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Original Article

FORMULATION AND EVALUATION OF LIQUID CRYSTAL EMULSION CONTAINING EXTRA VIRGIN OLIVE OIL AS SKIN ANTI-AGING

ULVA DWI AYU SEPTIANINGSIH, JULIA REVENY, ANAYANTI ARIANTO*

Department of Pharmaceutical Technology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan 20155, Indonesia
Email: anayanti@usu.ac.id

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ABSTRACT

Objective: The purpose of this research was to formulate and evaluate the and anti-aging activity and the characteristic of liquid crystal emulsion of extra virgin olive oil (EVOO).

Methods: Preparation of liquid crystal emulsion was made using the variation of alkyl polyglycoside and caprylic triglyceride as surfactant with concentration ratio was 3:5 (F1), 4:4 (F2), 5:3 (F3) and 5% extra virgin olive oil as an anti-aging substance. The liquid crystal emulsions were evaluated for an organoleptic test, microscopic, pH value, viscosity, particle size, zeta potential during 12 w storage at room temperature and centrifugation test. The selected liquid crystal emulsion was examined with anti-aging activity (moisture, pore, spot, and wrinkle) using a skin analyzer to volunteer's skin.

Results: The result showed that all formulas had stable storage for 12 w at room temperature (25±1 °C). Liquid crystal emulsion with a surfactant concentration of alkyl polyglycosides and caprylic triglycerides 4:4 (F2) showed the presence of liquid crystal, the stable value of pH (6.9) and viscosity (4995 mPa), smallest particle size 5.727 µm, and highest zeta potential-34.5 mV after 12 w storage at room temperature.

Conclusion: EVOO liquid crystal emulsion (F2) showed better skin anti-aging activity (condition of moisture content, pore, spot, and wrinkle) than conventional emulsion. It concluded that the formula was stable physically and showed better skin anti-aging activity than conventional emulsion.

Keywords: Liquid crystal emulsion, Extra virgin olive oil, Skin anti-aging

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INTRODUCTION

Skin aging is a complex biological activity that causes skin loses the elasticity, the presence of fine lines, ridges, creases, and discoloration of the skin with time, due to intrinsic (genetic) and environmental factors playing an important role in the aging process (eg; Ultra-violet (UV) exposure or weather) [1, 2]. Skin aging occurs just like the aging of body cells in general, which is the accumulation of endogenous damage due to the formation of Reactive Oxygen Species (ROS) during cellular oxidation metabolism [3]. ROS causes oxidative stress on biological structures, which ultimately has an impact on physical appearance and biochemical changes causing phenotypic consequences such as expression lines, low hydration, and pigmentation [4].

Extra Virgin Olive Oil (EVOO) is an oil produced from olive trees (*Olea europae* L.), which is still classified in the family Oleaceae was originally bred in the Mediterranean Basin [5]. EVOO contains 98-99% of triglycerides, the main components in this triglycerides is monounsaturated (oleic) with a slight amount of monosaturated (palmitic, stearic) and the presence of polyunsaturated (linoleic and α-linolenic). The minor component is 1-2% consists of α-tocopherol, phenol component, carotenoid (ß-carotene and lutein), squalene, phytosterols, and chlorophyll. The linoleic and linolenic content of extra virgin olive oil has an essential function as a moisturizer and protection for the skin [6]. According to Orey [7], α-tocopherol in extra virgin olive oil is a natural antioxidant that can prevent free radicals. Squalene in extra virgin olive oil can bind to singlet oxygen species from free radicals that formed by exposure to UV light on the skin. Consumption of extra virgin olive oil is proven to protect against aging by inhibiting oxidative stress because of its contain [6, 8].

Several innovative cosmetic delivery systems that are used in cosmetic products, one of which is a liquid crystal emulsion. Liquid crystal emulsions show mechanical characteristics such as liquids and optical characteristics such as solid crystals (optical anisotropy). The liquid crystal emulsion is a new type of emulsion which is different from conventional emulsion systems. This system is arranged regularly from surfactant and water molecules formed by the oil-water interface; this arrangement forms an emulsion with a liquid crystal structure which shows better usage than conventional emulsion systems in terms of stability, which is more stable compared to other emulsions because it offers some protection against photo or thermal degradation and increases high cutaneous hydration by increasing water intake in the corneal layer and also can delivering active ingredients with delayed-release in the target area [9]. The formation does not only depend on the emulsion formulation, but also the manufacturing process [10]. In a previous study, Bernegossi *et al.* [9], had formulated green tea extract into a liquid crystal system using silicone glycol copolymer as a surfactant. The result of the study showed that formulation with a high concentration of surfactants could produce a liquid crystal system with enhanced efficacy of the green tea extract. The study aims to formulate liquid crystal emulsion containing extra virgin olive oil then evaluate the effects of skin anti-aging was examined.

MATERIALS AND METHODS

The emulsion and liquid crystal emulsions were formulated using extra virgin olive oil (PT. Sari Bunga Alam Lestari Al Arobi) as the active substance. Alkyl polyglycosides, caprylic triglycerides, stearyl alcohol, dimethicone, glycerine were purchased from CV. Dendelion. Sodium carboxymethyl cellulose (CMC), Tween 80, Span 80, propylene glycol, methylparaben, and distilled water were purchased from CV. Rudang Jaya. All ingredients were analytical grade.

Extra Virgin Olive Oil (EVOO) liquid crystal emulsion was formulated by using variations of alkyl polyglycosides and caprylic triglycerides as a surfactant. The liquid crystal emulsion was prepared by using the phase inversion method. The system consists of the oil and the water phase. The phase inversion system was done by adding the water phase to the oil phase dropwise. The oil phase consists of stearyl alcohol, dimethicone, and extra virgin olive oil; this mixture was heated at 80 °C until it melts while stirred, after that alkyl polyglycosides and caprylic triglycerides as surfactants were added. In the water phase, methylparaben (preservative) was dissolved in distilled water, then heated by using the hot plate (Thermo Scientific). This solution was then cooled down and added glycerine. The water phase was inserted into the oil phase and was stirred

until homogeneous for 4 h for obtained milky-white in the color of EVOO liquid crystal emulsion [11].

EVOO emulsion was formulated using spontaneous emulsification. The system consists of an oil phase and a water phase. First, sodium CMC was swelled with hot distilled water in a mortar. The oil phase, EVOO and Span 80 mixture were heated at 60 °C. In the water phase, methylparaben (preservative) was dissolved in distilled water, then heated by using the hot plate (Thermo Scientific). This solution was then cooled down and added propylene glycol, glycerin, Tween 80. This water phase mixture was added to the swelled sodium CMC and was stirred. Then the oil phase also added to the mixture and stirred quickly to avoid the formation of air bubble until obtained homogenous emulsion.

EVOO emulsion and liquid crystal emulsions were stored at room temperature (25±1 °C) for 12 w. The organoleptic test was done by visual observation, including color, odor, and phase separation. The pH value of emulsion and liquid crystal emulsions was measured using a pH meter (Hanna) and viscosity by using NDG-8s viscometer with spindle number 2 and speed 6 rpm every four weeks for 12 w.

The microscopic test of EVOO emulsion and liquid crystal emulsion was done with a light microscope (Primo Star) using a polarizing filter and observed the presence of a liquid crystal phase. The globule size of EVOO emulsion and liquid crystal emulsions were determined using a Particle Size Analyzer (FRITSCH Analysette 22) and zeta potential determined using the HORIBA SZ-100 Zetasizer at room temperature.

Centrifugation test of emulsion and liquid crystal emulsions were done by using a centrifuge (HITACHI) at 3750 rpm for 5 h and observed for phase separation.

The effectivity of skin anti-aging was performed on 6 volunteers. The initial condition of the volunteer's skin was determined on face skin with various parameters, such as moisture, pore, spot (melanin), and wrinkles using Aram HUVIS ASW-100 skin analyzer. The changes in skin condition were determined every week for 4 w.

RESULTS

EVOO liquid crystal emulsions were formulated in 3 formulas the variation of alkyl polyglycosides and caprylic triglycerides. The composition of the formulas can be seen in table 1. All the EVOO liquid crystal emulsions were formed milky-white in color and were not transparent.

Table 1: Composition of EVOO liquid crystal emulsions

Materials	Quantity of 100 ml (%)		
	F1	F2	F3
Alkyl polyglycoside	3	4	5
Stearyl alcohol	2	2	2
Caprylic triglyceride	5	4	3
Extra virgin olive oil	5	5	5
Dimethicone	5	5	5
Glycerine	5	5	5
Methylparaben	0.2	0.2	0.2
Distilled water	Up to100 ml	Up to 100 ml	Up to 100 ml

The composition of EVOO emulsion preparation shows in table 2. The emulsion was formed milky-white in color and was not transparent.

Table 2: Composition of EVOO emulsion

Materials	Quantity of 100 ml (%)
Extra Virgin Olive Oil	5
Tween 80	1.26
Span 80	3.73
Methylparaben	0.1
Propylene glycol	10
Glycerine	15
Sodium CMC	1
Distilled water	Up to 100 ml

The evaluation result of the physical stability of EVOO emulsion and liquid crystal emulsions after 12 w storage at room temperature shows in fig. 1. The liquid crystal emulsions were stable. There is no change in color, odor, and no phase separation. The emulsion was not stable; there was a phase separation during 12 w storage (within week 10) at room temperature.

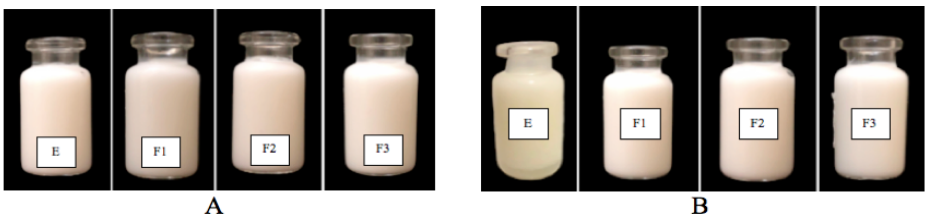

Fig. 1:Appearance of EVOO emulsion (E) and liquid crystal emulsions (F1, F2, F3) (A: Before storage. B: After storage for 12 w at room temperature)

The pH value and viscosity from EVOO emulsion and liquid crystal emulsion were decreased during 12 w storage at room temperature (table 3).

The microscopic results of EVOO emulsion and liquid crystal emulsion can is seen in fig. 2. The micrograph indicated the presence of the shining halos in F1, F2, and F3, while there is no shining halos presence in an emulsion.

EVOO liquid crystal (F2) has the smallest particle size (5.727 µm) and highest zeta potential value (-34.5 mV) among all of the liquid crystal emulsion after 12 w storage. In an emulsion, the particle size was

13.97 µm, and zeta potential was -40.07 mV after 12 w storage. The result of the particle size and zeta potential can be seen in table 4.

Table 3: pH of EVOO emulsion and liquid crystal emulsions

Formula	Time (week)	pH ± SD	Viscosity (± SD)(cP)
Emulsion	0	7.00 ± 0.00	14000±5.77
	4	6.60 ± 0.00	8500±0.58
	8	6.20 ± 0.06	5000±69.28
	12	5.80 ± 0.00	4250±86.61
F1	0	7.30 ± 0.10	6300±28.89
	4	7.00 ± 0.00	5500±28.89
	8	6.90 ± 0.06	4600±57.74
	12	6.60 ± 0.06	4100±86.61
F2	0	7.30 ± 0.00	4995±0.00
	4	7.10 ± 0.00	4995±0.00
	8	7.00 ± 0.06	4995±0.00
	12	6.90 ± 0.06	4995±0.00
F3	0	7.30 ± 0.06	6350±28.87
	4	7.10 ± 0.06	5675±69.28
	8	6.90 ± 0.00	5000±0.58
	12	6.70 ± 0.00	4250±86.60

Note: Data given in mean±SD

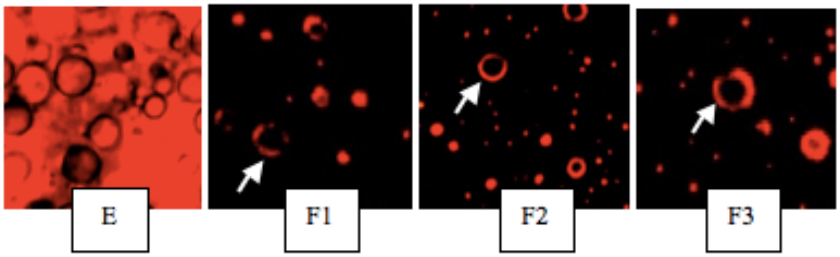

Fig. 2: Micrograph results of EVOO emulsion (E) and liquid crystal emulsions (F1, F2, F3)

Table 4: Particle size and zeta potential of EVOO emulsion and liquid crystal emulsions

Formula	Time (week)	Particle size (µm)	Zeta potential (mV)
Emulsion	0	9.788	-14.0
	12	13.97	-40.7
F1	0	4.011	-28.0
	12	7.024	-31.0
F2	0	4.593	-33.9
	12	5.727	-34.5
F3	0	4.915	-31.5
	12	7.103	-31.9

The results of centrifugation showed the EVOO liquid crystal emulsions were stable; there is no phase separation. In the EVOO emulsion, there is phase separation, that is indicated that the emulsion not stable (fig. 3).

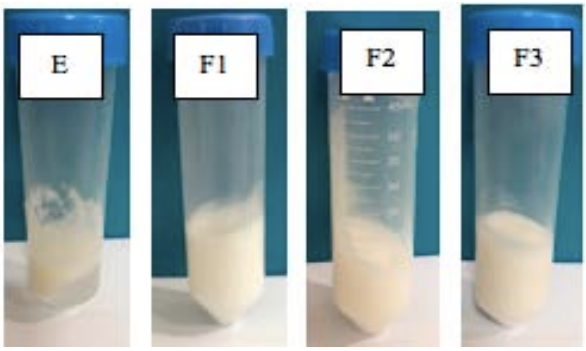

Fig. 3: Centrifugation result of EVOO emulsion (E) and liquid crystal emulsions (F1, F2, F3)

The effectivity study of anti-aging skin was carried out using Aram HUVIS ASW-100 skin analyzer. The parameters of the test include measurement of moisture content, pore size, number of the spot (melanin), and wrinkles. The F2 formula of EVOO liquid crystal emulsion was selected to be measured for skin anti-aging activity based on the most stable of viscosity, particle size, and highest zeta potential. The skin anti-aging measurement results can be seen in table 5 and table 6.

Table 5: Moisture content results on volunteers

Formulas	Average of moisture content (%)				
	Week				
	0	1	2	3	4
Emulsion	21.5±1.2	26.7±1.9	30.7±4.1	35.5±4.8	41.8±3.9
Liquid Crystal Emulsion (F2)	21.2±1.7	30.7±4.3	38.8±3.9	48.8±3.7	56.7±3.0

Note: Data given in mean±SD, Dry/low<36, Dry/Normal 37-39, Normal/Normal>39-54, Hydration>55

Table 6: Pore, spot, and wrinkle results on volunteers

Formulas	Time (Week)	Average of score		
		Pore	Spot	Wrinkle
Emulsion	0	44.8±4.4***	55.8±3.9**	63.7±2.3**
	1	41.7±4.8***	45.2±3.3***	53.3±4.1***
	2	36.7±2.3****	41.5±4.1***	44.2±7.5***
	3	32±4.3****	35.7±3.5****	34.0±5.7****
	4	30±3.2****	34.7±2.4****	25±3.8****
Liquid Crystal Emulsion (F2)	0	45±3.5***	58.5±3.5**	64.7±4.1**
	1	37±3.9****	46.7±4.7***	51.7±2.2***
	2	30.1±4.5****	36.7±3.1****	36.2±6.6****
	3	22.3±2.1****	29.2±2.9****	25.8±4.3****
	4	15.7±2.8*****	22.2±2.5****	14.8±1.8*****

Note: Data given in mean±SD, **Intensive care (>60), ***Need care (41-60), ****Good (21-40), *****Excellent (<20)

DISCUSSION

The preparation of Extra Virgin Olive Oil (EVOO) liquid crystal emulsions was using the phase inversion method. Phase inversion is a phenomenon in which the dispersed phase becomes a continuous phase that causing spontaneous arrangement changes in the oil-water interface [12]. Alkyl polyglycosides and caprylic triglyceride which are non-ionic surfactants that have an important role in the formation of these liquid crystal emulsions. These non-ionic surfactants are very temperature-dependent. The liquid crystal phase can be achieved by changing temperature in the system, forcing the transition from the oil-in-water emulsion at low temperatures to the water-in-oil emulsion at high temperatures. As the temperature increases, the ability of hydrophilic decreases, and hydrophobicity become increases. After cooling, the system is formed, then decreases the surface tension and forms a liquid crystal system [13, 14].

All the EVOO liquid crystal emulsion did not show the changes in color, odor, and no phase separation at room temperature for 12 w storage. This is because the surfactants that used in the EVOO liquid crystal emulsion (alkyl polyglycosides and caprylic triglycerides) are forming a lamellar liquid crystal structure. The structure of the lamellar liquid crystal is very dependent on the structure of the alkyl polyglycoside [4, 15]. The liquid crystal phase has some characteristics like solid and more organized like liquid, characterized by the ability to flow [16], it made the emulsions to be stable.

The micrographs result of the EVOO liquid crystal indicated the presence of the shining halos. These halos were only visible when viewed on a microscope with polarized light [17]. In emulsion micrograph results, there was only the showed droplet of the emulsion. The formation of liquid crystal structures is very dependent on the regular arrangement of surfactant molecules and oils at dynamic equilibrium with thermal motion. The thermal movement between the surfactant and the oil can reduce the arrangement of liquid crystals arranged during storage, thus leading to a gradual decomposition [11].

The pH value of all formulas of EVOO liquid crystal emulsion and emulsion stored at room temperature for 12 w showed a slight decrease in pH, but the pH of the preparation was still following skin pH, which was between 5.0-8.0, so it is safe to use and did not cause skin irritation [18].

EVOO liquid crystal emulsion and emulsion had micromolecular size. The particle size of EVOO liquid crystal emulsion and emulsion was increased during 12 w of storage. EVOO liquid crystal emulsions (F2) had the smallest particle size and showed a slight increase during storage (4.593 µm to 5.727 µm). This is because the F2 formula has the same amount of alkyl polyglycosides and caprylic triglycerides in the formula, unlike other liquid crystal emulsion formulas. The emulsion and liquid crystal emulsion tend to coalesce, which can increase particle size [19]. The particle size that has a liquid crystal phase is usually less than 5 µm [20].

The viscosity results of EVOO emulsion and liquid crystal emulsions decrease during storage, except the liquid crystal emulsion F2. This is because liquid crystal emulsions maintain their shape and reduce coalescence and creaming, which increases the stability of the emulsion [19].

The result zeta potential of the liquid crystals emulsion was below-30 mV, except the emulsion was-14.0 mV. EVOO liquid crystal emulsions (F2) had the highest zeta potential (-34.5 mV) during storage. Zeta potential values (<-30 mV and>+30 mV) can stabilize the preparation sterically by the presence of non-ionic surfactants in liquid crystal emulsions by inhibiting droplets to coalesce and increasing electrostatic repulsion between liquid crystal emulsion droplets and surfaces [21, 10], this causes the liquid crystal emulsions to be stable. Whereas after storage, the zeta potential value of all the liquid crystal decreased and still bellow-30 mV. In the emulsion, the zeta potential was-40.7 mV after storage for 12 w. The zeta potential value increased in line (more negative) with a decrease in the viscosity value of the preparation [22].

The results of the centrifugation test showed that there is no phase separation in F1, F2, and F3 liquid crystal emulsions. This means that the EVOO liquid crystal was stable against the gravitational force for 1 y [23]. The emulsion was not stable; there was a phase separation after centrifugation.

Based on the results of the effectivity of skin anti-aging, it showed that the EVOO liquid crystal emulsion could increase water content

so that the skin became hydration, reduces pore size, spots, and skin wrinkles. This is because oil, in this research, was extra virgin olive oil can act as a protective bridge on the skin by providing occlusive properties that can prevent loss of water so that the skin became hydration [24]. The component of extra virgin olive oil, such as (linoleic and linolenic content, α-tocopherol, and squalene) is a natural antioxidant that can prevent free radicals that can make the skin become moist and protecting skin [6-8]. According to Iwai, *et al.* [25], water is quickly evaporated because there is a continuous phase in the liquid crystal emulsion released under dry skin conditions after application, which can increase the water content that enters the skin. The liquid crystal phase in a preparation that resembles the structure of the lamellar stratum corneum that works by stimulating the natural regeneration function of the skin in the intercellular lipid structure and causing rapid replacement and stimulation of skin formation that prevents skin aging [26].

CONCLUSION

Extra Virgin Olive Oil (EVOO) can be formulated into liquid crystal emulsions using surfactants, such as alkyl polyglycosides and caprylic triglyceride. The EVOO liquid crystals are stable during 12 w storage at room temperature and centrifugation test. The best formula is F2 formula with 4% alkyl polyglycosides and 4% caprylate triglycerides, based on evaluation performed and effective in preventing skin aging.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

All authors declare that there is no conflict of interest among the authors.

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