Characterization of nanoemulsion gotukola, mangosteen rind, cucumber and tomato extract for cosmetic raw material

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Abstract. Aging is related to defacement caused by free radical and the most common signs are fine lines and wrinkles. Wrinkles occur in skin layer called dermis-contained collagen that is responsible in preventing wrinkles. This physiological change on skin made an individual look old, and this is a troubling issue especially for women. Natural substances have an excellent anti-aging remedy to improve skin defec tion caused by free radical and maintain skin rejuvenation. It’s already proved that gotukola, mangosteen rind, cucumber and tomato extract has antioxidant activity. In this study, these extracts were formulated in nanoemulsion which was prepared in anti-aging topical preparation. This nanoemulsion system was expected to accelerate active agent absorption into skin tissue. It was also investigated the the emulsion stability system by pH, refractive index, viscosity and particle size parameter. It was shown that the anti-aging topical preparation with a composition of 2% Gotukola extract, 2% mangosteen rind extract, 2% tomato extract and 4% cucumber extract was stable for 2 months of observation with average pH 6.16 ± 0.33; 6.11 ± 0.351, refractive index 1.38817 ± 0.00863; 1.37 ± 0.00453, viscosity 3148 cP ± 1068; 1638 cP ± 1294 and particle size 1.14 µm ± 0.84; 1.64 µm ± 0.99 in 5 °C and ambient temperature respectively. Overall, the emulsion system was relatively stable in both storage temperature 5 °C and ambient temperature. It was also proven to be safe for human application.

Keywords: cosmetic, anti-aging, antioxidant, nanoemulsion, stability test

1. Introduction
Cosmeceutical is a combination between cosmetic and pharmaceutical product, such as anti-acne, sun screen and anti-aging [1]. Aging is related to defacement caused by free radical on cell component and tissue [2]. The common sign of aging is fine line and wrinkle. Wrinkle appears on skin layer called dermis where collagen contained in this layer, which responsible to prevent wrinkle. In time, collagen will be damaged [3]. It decreases skin moisture and firm looks which leads to wrinkle forming [4].

Gotukola is a herb which has a potency as natural antioxidant that can be used to overcome skin problem and heal wound [5]. Mangosteen rind is a by-product which contained lots of water dissolved antioxidant [6]. It contains varies types of xantons including alfa mangostin which has been proven has strong antioxidant activity [7]. Lycopene is carotenoid group, which is contained in tomato. That active agent is the most potential antioxidant among all the carotenoids groups [8]. Cucumber extract is rich in vitamin C and A [9,10]. In the cosmetic field, cucumber extract has a big potency as coolant, healer and skin irrigation caused by sun light [10]. Furthermore, cucumber extract is often used to overcome skin problem such as wrinkle and sunburn and also as antioxidant [10,11]. There are so many herbal extracts, which are used as the anti-aging active agent. Previous study used Moringa oleifera seed oil which is rich of antioxidant [12], Punica granatum extract which is also rich in...
antioxidant [13] and mangosteen rind extract as anti-aging cream [14]. In this study, besides mangosteen rind extract, it was also used three types herbal extract, gotukola, cucumber and tomato to enhance the antioxidant effect. The combinations of these four natural resources was formulated as a new anti-aging formulation, which could repair skin damage caused by free radicals, and maintain skin elasticity.

Those natural products were formulated into a nanoemulsion system in order to enhance active agent absorption into skin tissue. Emulsion is categorized in nano scale if the droplet size ranged between 10-1000 nm [15]. The small size of the emulsion could restrain physical separation caused by gravitational force or coagulation, and also prevent creaming phenomena because it has sufficient brownian force to overcome gravitational separation [16,17]. Anti-aging skincare was made in topical preparation, which was applied, to human skin in cream shape.

Stability is very important factor in emulsion because as time goes by creaming, flocculation, Ostwald ripening, coalescence and phase inversion is possible to occur [18]. Thus, this study aimed to perform stability test at low temperature (5 °C), ambient temperature, high temperature (42 °C) and also to evaluate viscosity, pH, refractive, particle size parameter of mangosteen rind, gotukola, tomato, and cucumber extract combination as potential cosmetic raw material for anti-aging.

### 2. Material and methods

#### 2.1. Nanoemulsion material

Extracts used in this study were a gotukola extract from Tawangmangu by PT. Java Plant, mangosteen rind, tomato and cucumber glycolic extract from PT. Haldin. Euxyl PE 9010 by Schulke, nanosilver, Tween 80, Span 80, Glycerin, Stearic Acid and Ethanolamine from Merck, Cetyl Alcohol and Propylene Glycol from PT. Bratachem, Vitamin Soluvit Richter by CLR and Deionized water.

#### 2.2. Nanoemulsion formulation

In this study there were four formulations, which were compared in all parameter (table 1). The formulation contained two phases. Water and propylene glycol were water phase and the rest were oil phase. Water phase was mixed with magnetic stirrer at 50 °C and oil phase was mixed at 60 °C. The oil phase was added into water phase and then homogenized using Ultra Turrax homogenizer, 15000 rpm for 10 minutes.

#### 2.3. Base cream formulation

Base cream formulation consisted of oil phase and water phase. Oil phase compositions were tween 20 for synthesis Merck 7 %, stearic acid Merck 2.8 %, glycerine Merck 2.1 %, cetyl alcohol PT. Bratachem 2.1 % and avocado oil PT. Bratachem 3.5 %. Oil phase was stirred using magnetic stirrer at temperature 60 °C until homogenous.

| Material                              | F1  | F2  | F3  | F4  |
|---------------------------------------|-----|-----|-----|-----|
| Gotukola extract (in %)               | 4   | 2   | 2   | 2   |
| Mangosteen rind extract (in %)        | 2   | 4   | 2   | 2   |
| Tomato extract (in %)                 | 2   | 2   | 4   | 2   |
| Cucumber extract (in %)               | 2   | 2   | 2   | 4   |
| Euxyl (in %)                          | 1   | 1   | 1   | 1   |
| Nanosilver (in %)                     | 0.05| 0.05| 0.05| 0.05|
| Tween 80 (in %)                       | 3   | 3   | 3   | 3   |
| Span 80 (in %)                        | 3   | 3   | 3   | 3   |
| Glycerin (in %)                       | 4   | 4   | 4   | 4   |
| Stearic acid (in %)                   | 2   | 2   | 2   | 2   |
| Cetyl Alcohol (in %)                  | 7   | 7   | 7   | 7   |
| Avocado oil (in %)                    | 2   | 2   | 2   | 2   |
| Vitamin (in %)                        | 5   | 5   | 5   | 5   |
| Ethanolamine (in %)                   | 0.05| 0.05| 0.05| 0.05|
| Water (in %)                          | 52.9| 52.9| 52.9| 52.9|
| Propylene Glycol (in %)               | 10  | 10  | 10  | 10  |
Table 2. Anti-aging cream pH measurement

| Formula | Temperature | Day 7 | Day 14 | Day 21 | Day 30 | Day 60 |
|---------|-------------|-------|--------|--------|--------|--------|
| F1-BC   | 5°C         | 5.44  | 5.89   | 5.82   | 5.66   | 5.57   |
|         | Ambient     | 5.3   | 5.72   | 5.71   | 5.6    | 5.82   |
|         | 40°C        | 5.5   | 5.64   | 5.53   | 4.59   | 5.39   |
|         | 5°C         | 5.58  | 6.07   | 5.77   | 6.03   | 5.85   |
| F2-BC   | Ambient     | 5.61  | 5.97   | 5.73   | 6.06   | 5.71   |
|         | 40°C        | 5.49  | 5.78   | 5.68   | 5.08   | 5.33   |
|         | 5°C         | 5.63  | 5.81   | 5.75   | 6.12   | 5.71   |
| F3-BC   | Ambient     | 5.55  | 6.03   | 5.74   | 6.06   | 6.05   |
|         | 40°C        | 5.28  | 5.86   | 5.61   | 4.93   | 5.55   |
|         | 5°C         | 5.94  | 5.88   | 5.87   | 6.27   | 6.3    |
| F4-BC   | Ambient     | 5.97  | 5.98   | 5.77   | 6.28   | 5.91   |
|         | 40°C        | 5.64  | 5.72   | 5.61   | 4.96   | 5.35   |
|         | 5°C         | 5.67  | 5.88   | 5.75   | 5.87   | 5.56   |
| BC      | Ambient     | 5.68  | 5.84   | 5.64   | 5.76   | 5.59   |
|         | 40°C        | 5.6   | 5.81   | 4.66   | 3.56   | 4.13   |

Water phase compositions were Propylene glycol PT. Bratachem 10.5 % and deionized water 70.6 %. These two materials were stirred using magnetic stirrer at temperature 40 °C. Oil phase was slowly added into the water phase while stirred using mechanical stirrer, with 500 rpm of rotation speed for 15 minutes.

2.4. Anti-aging cream formulation

Nanoemulsion formulation was mixed with base cream with a concentration of 10 %wt. Nanoemulsion was added into base cream while stirred using mechanical stirrer with 500 rpm of rotation speed for 15 minutes.

2.5. Characterization

Four formulations of anti-aging cream was stored at temperature 5 °C, ambient and 40 °C. The parameter characterized were pH using pH Meter Eutech 700 Series, refractive index using Refractometer Atago RX-5000α, particle size using Particle Size Analyzer Horiba Nano Partica S-100 and viscosity using Brookfield DV-E Viscometer, spindle 05 with stirring speed 50 rpm. pH measurement was done by diluting 5 mg sample into 5 ml deionization water and then stirred vigorously. pH was measured by immersing the electrode into the sample. Refractive index measurement was performed by picking up 5 mg of samples and putting it on refractometer prism. Particle size analysis was done by dispersing 1 mg of sample into 3 ml of deionization water.

3. Results and discussion

3.1. Anti-aging cream pH measurement

pH measurement was aimed to test the stability of emulsion’s acidity. This characterization was done to anti-aging cream formula 1-4 and base cream. According to Blaak et al. [19] elder human skin pH is approximately 6. Therefore, pH of skincare product that is suitable for anti-aging treatment was 4 to neutralized natural skin pH. The result is shown in table 2.

In this observation the pH range for anti-aging cream F1 was 4.59–5.89, F2 was 5.49–6.07, F3 was 4.93–6.06, F4 was 5.35–6.3 and base cream was 3.56–5.88. The emulsions were stored at different temperature condition at 5 °C, ambient and 40 °C. At 5 °C temperature and ambient pH remained at value range 5–6. At 40 °C temperature, the pH decreased in one month observation and then increased on day 60. Elevated temperature could quicken chemical reaction which could change the activity and the properties of the components [20]. The high temperature caused destabilization by hydrolysis which contributed to pH decrease [21]. Increasing pH in day 60 may occur because of oxidation reaction where the rate of oxidation had been found in line with the increase of pH [22]. Emulsion in this work had average pH value 5.64 ± 0.44 which was still in allowed pH value. Measurement result
for 2 months was still in the range of pH that was suitable for skin care product, which commonly was 4.5–6 [23]. Therefore, the product resulted in this experiment is still safe for human skin and also acid pH range optimize preservatives activity in inhibiting microorganism growth [24]. Although the pH was acceptable, yet the pH was slightly above the anti-aging treatment skincare pH. However, the pH could be adjusted into desirable value by using citric acid-sodium citrate buffers to adjust acidic pH and sodium phosphate buffers to adjust neutral pH [25].

3.2. Anti-aging cream refractive index measurement

Refractive index measurement aimed to test the stability of physical and color appearance for all anti-aging formulation and base cream at temperature 5 °C, ambient and 40 °C. The measurements were done in dark-condition using refractometer instrument. The color of base cream was milky white and for anti-aging cream was light brown.

In this observation the range of refractive index for anti-aging F1 was 1.36611-1.41164, F2 was 1.36514-1.40408, F3 was 1.36823-1.41535, F4 was 1.36588-1.42616 and base cream was 1.3662-1.43816 (table 3). The refractive index could give information about light behavior. When the light passed different substances, the velocity decrease so that the refractive index increase [26]. This phenomenon was seen at temperature 40 °C. The refractive index in day 30 and day 60 increased significantly as it indicated the change of the chemical properties of the substance. This was supported by the change of pH in the previous discussion. The other samples at temperature 5 °C and ambient relatively perform stable refractive index value. Therefore, it could be said that samples in this temperature condition were chemically stable and homogenous based on its physical appearance through refractive index parameter [13].

3.3. Anti-aging cream viscosity

Rheology was one of the important factors in the topical product. It was because the topical product should flow easily from its packaging when the user applies force and spreads on the skin. It should also have enough viscosity to remain on the skin after the application. The rheological properties were time-dependent due to some factors such as excipient interaction, crosslinking and polymer thermodynamic mobility [27].

### Table 3. Anti-aging cream refractive index measurement.

| Formula | Temperature | Day |
|---------|-------------|-----|
|         |             | 7   | 14  | 21  | 30  | 60  |
| F1-BC   | Ambient     | 1.37531 | 1.38594 | 1.3813 | 1.38376 | 1.38928 |
|         | 40°C        | 1.38956 | 1.37441 | 1.37549 | 1.39448 | 1.41164 |
|         | 5°C         | 1.37541 | 1.37669 | 1.37977 | 1.38663 | 1.38736 |
| F2-BC   | Ambient     | 1.37704 | 1.36514 | 1.37012 | 1.36556 | 1.36629 |
|         | 40°C        | 1.37706 | 1.37981 | 1.37392 | 1.39427 | 1.40408 |
|         | 5°C         | 1.37492 | 1.38607 | 1.38727 | 1.38605 | 1.39538 |
| F3-BC   | Ambient     | 1.37553 | 1.36705 | 1.37248 | 1.37176 | 1.36823 |
|         | 40°C        | 1.37869 | 1.38486 | 1.37802 | 1.40258 | 1.41535 |
|         | 5°C         | 1.3766  | 1.38177 | 1.38471 | 1.39581 | 1.39907 |
| F4-BC   | Ambient     | 1.37801 | 1.36735 | 1.36709 | 1.36588 | 1.36935 |
|         | 40°C        | 1.38414 | 1.38476 | 1.39287 | 1.40667 | 1.42616 |
|         | 5°C         | 1.37801 | 1.38567 | 1.38987 | 1.39243 | 1.39297 |
| BC      | Ambient     | 1.37157 | 1.3762  | 1.3662  | 1.3673  | 1.36778 |
|         | 40°C        | 1.37624 | 1.37503 | 1.37927 | 1.41646 | 1.43816 |

Table 3. Anti-aging cream refractive index measurement.
Table 4. Anti-aging viscosity measurement (cP)

| Formula | Temperature | Day     |
|---------|-------------|---------|
|         |             | 7       | 14    | 21    | 30    | 60    |
| F1-BC   | 5 °C        | 2370    | 3200  | 2500  | 2820  | 2690  |
|         | Ambient     | 2180    | 770   | 1020  | 580   | 580   |
|         | 40 °C       | 14270   | 2750  | 1920  | -     | -     |
| F2-BC   | 5 °C        | 2820    | 3260  | 3140  | 2820  | 2690  |
|         | Ambient     | 13500   | 1470  | 1150  | 770   | 640   |
|         | 40 °C       | 16000   | 5060  | 3200  | -     | -     |
| F3-BC   | 5 °C        | 4030    | 2880  | 3200  | 2820  | 2690  |
|         | Ambient     | 9920    | 580   | 1150  | 450   | 450   |
|         | 40 °C       | 17280   | 6020  | 3710  | -     | -     |
| F4-BC   | 5 °C        | 14270   | 2750  | 1920  | -     | -     |
|         | Ambient     | 3900    | 1540  | 1020  | 900   | 830   |
|         | 40 °C       | 16000   | 5060  | 3200  | -     | -     |

As seen in table 4, for all formulation there was a tendency the viscosity decrease from time to time. This was corellated with the increasing particle size due to Ostwald ripening phenomena. As the droplets size became smaller, the surface area is increasing [28]. So, if the droplets size increases, it will affect the viscosity to become lower. The similar result was also shown in the previous study by Juntarasakul et al. [29]. The viscosity of emulsion decreases at higher temperature, because the
molecules got higher energy from heat which made the emulsion less viscous [29]. High temperature also increased the volume fraction of the dispersed phase by wax precipitation [30]. This phenomenon also occurred in this study where the wax was precipitate on the top part and water on the bottom part. This condition happened in day 30 and 60 so that the viscosity measurement couldn’t be performed.

3.4. Anti-aging cream particle size analysis
Observation of anti-aging cream of four formulas and base cream is shown in Table 5. Particle size result was ranged from nano until micro sized emulsion. Microemulsion could be visually noticed by the white colored physical appearance. This happened because the micro particle had strong light scattering which gave white colored visual appearance [31].

The initial particle size of F1, F2 and F3 were seen on the table, in micrometer size. Different extract concentration required different composition and types of surfactant to meet smaller particle size and more stable emulsion. It was known that surfactant had an important role to the stabilized emulsion and also the combination of two or more surfactant was required to reduce surface tension, reduce particle size and narrow the size distribution [32]. Therefore, it could be concluded that suitable surfactant combination and composition should be optimized to reach nanometer droplets. Initial particle size on F4 showed droplet size in nanometer range yet it changed through storage time and higher temperature. The difference of the particle size on each measurement caused by destabilization emulsion which involved flocculation, coalescence and separation [33]. The Ostwald ripening phenomenon also contributed to increasing droplet size which could lead to coalescence and creaming [34]. Particle properties, such as shape, was an important factor in particle size measurement. The particle size measurement was based on Dynamic Light Scattering principle where the measurement result was highly sensitive with the presence of big molecule due to flocculation and coalescence. This big molecule would dominate the light scattering and it would be detected as the result of the measurement [35]. The similar result was shown in the previous study by Ševčíková et al. [36] where the particle size increased due to storage time and temperature. This study proved that the stability of emulsion was affected by storage temperature [36].

4. Conclusions
The nanoemulsion formulation with the combination of gotukula, mangosteen rind, cucumber and tomato in 60 days observation was safe for human application. According to parameter pH, refractive index, viscosity and particle size, the most stable formulation was Formula 4. It needs further study to improve the stability in higher temperature. However, this topical preparation formulation had the potential to be cosmetics material for anti-aging treatment.

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