthermore,

this oil also reduces the risk of diabetes², normalizes blood cholesterol levels⁶, improves liver metabolism⁷, and provides skin health benefits⁸. Studies have shown that avocado oil has the same total cholesterol-lowering ability as olive oil. It also reduces inflammation associated with metabolic syndrome⁹.

Apart from avocado oil, lemon oil also has many health benefits. In its essential form, it is a low molecular weight compound that easily penetrates cells and is involved in the body's biochemical reactions¹⁰. The bioactive compounds contained in lemon essential oil are limonene, β -pinene, and γ -terpinene. There are small amounts of compounds present which include α -pinene, myrcene and geraniol¹¹. Furthermore, the lemon essential oil has several health benefits which include antioxidant¹², anti-inflammatory¹³, cholesterol-lowering¹⁴, and anti-diabetic¹⁵. Studies carried out showed a decrease in cholesterol, triglyceride, and LDL levels in mice at doses of 50 and 100 g/Kg BW¹⁶. Other studies have also shown that lemon essential oil significantly reduces cholesterol levels better than limonene¹⁷.

There are enormous benefits of avocado and essential oils, it is necessary to formulate them into safe, convenient, and effective dosage forms which can be consumed. The two active ingredients are non-polar and insoluble in water which requires, a good delivery system, such as a microemulsion dosage formulation. A microemulsion is a bicontinuous system that contains two phases which include, water and oil separated by surfactants and co-surfactants¹⁹. Furthermore, it has a small globule size ranging from 50-200 nm¹⁸ and is used to deliver compounds with low solubility and permeability. Therefore this study aims to examine the effect of the surfactants and co-surfactants ratio on the characteristics and stability of a formulated microemulsion containing a mixture of olive and lemon essential oil.

Materials and Methods

A. Tools and materials

The tools used in this study include analytical balance (ADAM), electric stirrer (IKA Labortechnik), viscometer (Brookfield DVI), pH meter (Beckman), particle size analyzer (DelsaTM Nano C, Beckman Coulter), and digital microscope (Optima).

All materials used were of pharmaceutical grade. The ingredients used include avocado oil, lemon essential oil, tween 80, span 80, ethanol, 2-Propanol, and glycerin.

B. Method

Surfactant: Co-surfactant Optimization

The micro-formulation of O/W type emulsions was made by the probe sonication method. A mixture of avocado and 10% olive oil was added to Tween 80 at various concentrations of 10%, 15%, and 20%. Furthermore, the water phase (a mixture of water and propylene glycol) was used in a ratio of 1:1, 1:2, and 2:1. Finally, the mixture was sonicated for 30 minutes.

Table 1. The formula of a microemulsion containing avocado and olive oil

No	Formula Code	Surfactant (Tween 80)	Water	Propylene glycol
1	MAZ 1	20 %	1	2
2	MAZ 2	20 %	2	1
3	MAZ 3	20 %	1	1
4	MAZ 4	15%	1	2
5	MAZ 5	15 %	2	1
6	MAZ 6	15 %	1	1
7	MAZ 7	10 %	1	2
8	MAZ 8	10 %	2	1
9	MAZ 9	10%	1	1

Evaluation

pH

The measurement of pH was carried out using a pH meter by dipping the electrode into the microemulsion till the instrument showed a stable number. The pH meter was calibrated using a calibrating solution at pH 4.9 to 7.9 before it was used. The suitable pH range for

oral microemulsion is from 4.5 to 7.5. This is because in that range the emulsion is neither too acidic nor basic.

Determination of Specific Density

A clean and dry empty pycnometer with a volume of 25 mL was carefully weighed 3 times until it stabilized (A gram). It was then filled with distilled water to reach the cavity in the lid and then weighed (A1 gram). The difference in weight between the empty pycnometer and the other containing distilled water was calculated. The pycnometer was then emptied and dried again. Furthermore, several microemulsions were used to fill the cavity in the pycnometer lid and then weighed (A2 gram). The difference in weight between the empty pycnometer and the other containing the microemulsion was calculated. This value was then divided by the difference in weight between the empty pycnometer and the other pycnometer filled with distilled water to obtain the specific gravity of the microemulsion.

$$\text{Density (gram/mL)} = \frac{A2 - A}{A1 - A}$$

Description:

A= Empty pycnometer

A1= Pycnometer filled with water

A2= Pycnometer filled with sample

Globule Measurement

Particle size was measured with a Lab 3-Delsa Particle Analyzer (Beckman Coulter). A total of 1 mL of microemulsion was dissolved in 100 mL of distilled water in a glass beaker or volumetric flask. Furthermore, 10 mL of the solution was added into a cuvette that has been clean of foam and fat. Assuming there is a presence of fat, the cuvette will be cleaned with toluene or another solvent capable of dissolving it. The cuvette filled with the sample is inserted into the sample holder. Furthermore, this tool is turned on and the particle size menu is selected. The tool will measure the sample for 15 minutes and it will then generate

a particle size and distribution curve. The cuvette should be cleaned again making it fat-free(Budiman, 2008).

Viscosity Test

The microemulsion was placed in a beaker until it reached a volume of 350 mL. Then a spindle was inserted into the microemulsion to the specified limit and measurements were obtained with a Brookfield viscometer at speeds of 0.5; 1; 2; 2.5; 4; 10; and 20 rpm.

Results and Discussion

The microemulsion is a dispersion system developed from oil in water (o/w) or water in oil (w/o) emulsion preparations that are stable, clear, transparent, and have low viscosity. In this study, a microemulsion preparation of an avocado and olive oil mixture was made. The purpose of formulating microemulsion is to obtain compatibility with the digestive tract. Furthermore, in the manufacturing process of the microemulsion, a physical evaluation which includes the examination of pH, globule size, polydispersity index, zeta potential, viscosity test, and density was carried out. Observational data are shown in Table 2.

Table 2. The formula of a microemulsion containing avocado and olive oil

No	Formula	Globule Size (nm)	Polydispersity Index	Zeta Potential (mV)	Density	pH	Viscosity (Cps)
1	MAZ 1	133,3	0,199	-45,6	1,05	5,24	60
2	MAZ 2	132,9	0,202	-44,2	1,05	4,72	55
3	MAZ 3	134,0	0,211	-45,3	1,03	5,06	67
4	MAZ 4	143,8	0,105	-32,3	1,04	4,81	30
5	MAZ 5	141,5	0,094	-33,0	1,03	4,74	36
6	MAZ 6	143,8	0,078	-33,9	1,03	4,36	75
7	MAZ 7	171,9	0,220	-37,1	1,03	4,49	95
8	MAZ 8	172,0	0,246	-39,6	1,03	3,92	77,5
9	MAZ 9	173,0	0,244	-39,2	1,03	4,65	55

The measurement of globule size distribution is an essential factor to determine the stability of a microemulsion preparation, which depends on the droplet size in the dispersed phase. This was measured using a Coulter Particle Size Analyzer. The measurement results on nine various preparations showed varying globule sizes. Formula 1, 2, and 3 had the lowest globule size due to the use of high surfactant concentration (Tween 80). Similarly, formulas 4,5, and 6 had lower globule sizes compared to 7,8 and 9. This is because the surfactant concentration is inversely proportional to the globule size. The droplet size was directly proportional to the rate of incorporation, therefore the microemulsion is not prone to creaming. Furthermore, the small particle size has characteristics that include the ability to be stored for long periods, durability, non-varying change in taste, and ease of absorbance into the body²⁶. The polydispersity index is a parameter that shows the uniformity of particle size in a sample. In this study, its value obtained ranged from 0.1 to 0.3. This indicated that particles in the suspension were homogeneous (0.0 was very homogeneous and 1.0 was very heterogeneous)²⁷. Also, its index value showed that all formulas have a homogeneous particle size distribution.

The zeta potential can be determined by electrophoretic movement due to an electric charge. This is a technique for the determination of surface charge on colloidal nanoparticles. These particles have a surface charge that attracts a thin layer of ions with an opposite charge to their surface. This double ion layer will be around the surface of the nanoparticle ion and diffuses throughout the solution. Furthermore, the electric potential at the boundary between the two layers is known as the zeta potential of the particle. The magnitude of the zeta potential of a particle can be used to predict its stability. The zeta potential value of a nanoparticle that is greater than +30 mV or less than -30 mV indicates a high degree of stability. Particles in suspension that have a zeta potential value greater than ± 30 mV will repel each other, therefore there is no tendency to flocculate. Furthermore, the dispersion with a low zeta potential value will form aggregates due to attractive forces through the Van der Waals bond²⁸. The test results showed that all formulas satisfied the requirements of the potential zeta value.

In determining the density, the results showed no significant difference in the nine formulas. The viscosity testing can be used as a parameter regarding the success of

microemulsion formulation in which, formulas with low viscosity tend to have small globule sizes which are not always followed by low viscosity. Therefore, viscosity is influenced by several factors which include mixing or stirring during the preparation process, selection of thickening substances and surfactants.

Conclusion

Based on the results of the study carried out, olive and avocado oil can be made into microemulsions using Tween 80 as a surfactant with a mixture of propylene glycol and water as the water phase.

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Conflict of Interest

All authors state that there is no potential conflict of interest with the study, authorship, and/or publication of this study.

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