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Formulation and Evaluation of Microemulsion From Chloroform Extract of Tomato (Solanum Lycopersicum L.)

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ABSTRACT

Tomato (Solanum lycopersicum L.) contains lycopene which has efficacy as antioxidant. Lycopene is poorly water soluble thus was extracted in chloroform. The objective of this study was to prepare and characterize microemulsion from chloroform extract of tomato for topical delivery. The optimization formula for microemulsion was done by varying the composition of surfactant, co-surfactant, oil, and water. Microemulsion was prepared spontaneously in a hot-plate (70°C) using magnetic stirrer at 700 rpm for 30 minutes. After six weeks of storage, the most stable formula (Formula G) was obtained by composition of 40% tween 80 as surfactant, 5% glycerol and 15% sorbitol as co-surfactant, 5% VCO as oil phase, and 35% water. The turbidity value was less than one (<1). The chloroform extract, as active ingredient, was added to this formula in a concentration of 1000 ppm. Physical evaluations were done by visual observation, turbidity test, surface tension test, cycling test, centrifugation test, globule size, pH, and viscosity test. The result showed that the chloroform extract in microemulsion was clear and stable during six weeks of storage, with a surface tension was 41.90 dyne/cm, average globules size was 3.284 µm, no separation after centrifugation at 3750 rpm for 5 hours, pH in a range of 6.1 – 6.8, and viscosity was 8.95 poise. The recovery percentage of lycopene was conducted by determining specific extension of 1% standardized lycopene in chloroform ($E^{1\%}_{1cm}$) measured using UV-Vis spectrometer at λ_{max} of lycopene 484 nm. The amount of lycopene in microemulsion was 76.26%.

***KEYWORDS:** microemulsion, tomato, chloroform extract, lycopene*

INTRODUCTION

Tomato is one of familiar vegetables that has been consumed for its beneficial to human health. One of important compounds in tomato is lycopene. Lycopene is a carotenoid pigment that gives the red color in tomatoes ripe [1] Moreover, lycopene is a powerful antioxidant that has a high potential for inhibiting free radicals [1]. The antioxidant power of lycopene as a catcher singlet oxygen is twice compared to β-carotene [2] and ten times compared to α-tocopherol [3].

Preparation of lycopene is relatively challenging due to the nature characteristic of lycopene, which is poorly water soluble (Spernath *et al.,* 2002). Therefore, microemulsion is an appropriate delivery system for anticipating the solubility of active component. Moreover, microemulsion has advantages as increase drug solubility, enhance bioavailability, better stability, and increase penetration through the skin and increase permeability of active substances [4], Studies on lycopene microemulsion has been carried out using variation surfactant and co-surfactant [1] variation of Whey Protein Isolate (WPI) [3] and Brij as surfactant.

In this study, the microemulsion was prepared from chloroform extract of tomatoes that intended for topical application which has not been investigated yet. Moreover, this study also aim to optimize the use of tomato into a relatively stable product due to the nature of tomato that easily perish after post-harvest. Microemulasion was formulated using virgin coconut oil, surfactant, co-surfactant, and water.

MATERIALS AND METHODS

Materials

Lycopene (Sigma Aldrich, USA), Virgin Coconut Oil (VCO) (Herba Bagoes, Indonesia), chloroform pro analysis (Merck, Germany), methanol pro analysis (Merck, Germany), tween 80 (PT. Brataco, Indonesia), glycerin (PT. Brataco, Indonesia,), sorbitol (PT. Brataco, Indonesia), ethyl acetate (xx), distilled water and tomatoes purchased from tomatoes farm in Alaham Panjang, West Sumatera, Indonesia.

Chloroform extract preparation

Prior to extraction process, tomatoes were washed with water and cut pieces to be mashed in a blender. The tomatoes juice was then extracted with chloroform and shake in a separator funnel. The chloroform layer then put in a rotary evaporator in order to make it concentrated. The chloroform extract was then identified by organoleptic observation, thin layer chromatography (TLC) using ethyl acetate: methanol (4:6) as mobile phase and silica gel as stationary phase, and determination of λ_{max} lycopene in chloroform extract.

Optimization of basis micro emulsion

Variation of oil phase and surfactant concentration were optimized to obtain the stable of basis micro emulsion using a three-phase diagram. The component and concentration of each formula can be seen in Table1. Basis micro emulsion was prepared by mixing VCO, surfactant and co-surfactant, then stirred at 700 rpm speed in a hotplate

magnetic stirrer 70°C for 10 minutes. Then distilled water was added where temperature and speed were kept constant for next 20 minutes [5].

Table-1: Composition of base micro emulsion

Materials	Formula									
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
VCO (%)	5	10	5	10	5	10	5	10	15	15
Tween 80 (%)	10	10	20	20	30	30	40	40	30	40
Glycerin (%)	5	5	5	5	5	5	5	5	5	5
Sorbitol (%)	15	15	15	15	15	15	15	15	15	15
Distilled water (%) (ad)	100	100	100	100	100	100	100	100	100	100

Evaluation of base micro emulsion

Each formula was then evaluated including:

Organoleptic observation by visual observation

Turbidity test

Turbidity is determined by measuring the absorbance of the sample using UV-Vis spectrophotometer at λ 502 nm. A clear or transparent micro emulsion has turbidity value < 1%. Turbidity is calculated by the equation:

$$Tubidity\ (\%) = \frac{2.303\ x\ absorbance}{width\ cuvette\ (cm)}$$

pH using a pH meter

pHmeter was calibrated with buffer solution. The electrodes were washed with distilled water and dried. Measurements carried out by one gram dosage is diluted with distilled water to 100 mL. Electrode was dipped into the container until the needle moves in a constant position. Figures shown pH meter is a pH value stocks.

Surface tension (γ)

Surface tension was determined using a Du Nouy ring tensiometer. An iridium-platinum was embedded into a proper dish about 5 mm depth. The force to detach can be read on the scale and surface tension is calculated using the following equation:

$$\gamma = \frac{number\ on\ tensiometer}{2xdiameter\ Du\ Noy\ ring}\ x\ correction\ factor$$

Viscosity

Measurements were performed with a viscometer Hoeppler. The appropriate balls were choosing and when the ball has been dropped beyond the start line, return the ball to its original position by means of reversing the tube. Please note the travel time of a ball through the tube began to line start to the finish line in second.

Density

Specific gravity is determined by a Pycnometer was cleaned with distilled aqua, uses rinsed, then dried. Then the empty pycnometer weighed (W0). Furthermore, pycnometer filled with distilled aqua, weighed and recording the results (W1). Drain back then pycnometer filled with the test preparation, and record the results weighed (W2). The specific gravity calculated by the formula:

$$density = \frac{W2 - W0}{W1 - W0}$$

Preparation and evaluation of microemulsion from chloroform extract

Based on the results of the optimization base that has been done, have a formula basis to produce a clear and stable microemulsion then the chosen formula was added the chloroform extract of tomato (lycopene) as an oil phase as well as the active ingredient at a concentration of 1000 ppm. Evaluations were done as the base microemulsion

Lycopene assay in microemulsion

A total of 1mL microemulsion chloroform extract of tomato fruit is taken then added 4 ml of chloroform. This mixture was sonicated for 10 minutes and then divortex to chloroform miscible with the microemulsion. This mixture is centrifuged for 30 minutes at a speed of 6000 rpm. Take the bottom (chloroform) of 2.5 ml and 2.5 ml of chloroform added. This solution was then measured at a wavelength of maximum absorbannya lycopene. Absorbance obtained is inserted into the calibration standard curve and calculated levels of lycopene in the preparation.

RESULTS

The identification results show the tomatoes used his family is Solanaceae, species Solanum lycopersicum L. extract obtained by ramdemen 0.0611%. Extract maroon, have a thick consistency, a characteristic odor. Extract and lycopene comparison is practically insoluble in water and in ethanol, soluble in chloroform, and sparingly soluble in n-hexane. Identification of the extract by thin layer chromatography (TLC) produces the same Rf value comparison lycopene is 0.666 and the maximum absorption of lycopene in the extract is 456.80 nm, 482.20 nm, and 515.20 nm.

The results of organoleptic inspection for microemulsion base formula A, B, C, D, E, F, I, and J changed visually for 6 weeks of storage. But for the formula G has not changed visually for 6 weeks of storage. Formula H experience began to change at week 4 storage [6].

Table-2: Turbidity test of G and H formulas at weeks 0,2,4, and 6 at a wavelength of 502nm

No	Formula	Weeks	Abs	Turbidity (%)	
1	G	0	0.023	0.0530	< 1
		2	0.039	0.0898	< 1
		4	0.047	0.1082	< 1
		6	0.040	0.0921	< 1
2	H	0	0.046	0.1059	< 1
		2	0.047	0.1082	< 1
		4	0.692	1.5937	> 1
		6	1.856	4.2744	>1

Table-3: Results of the determination of the surface tension of the formula G and H.

No	Samples	Surface tension ± SD (dyne/cm)
1	Distilled water	72.80 ± 0.31
2	Formula G	41.01 ± 0.31
3	Formula H	42.29 ± 0.53

Globule size determination was done by using optilab viewer shows of formula G an average globule size is 16.5412 13.6566 µm and formula H lm. Examination of the viscosity obtained is known that the formula G and H has a higher viscosity than the glycerin is 8.95 poise to formula G and 9.92 poise to the formula H and tend to decrease the viscosity of the initial deposit. Examination of the specific gravity of the tenth formula shows the value that is not too big that is in the range of 1.068 to 1.107 g / ml. The results of organoleptic inspection for microemulsion chloroform extract of tomato (Solanum lycopersicum L.) with a concentration of 1000 ppm extract visually unchanged during 6 weeks of storage [7].

Figure-1: Microemulsion chloroform extract tomatoes 0-6 weeks no phase separation occurs

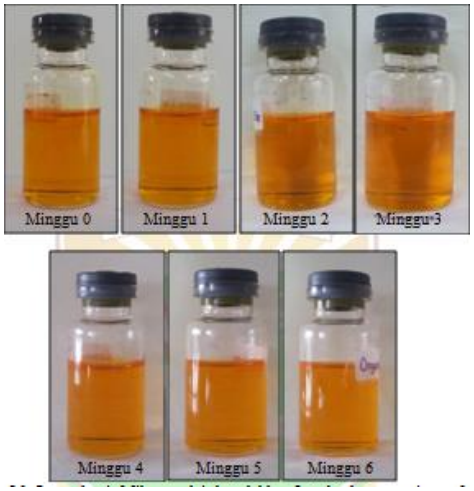
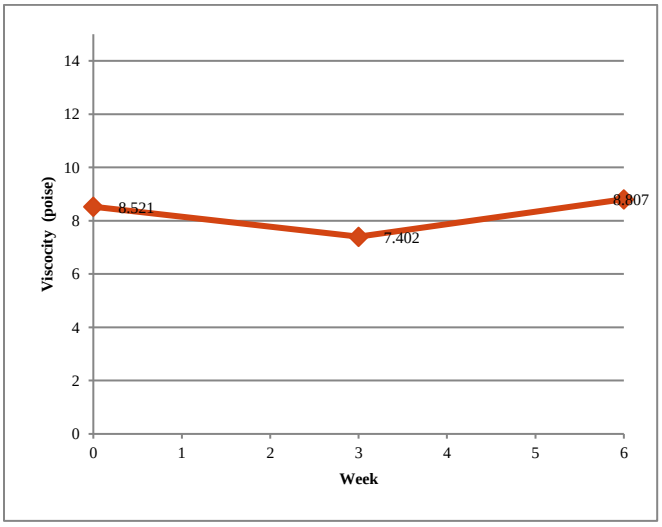


Table 4: Determination of the surface tension

	Samples	**Surface tension ± SD (dyne/cm)**
1	*A* Distilled water	72.80 ± 0.17
2	Microemulsion chloroform extract	41.90 ± 0.25
3	Emulsion *'SE'*	61.91 ± 0.27
4	Syrup *'T'*	67,85 ± 0,23

Results showed that the type of preparation of the microemulsion chloroform extract of tomato fruit is the type of M/A. Examination cycling test showed that the chloroform extract microemulsion tomatoes remained unchanged for 7 cycles. Centrifugation test showed that the chloroform extract microemulsion tomatoes do not undergo the process of separation. Examination globule size by using optilab viewer shows an average globule size is 3,284 lm. Examination of the specific gravity of the microemulsion tomatoes demonstrate the value of 1.106 g / ml

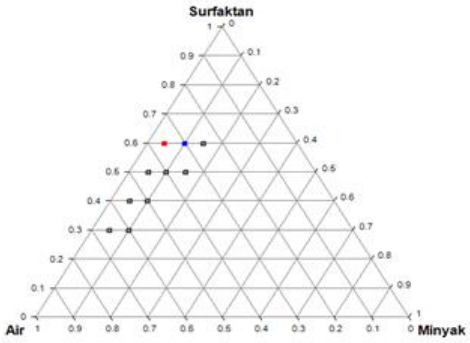
Figure 2: The viscosity of microemulsion chloroform extract tomatoes



DISCUSSION

Prior made the microemulsion, conducted preliminary experiments to determine the best conditions and the best composition of the materials in the manufacture of the microemulsion so obtained microemulsion is clear and stable. Microemulsion boundaries can be determined with the aid of the phase diagram. By way of plotting the concentration data preparation materials that have been made, it will show which areas of the microemulsion.

Figure-2: The phase diagram of the microemulsion



Note: = Emulsion = Microemulsion

= Microemulsion is unstable

Microemulsion chloroform extract of tomato (Solanum lycopersicum L.) is made using virgin coconut oil (virgin coconut oil) as the oil phase. Virgin coconut oil has the properties and benefits for skin health, nature softens the skin so that it can be used as carrier materials pharmaceutical preparations, such as penetration enhancers (Lucida, 2008). The surfactant used is a nonionic suraktan tween 80. The nonionic surfactant which has been used extensively in the preparation of topical and known as the polyoxyethylene derivative that is not toxic and does not irritate the skin. The addition of cosurfactant in the microemulsion aims to maintain the stability of oil and water [8].

In preliminary experiments, it is known that the formula A, B, C, D, E, F, I, J changes visually for 6 weeks of storage. The formula Eighth tend murky and had a turbidity of greater than 1%. This is probably caused by a lack of surfactant concentration so as not strong enough to block the merger in phase droplets. Formula G and H form the preparation is clear and transparent, has a turbidity of less than 1%. Microemulsion is formed by a surface-active agent or surfactant lowering the interfacial tension of two mutually immiscible liquids, by reducing the repulsive force between the two fluids refuse and reduce the attractive forces between like molecules, so that oil and water can mix. Turbidity measurements showed turbidity levels performed microemulsion formed. According to Cho et al., (2008) microemulsion has the appearance of a clear (transparent) and turbidities value of less than 1%. This is due to the small size of the droplets that tend to produce the physical appearance transparent. Formula G has a turbidity of <1% for 6 weeks of storage.

The surface tension was measured using a tensiometer is by Du Nouy Ring method where a low surface tension microemulsion resulting from the surfactant and cosurfactant which can lower the interfacial tension of oil and water. The stability of the microemulsion be higher if the microemulsion has a smaller surface tension of water is 72.8 dyne / cm. The surface tension microemulsion obtained by the 41.01 dyne / cm for formula G and 42.29 dyne /

cm for formula H. This shows that the formula G has greater stability than the formula H due to surface tension values much lower than the water.

Cycling test is used to look at the stability of the emulsion preparations, creams, and solutions, whether to crystallization and precipitation. Centrifugation test conducted by centrifuge at a speed of 3750 rpm for five hours to examine the possibility of instability caused by the force of gravity. Centrifugation for five hours at a speed of 3750 rpm will be equivalent to the effects of gravity caused during one year. Results of centrifugation show the formula A, B, C, D, E, F, I, and J undergo a process of separation or clumping onto the surface and this event is called creaming.

Globule size distribution measurement bases microemulsion performed using a microscope with a magnification of 10x. Measurements were made in the first week preparations were made, and the results of the measurements show the formula G and H has a size of micron-sized globules. Formula G 16.5412 13.6566 μm and formula H lm. Flanagan and Singh [4], mentions that the microemulsion has a droplet size of the dispersed phase is less than 1μm. The results are not in accordance with the literature, this is likely due to stirring with a magnetic stirrer included in the method of making the microemulsion spontaneously where the energy required is low so the resulting droplet size is less uniform.

Type rheological on microemulsion form newton flow system. Formula G and H formula tend to decrease the viscosity at the 3rd week but increased viscosity again at week 6. The value can be quite large compared to the microemulsion in general. This is probably due to the use of the materials used. Tween 80 and glycerin is used basically does have a viscosity so great that when he became preparations also have a large viscosity. Formula G has a lower viscosity than the formula H because of the amount of water in the formula G is greater than the formula H, causing stocks to be more fluid so it flows more easily. The greater the concentration of surfactant is added to the weight of the larger type of preparation. The specific gravity of the entire formula is not too large so that preparations can flow properly and easily pourable.

From the organoleptic examination showed that the chloroform extract microemulsion tomatoes tend not changed during the six weeks of storage. The pH value of the preparation for 6 weeks ranged from 6.1 to 6.8 and the value is still within the range of normal human skin pH. In the examination of the surface tension, the chloroform extract microemulsion tomatoes than water and preparations on the market, namely emulsion 'SE' and elixir 'T'. It aims to see whether the microemulsion made already have a lower surface tension than water, emulsions, and elixirs. The stability of the microemulsion was higher for smaller surface tension than water that is 72.8 dyne / cm. From the observation found that the surface tension of the microemulsion chloroform extract of tomato was 41.90 dyne / cm. This value is slightly larger than the surface tension of the base without active substance but smaller than the emulsion is 61.91 dyne / cm and is also smaller than the elixir that is 67.85 dyne / cm. The existence of a solute in a liquid will affect the surface tension. The addition of the solute will increase the viscosity of the solution, so that the

surface tension will increase. Along with the increase of the solute, then the viscosity will be higher and the force required to detach the ring submerged in liquids increases.

Test cycling test showed that the preparation does not change after going through 7 cycles of this show remains a thermodynamically stable microemulsion. Centrifugation test results showed that the preparation does not undergo the separation process. The average size of the globules microemulsion chloroform extract of tomato fruit is 3,284 lm. Size small globules or droplets that showed more stable emulsion. Examination viscosity microemulsion chloroform extract of tomato fruit preparations showed a tendency to decrease the viscosity at week 3 and increased viscosity again at week 6. Increased viscosity can improve the stability of the preparation [5]. This is because the increase in viscosity can inhibit dispersed phase droplets to join each other to form larger droplets.

Determination of levels of lycopene in the preparations done at the 7th week of storage. This is done to determine the percent recovery levels of lycopene in the preparation. Determination of levels of lycopene in the microemulsion was determined by spectrophotometry using chloroform. Chloroform chosen because it can break the microemulsion and lycopene can be completely soluble in chloroform. Sonication process is carried out to assist the process of solving the microemulsion so that chloroform can be dissolved in the preparation. Centrifugation process is performed to separate the chloroform with carbomers or water phase. Percent recovery of lycopene in the microemulsion week 7 was 76.26%.

CONCLUSION

Extract the chloroform tomato (Solanum lycopersicum L.) can be made into microemulsion is clear and stable for 6 weeks of storage using a magnetic stirrer speed of 700 rpm, for 30 minutes at a temperature of 700C stirring. Microemulsion chloroform extract of tomatoes 1000 ppm created by the combination of surfactant (tween 80 40%), cosurfactant (5% glycerin, sorbitol 15%), the oil phase (VCO 5%) aqueous phase (35% distilled water).

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