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THE EFFECT OF ANTIOXIDANT OF GRAPESEED OIL AS SKIN ANTI-AGING IN NANOEMULSION AND EMULSION PREPARATIONS

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ABSTRACT

Grapeseed oil is rich in antioxidants and vitamin E which prevent the occurrence of skin aging. Nanoemulsion is an effective technology for delivering grapeseed oil as an active ingredient due to its small droplet size, and can easily penetrate through the skin layer. The objective of this study was to formulate grapeseed oil by variating its concentration as an active ingredient in nanoemulsion as an anti-aging product and to find out the stability of nanoemulsion during 8 weeks storage at different conditions. Nanoemulsion was made from three formulas using the variation of grapeseed oil concentration (2%; 4%; 6%), and 30% of tween 80 and 15% of PEG 400 as surfactant and co-surfactant. The stability evaluation was observed during 8 weeks of storage. The anti-aging activity was conducted to 18 volunteers. The result showed that all nanoemulsion formulas were stable during 8 weeks storage. Nanoemulsions were also stable during cycling test for 12 days. The particle size of nanoemulsion containing grapeseed oil 2%, 4%, and 6%, in the beginning, were 137.49 nm, 160.01 nm, and 163.82, respectively, and had increased after 8 weeks storage. The result of the anti-aging activity of nanoemulsion had higher effect compared to the emulsion in all parameters of skin condition. Grapeseed oil 2%, 4% and 6% can be formulated as nanoemulsion as an anti-aging product and nanoemulsion is the most stable preparations compared to emulsion during 8 weeks storage and nanoemulsion has better anti-aging activity compared to the emulsion.

Keywords: Grapeseed Oil, Antioxidant, Nanoemulsion, Anti-aging.

INTRODUCTION

Skin is one of the body organ located outside the human body. This organ will be in direct contact with the environment. It acts by protecting the body from damage or bad environmental impact. The skin has an important role in protecting the internal part of the body from direct contact with the external environment, physically or mechanically, chemically, sun (ultraviolet) and microbial[1].

Signs and symptoms of premature aging can be happened on all over human body organ, mainly on the skin[2]. This process including skin becomes dry and thin, fine wrinkles, and skin pigmentation (age spot) showed up. Otherwise, photo aging process is related to decreasing of collagen and elastin fibers due to excessive UV light exposure. This can cause skin damage because of the occurrence of a proteolytic enzyme from free radical that formed. Furthermore, this enzyme breaks the collagen and also connecting tissue under the dermis skin[3].

Anti-aging treatment will be done better as soon as possible when all of the cell function is still healthy and well-functioned. With the advances of technology and cosmetology sciences, reduction and prevention of aging can be done and skin will look younger[4].

Grapeseed oil contains beneficial antioxidant for skin care. Antioxidants contained in it are vitamin E and oligomeric proanthocianidins (OPC). OPC has a function to prevent free radical which damages the skin. It also repairs collagens damaged by free radical, so it prevents wrinkles[5]. Vitamin E in grapeseed oil is

also beneficial for skin, whereas vitamin E helps to moisturize the skin, improving skin elasticity, and reducing wrinkles[6].

Nanoemulsion is oil in water dispersion system which is stabilized by interfacial layer from surfactant molecule. The dispersion between two transparent insoluble fluid is stable thermodynamically with the usual particle size in the range of 10-500 nm, has extensive stability, easy to make, and the high solubility of this drug molecule made this technique as a promising system in drug delivery[7]. Nanoemulsion does not have creaming, sedimentation, and flocculation or coalescence compare to the macroemulsion[8]. This also potentially acts as a carrier in topical medicine because it optimizes the dispersion of active substance in skin layers[9].

Grapeseed oil is a yellowish colored and deodorized oil which high in linoleic acid (omega 6) in the range of 60% - 70%, 12% - 27% of oleic acid, 3% - 6% of stearic acid, 6% - 8% of palmitic acid, and also rich in antioxidant so it is well used in cosmetic formulation[10]. Grapeseed oil has a great content of vitamin E, ranging from 1 to 53 mg per 100 g of oil and 148 - 358 α-tocopherol equivalents which are higher than that of soybean oil and olive oil[11]. Based on the background stated above, the researcher was interested to do a study in comparing effectivity of nanoemulsion and emulsion containing antioxidant of grapeseed oil as an anti-aging product.

EXPERIMENTAL

Materials and Methods

Materials used in this study are grapeseed oil (Pietro Coricelli), tween 80, PEG 400, methyl paraben, propyl paraben, distilled water, acid pH buffer 4.01 (Hanna Instrument), neutral pH buffer 7.01 (Hanna Instrument), span 80, propylene glycol, CMC Na, and glycerin.

Preparation of Nanoemulsion

Nanoemulsion containing grapeseed oil was made using spontaneous emulsification method, and the formula was modified by variating the concentration of active ingredient which was grapeseed oil (Table-1). All materials were prepared and weighed. Methyl paraben and propyl paraben were then diluted into distilled water and heated on the hotplate until all were fully diluted. After that, tween 80 and PEG 400 were added into the mixture of methyl paraben and propyl paraben after the solution was cooled down. This was called as water phase. The water phase was stirred manually using a stirring bar and continued by using magnetic stirrer until it was homogeneous. Grapeseed oil as the oil phase was added into water phase by dripping it little by little using drop pipette, then the mixture was homogenized using magnetic stirrer for 6 hours until a clear and transparent nanoemulsion was formed. After that, nanoemulsion was sonicated for 30 minutes.

Table-1: Formula of Nanoemulsion Containing Grapeseed Oil

Materials	Formula		
	F1A	F2A	F3A
Grapeseed Oil (%)	2	4	6
Tween 80 (%)	30	30	30
PEG 400 (%)	15	15	15
Methly Paraben (%)	0.3	0.3	0.3
Propyl Paraben (%)	0.6	0.6	0.6
Distilled water ad	100	100	100

Notes:
F1A: Nanoemulsion containing 2% of Grapeseed oil
F2A: Nanoemulsion containing 4% of Grapeseed oil
F3A: Nanoemulsion containing 6% of Grapeseed oil

Preparation of Emulsion

The formula of the emulsion was modified by variating the concentration of active ingredient which was grapeseed oil (Table-2). All materials were prepared and weighed. Methyl paraben and propyl paraben were added into beaker glass containing distilled water. Those were then heated until all of the materials were diluted. The mixture was cooled down, and then added into porcelain cup containing tween 80,

propylene glycol, and glycerin. This was called as water phase. After that, grapeseed oil and span 80 were weighed inside another porcelain cup and then heated. This was called as oil phase.
CMC Na was poured into a hot mortar containing hot water and let it stored for approximately 20 minutes, then it was crushed until homogeneous. After that, the water phase was added into the mortar and crushed until homogen, and followed by the addition of oil phase little by little and crushed until the emulsion was formed.

Table-2: Formula of Emulsion Containing Grapeseed Oil

Materials	Formula		
	F1B	F2B	F3B
Grapeseed Oil (%)	2	4	6
Tween 80 (%)	1.26	1.26	1.26
Span 80 (%)	3.73	3.73	3.73
Methly Paraben (%)	0.1	0.1	0.1
Propyl Paraben (%)	0.02	0.02	0.02
Propylene glycol (%)	10	10	10
Glycerin (%)	13	13	13
CMC Na (%)	1	1	1
Distilled water ad	100	100	100

F1B: emulsion containing 2% of Grapeseed oil, F2B: emulsion containing 4% of Grapeseed oil, F3B: emulsion containing 6% of Grapeseed oil

Physical Stability Test
The physical stability test was done by storing the preparation in low temperature, room temperature, and high temperature for 8 weeks. Then observed organoleptic (color changes, odor, phase separation, clarity)[12], homogeneity test[13], pH measurement[14], viscosity, emulsion type test[15], centrifuge test[16], and particle size measurement.

Skin Irritation Test
This experiment was conducted on 18 volunteers to find out whether the preparations that were made can cause skin redness, itchy or swollen skin. Cosmetics were applied behind the ear, then left for 24 hours and the changes that occur in the form of skin redness, itchy or swollen skin were observed[17].

Anti-aging Activity Test
Evaluation of Anti-aging Activity was done to 18 female volunteers.The test was done on the face skin. Volunteers were divided into 6 groups. Firstly, initial skin condition was examined using skin analyzer (moisture, evenness, skin pore, spot, and wrinkle). Application of nanoemulsion and emulsion were done by applying nanoemulsion and emulsion into face evenly. Nanoemulsion and emulsion were applied two times (day and night) per day for 4 weeks. Skin condition was measured every week for 4 weeks using skin analyzer.

Data Analysis
Data result of the research is analyzed using SPSS (Statistical Product and Service Solution) 21. The distribution of data is firstly analyzed using Kruskal-Wallis Test to know the anti-aging effectivity of skin between all of the formula. After that, for knowing the effect of formulas towards skin condition for 4 weeks treatment, Mann-Whitney Test is used.

RESULTS AND DISCUSSION

Nanoemulsion Preparations
Nanoemulsion containing grapeseed oil was made in this research using modified standard formula[18]. The modified formula is made by variating the concentration of grapeseed oil in 1%, 2%, 3%, 4%, 5%, 6 %, and when it came to 7% and 8%, a clear and transparent nanoemulsion was not obtained. So in this

research, the concentrations of 2%, 4%, and 6% were used. The preparations obtained have a soft yellow color, clear and transparent solution, and had no odor.

Emulsion Preparations

The emulsion was made based on a formula from previous research[19], which was then modified by variating the concentrations of grapeseed oil into 2%, 4% and 6%. Samples obtained were having white color and had a specific odor.

Physical Stability

All of the formulations were evaluated starts from week 0, in order to compare the physical condition of nanoemulsion before and after through stability evaluation using physical parameters. Based on the evaluation, all of the formulas could be seen as stable preparations at low temperature, room temperature, and high temperature. Physical appearance did not show any changes and there were no phase separation and changes of preparation becoming cloudy occur. This showed that surfactant and co-surfactant concentrations were enough to make a stable nanoemulsion[20]. Meanwhile, emulsion showed some physical changes that mean emulsion was not stable[21].

Homogeneity Test

A certain amount of sample is applied on a piece of glass or other suitable transparent material must show a homogeneous composition and no visible coarse grain of the sample[13]. In both nanoemulsion and emulsion preparations showed none of the coarse grain, so it could be concluded that both preparations obtained were homogeneous.

pH Measurement

pH value of a topical preparation must be in a range of pH that is suitable for skin, which is 4.5-7.0. the pH of preparations can't be too acid because it can cause skin irritation and also can't be too base because it can lead to flaky skin[17]. Based on the result of pH measurement for 8 weeks at room temperature (Table-3) were changed. However, those pH changes were not changing significantly and still on the range of skin pH. This showed that the pH of these 4 formulations was relatively stable.

Table-3: pH Result of Nanoemulsion and Emulsion Containing Grapeseed Oil at Room Temperature for 8 Weeks

	Formula	Period (Weeks)								
		0	1	2	3	4	5	6	7	8
pH	F1A	6.8	6.8	6.7	6.6	6.5	6.4	6.3	6.2	6.2
	F2A	6.9	6.9	6.8	6.8	6.7	6.5	6.4	6.4	6.3
	F3A	6.7	6.7	6.7	6.6	6.5	6.5	6.4	6.4	6.4
	F1B	6.9	6.9	6.8	6.8	6.8	6.7	6.7	6.5	6.4
	F2B	6.8	6.8	6.7	6.7	6.7	6.6	6.6	6.5	6.5
	F3B	7.0	7.0	6.9	6.9	6.9	6.8	6.7	6.6	6.6

F1A: Nanoemulsion containing grapeseed oil 2%, F2A: Nanoemulsion containing grapeseed oil 4%, F3A: Nanoemulsion containing grapeseed oil 6%, F1B: Emulsion containing grapeseed oil 2%, F2B: Emulsion containing grapeseed oil 4%, F3B: Emulsion containing grapeseed oil 6%

Viscosity Test

Viscosity test for nanoemulsion in room temperature showed an increasing value on week 8 which showed that the formula became thicker as the length of storage time (Table-4). The viscosity of preparation was affected by several factors, there were factors of mixing process during the making of preparations, choosing thickening agent and surfactant, the proportion of dispersed phase and particle

size. The increasing of viscosity on nanoemulsions might happen because the room temperature was quite low. Viscosity was inversely proportional with temperature[15]. If the temperature was decreased then the viscosity will increase and preparations becoming thick, and vice versa. In the other hand, the viscosity of emulsion during 8 weeks storage was decreased. This showed that emulsion was not stable kinetically where the droplets can freely moving will collide each other and tend to fuse[16].

Table-4: Nanoemulsion and Emulsion Viscosity Test Result

	Formula	Period (Weeks)								
		0	1	2	3	4	5	6	7	8
Viscosity (cp)	F1A	125	125	155	155	175	175	225	250	250
	F2A	150	150	175	225	225	325	325	375	400
	F3A	210	210	275	275	325	350	425	500	550
	F1B	6650	6650	6500	6450	6350	6150	6050	5850	5750
	F2B	6750	6750	6650	6600	6400	6250	5950	5800	5800
	F3B	6800	6800	6700	6650	6450	6250	6000	5900	5850

F1A: Nanoemulsion containing grapeseed oil 2%, F2A: Nanoemulsion containing grapeseed oil 4%, F3A: Nanoemulsion containing grapeseed oil 6%, F1B: Emulsion containing grapeseed oil 2%, F2B: Emulsion containing grapeseed oil 4%, F3B: Emulsion containing grapeseed oil 6%

Emulsion Type Testing

Determining emulsion type of preparation was done by adding methylene blue into the sample little by little, if it was diluted during stirring process, means that was an oil in water (o/w) emulsion type[22]. Nanoemulsion and emulsion showed a homogeneous blue color of methylene blue which means they were oil in water (o/w) preparations. This can because of the most of compounds contained in the formula were hydrophilic or polar, so when there was a hydrophobic compound, the type of both nanoemulsion and emulsion were oil in water (o/w) type.

Centrifuge Test

Centrifuge test is intended to know the stability of nanoemulsion and emulsion preparations. Centrifuge process on the speed of 3800 rpm for 5 hours is equivalent with gravitation effect for approximately 1 year. Nanoemulsion and emulsion were centrifuged on 3800 rpm for 5 hours. After the test had been done, there were no changes in nanoemulsion (Fig.-1) which meant nanoemulsion was stable for 1 year. Meanwhile, the physical properties of emulsion were changed after the centrifuge process. Phase separation was showing on emulsion that meant it was not stable physically.

Particle Size Measurement

Based on particle size measurement using Particle Size Analyzer, the particle size of nanoemulsion containing grapeseed oil 2%, 4%, and 6%, in the beginning, were 137.49 nm, 160.01 nm, and 163.82 and had increased after 8 weeks storage (Table-5).

Table-5: Determination of Average Nanoemulsion Particle Size

No	Formula	Particle Size Distribution (nm)		
		Week 0	Week 4	Week 8
1	F1A	137.49	230.37	378.42
2	F2A	160.01	251.92	410.51
3	F3A	163.82	343.60	438.50

F1A: Nanoemulsion containing grapeseed oil 2%, F2A: Nanoemulsion containing grapeseed oil 4%, F3A: Nanoemulsion containing grapeseed oil 6%

PEG 400 used as co-surfactant in the concentration of 10-20% could obtain a clear and stable nanoemulsion (Fig-1) and a droplet size < 100 nm[23]. The result showed that not all of the samples were

having particle size < 100 nm before and after they were stored, but the particle size was still regarding particle size standardization of nanoemulsion that was 2-500 nm[24].
This theory was also convinced by research that nanoemulsion was oil in water emulsion which had a range of droplet size from 100-500 nm. Commonly the average droplet size was in the range of 100-500 nm[25].

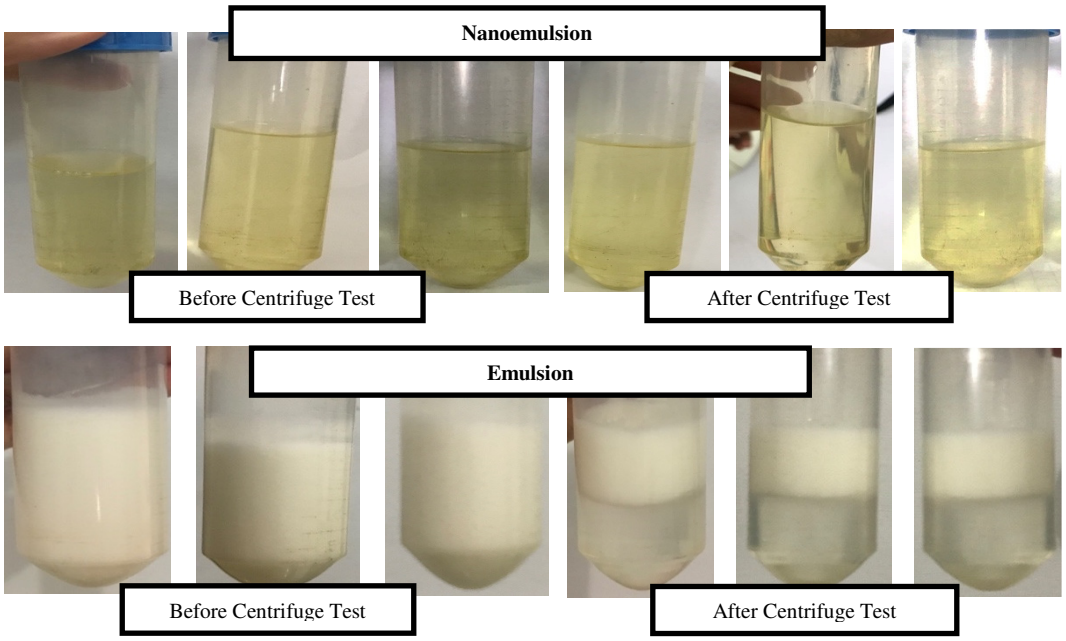

Fig.-1: Physical Evaluation of Nanoemulsion and Emulsion Containing Grapeseed Oil 2%; 4%; 6% after Centrifuge Test

Skin Irritation Test

Based on the result that was done to 18 volunteers by applying nanoemulsion and emulsion on the back of the ear, this showed a negative result on parameters of skin irritation. Those parameters are red, itchy, and swollen skin. This meant that both nanoemulsion and emulsion were safe to be used on human skin[17].

Anti-aging Activity Result

Moisture

Nanoemulsion containing 6% grapeseed oil (F3A) had the highest improvement from week 0 to week 4 compared to all formulations (Fig.-2). But if compared nanoemulsion and emulsion grouped in the same concentration, all of the nanoemulsion formulas always had a higher improvement compared to emulsion (F1A compared to F1B; F2A compared to F2B; F3A compared to F3B).

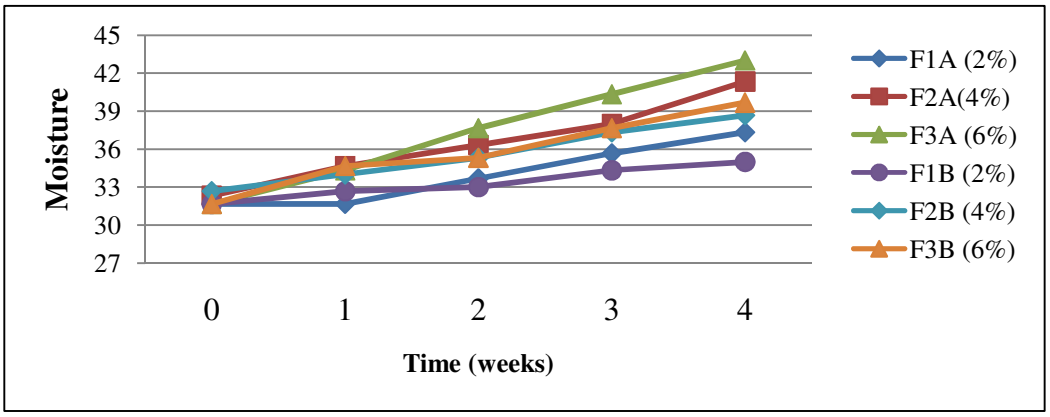

Fig.-2: Chart of Moisture Level Improvement Towards Volunteer's Skin

The moisture data were then analyzed using nonparametric Kruskal Wallis test to know the effectivity of each formula towards skin moisture. On the initial condition (Week 0), there was no significant improvement in moisture level ($p > 0.05$) but on the application in week 1, 2, 3, and 4 showed a significant improvement in skin moisture ($p < 0.05$). Next, a Mann-Whitney test was done to know the difference between nanoemulsion and emulsion grouped by the same concentration. Based on this test, application of sample on week 1, 2, and 3 did not have any significant difference ($p > 0.05$) while on the fourth week showed a significant difference ($p < 0.05$) between formula F1B emulsion and formula F1A nanoemulsion, as well as formula F2B emulsion and F2A nanoemulsion. Meanwhile, the application between formula F3B emulsion and F3A nanoemulsion showed a significant difference from the second week until the fourth week.

Evenness and Pore

F3A had the highest improvement from week 0 to week 4 compared to all formulations (Fig.-3 and Fig.-4). But if compared nanoemulsion and emulsion grouped in the same concentration, all of the nanoemulsion formulas had a higher improvement compared to emulsion (F1A compared to F1B; F2A compared to F2B; F3A compared to F3B). Dry and rough skin is a general sign of premature aging. When there is an overexposed skin to sunlight, collagen and elastin inside the skin layer will be damaged, causing an accumulation of dead skin, making skin looks rougher. Besides that, the skin will feel rough, dull, and scaly because of the ability of the skin to do cell regeneration is decreasing[17].

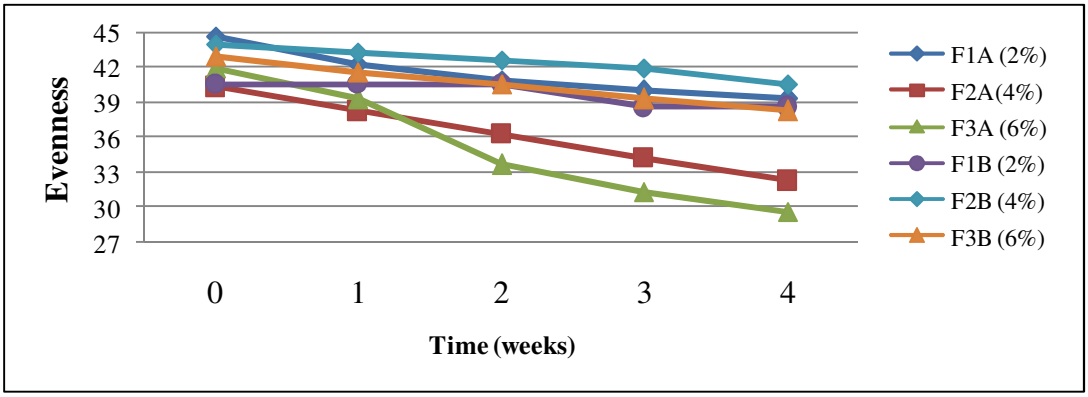

Fig.-3: Chart of Evenness Level Improvement Towards Volunteer's Skin

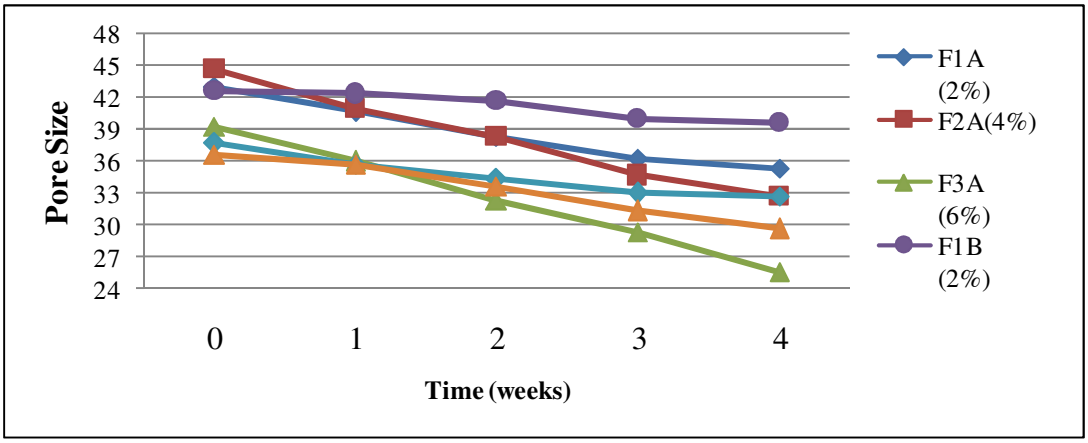

Fig.-4**:** Chart of Pore Size Improvement Towards Volunteer's Skin

The enlarged pore can be caused by too much sunlight exposure, increasing temperature causing damaged collagen at the same time which leads to decreasing of skin wall and pore enlargement[26,27]. Accumulation of dead skin cell will trigger the appearance of acne and also affecting pore size. Vitamin E in grapeseed

oil can release dead skin cell, stimulates cell regeneration then also catch free radicals, and smaller the skin pore size.

Spots

Hyperpigmentation is a condition in which melanin is synthesized excessively. This happens because of the amount of exposure to sunlight (UV light) so that melanocyte cells initiate melanin synthesis. The more sunlight exposure to the skin causes more active melanin formation and leads to the formation of dark brown stain spots on the skin[28]. F3A had the highest improvement from week 0 to week 4 compared to all formulations (Fig.-5). But if compared nanoemulsion and emulsion grouped in the same concentration, all of the nanoemulsion formula had a higher improvement compared to emulsion (F1A compared to F1B; F2A compared to F2B; F3A compared to F3B).

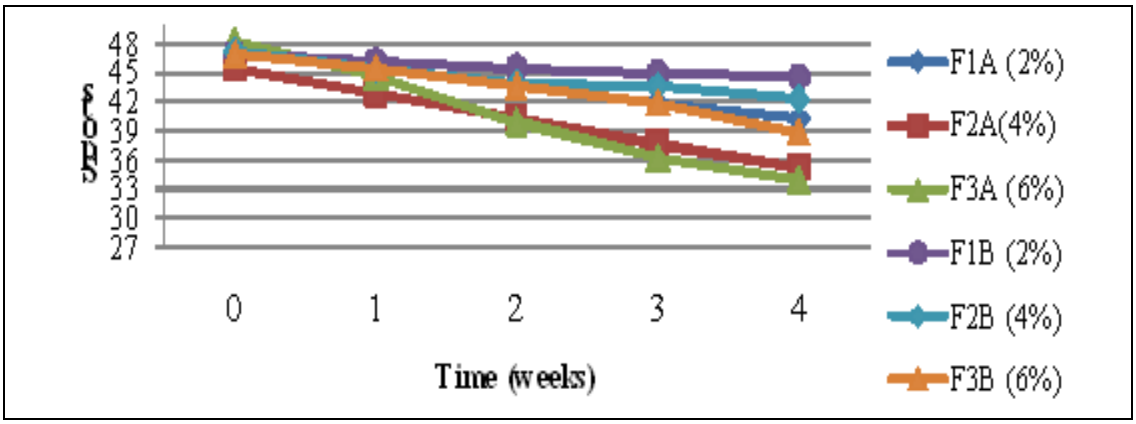

Fig.-5**:** Chart of Spots Improvement Towards Volunteer's Skin

Antioxidant activity of vitamin E from grapeseed oil prevents skin irritation and aging, which vitamin E inhibits tyrosinase in vitro and melanogenesis in epidermal melanocytes. The properties of vitamin E that interfere with the peroxidation of melanocyte lipid membranes can increase intracellular glutathione. According to *in vivo* studies, vitamin E inhibits melanogenesis of normal human melanocytes in culture media, although it does not affect the synthesis of melanin in the enzyme solution as a homogeneous cell. In addition, vitamin E stimulates the synthesis of intracellular glutathione (GSH) that can provide a depigmentation effect[29].

Wrinkles

The formation of wrinkles is caused by various internal and external factors. Sunlight, especially UV light A is known to be one of the biggest causes and contributors in the process of wrinkle formation, but there are also other causes such as environmental stresses on the skin including dryness, physical stress, and exposure to chemicals. The occurrence of wrinkles is thought to result from decreased skin strength and elasticity caused by reduced moisture content of the stratum corneum, thickening of the stratum corneum, epidermal atrophy, changes in the amount and quality of dermal collagen, elastin fibers, and collagen elasticity, as well as changes in the three dimensional structure of the dermis and other changes resulting from external and internal factors[30]. This chart below (Fig.-6) showed that F3A (nanoemulsion containing 6% grapeseed oil) had the highest improvement from week 0 to week 4 compared to all formulations. But if compared nanoemulsion and emulsion grouped in the same concentration, all of the nanoemulsion formulas had a higher improvement compared to emulsion (F1A compared to F1B; F2A compared to F2B; F3A compared to F3B).

Nanoemulsions have recently become increasingly important as potential vehicles for the controlled delivery of cosmetics and for the optimized dispersion of active ingredients in particular skin layers. Due to their lipophilic interior, nanoemulsions are more suitable for the transport of lipophilic compounds than

liposomes. Similar to liposomes, they support the skin penetration of active ingredients and thus increase their concentration in the skin. Another advantage is the small-sized droplet with its high surface area allowing effective transport of the active to the skin. Furthermore, nanoemulsions gain increasing interest due to their own bioactive effects. This may reduce trans-epidermal water loss (TEWL), indicating that the barrier function of the skin is strengthened. Nanoemulsions are acceptable in cosmetics because there are no inherent creaming, sedimentation, flocculation, or coalescence that are observed with macroemulsions. The incorporation of potentially irritating surfactants can often be avoided by using high energy equipment during manufacturing[31].

Nanoemulsion technology is also an effective method for delivering grapeseed oil as an active ingredient due to its small droplet size, nanoemulsion can easily penetrate through the skin layer and can increase penetration of active ingredients, resulting in grapeseed oil activity in reducing fine lines become more effective.

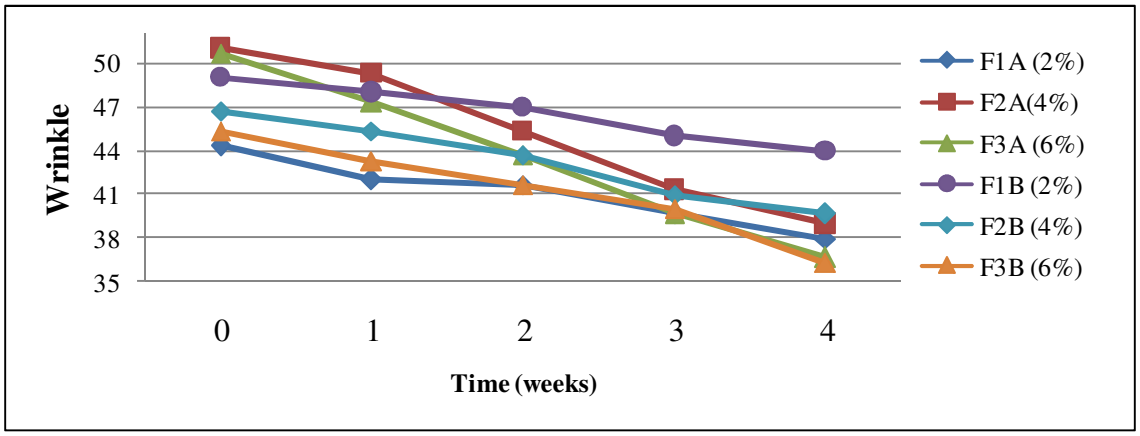

Fig.-6: Chart of Wrinkle Improvement Towards Volunteer's Skin

CONCLUSION

Grapeseed oil can be formulated as nanoemulsion and emulsion by variating the concentration of grapeseed oil into F1 (2%), F2 (4%), and F3 (6%). All of the formula of nanoemulsion showed a clear, soft yellow and stable form during 8 weeks storage process in three conditions (low, room, and high temperatures). Nanoemulsion was also stable during the cycling test process for 12 days. In the other hand, emulsion preparation was not stable during the physical evaluation for 8 weeks. Nanoemulsion containing 2%, 4% and 6% of grapeseed had the highest anti-aging effectivity compared to an emulsion containing grapeseed oil 2%, 4% and 6%, by the improvement of each skin aging parameters that was moisture level, evenness, pore size, amount of spots and wrinkles.

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