INTRODUCTION

The skin covers the entire surface of the body and is in contact with the environment. White skin has come to be regarded as a beautiful concept associated with a healthy skin and youthful appearance [1]. Thus, it becomes the dream of most Asian women. If the skin is exposed to sunlight for a lengthy period, it becomes darker and black spots appear, due to melanin hyperpigmentation. In human skin, melanogenesis is catalyzed by tyrosinase, and several tyrosinase inhibitors have been used as skin-whitening agents in the cosmetics industry; the use of ion or tyrosinase inhibitor molecules, such as arbutin, kojic acid, mercury, and hydroquinone, will inhibit melanogenesis [2].

Alpha (α) arbutin is a whitening agent that inhibits the action of tyrosinase in melanogenesis, and the o-glucoside bond in α arbutin results in greater efficacy than the β-glucoside bond in β arbutin. It has previously been shown that α arbutin inhibition of tyrosinase in melanoma cells is 10 times more potent than that of β arbutin [3]. Lactic acid, which is an alpha hydroxy acid, accelerates epidermis turnover or acts as a chemical peel. It can lighten skin by lifting the pigmented cells [4] and increasing the penetration of its active ingredients, α arbutin and niacinamide, which affects melanin. Niacinamide has a depigmentation effect, via the inhibition of melanosomes transfer from melanocytes to keratinocytes [5]. Niacinamide can reduce the content of melanin in skin keratinocytes, making skin appear brighter [6]. It is expected that the combination of a arbutin, lactic acid, and niacinamide, as three active substances that have different mechanisms of action, will produce a synergistic effect in skin lightening.

Whitening cosmetics are currently formed in cream preparations. However, innovation in the emulsion technology used to formulate skin-whitening cosmetics is required to improve the delivery of active substances and thereby increase whitening effectiveness. It is also expected that innovation will allow the improvement of stability and comfort compared with the usual cream preparation. Microemulsion has thermodynamically stable characteristics, a high solubilization rate that can increase the bioavailability of the active substance, and very small particle sizes, which can accelerate microemulsion penetration of human skin layers [7,8]. The use of microemulsion also improves the efficacy of a drug, allowing the total dose to be reduced and thus minimizing side effects [8]. The multiple emulsions system, known as “emulsion in the emulsion” is ideal for cosmetic preparation because it can dissolve substances into three separate compartments, prolong drug release, protect active ingredients, and produce the same consistency as cream by adding a thickener in the outer phase [9,10]. These two dosage forms have several advantages that are considered ideal for use in cosmetics.

The present study aimed to formulate and determine the physical stability of microemulsion and W/O/W multiple emulsions, containing α arbutin, lactic acid, and niacinamide, as skin-whitening cosmetics.

MATERIALS AND METHODS

Materials

Alpha arbutin (DSM Nutritional Product, Kaiseraugst, Switzerland), lactic acid (Purac Ltd., Rayong, Thailand), niacinamide (Bractco, Jakarta, Indonesia), tocotrienol (Davos Life Science, Synapse, Singapore), isopropyl myristate (Palm Oleo Sdn. Bhd., Rawang, Malaysia), Tween 80

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Preparation of W/O/W multiple emulsions

W/O/W multiple emulsions are generally prepared using a two-step procedure. In this study, the primary emulsion (W/O) was first prepared by adding 0.2 M NaCl solution containing a arbutin, niacinamide, lactic acids, and Na\textsubscript{2}EDTA. Methylparaben and propylparaben were then dissolved in propylene glycol, mixed with the first part of the water phase and heated. The resulting microemulsion had a yellow color (Pantone PMS 101), and appeared clear, yellow (Pantone PMS 101), and homogeneous, and had pH values of 3.45, 3.67, and 3.85, respectively. The tendency toward acidic pH was due to the use of lactic acid as an active ingredient, and pH value was also influenced by the amount of Tween 80; the higher concentration of Tween 80, the higher the pH value.

Initially, the viscosity of microemulsions F1, F2, and F3 was measured at spindle 1, with a speed of 2 rpm, was 2100 cps, 3500 cps, and 5650 cps, respectively. It suggested that a higher concentration of Tween 80 would be followed by an increase in viscosity value. The use of high concentrations of surfactants resulted in smaller dispersed phase globules, so the surface area and viscosity increased [12]. A rheogram showed that microemulsions F1, F2, and F3 had a pseudoplastic flow and viscosity measurement at weeks 4 and 8 showed declining viscosity grades. After storage for 8 weeks, the flow properties of microemulsions F1, F2, and F3 remained pseudoplastic. This indicated no change in flow properties, which meant that the microemulsions were stable for 8 weeks of storage at room temperature (28±2°C).

Measurement of the average diameter of microemulsion globules was performed with a Zetasizer Nano S PSA. Microemulsions that were stored at room temperature (28±2°C), low temperature (4±2°C), and high temperature (40±2°C), were taken every week. In general, all microemulsion formulas showed neither a constant value nor a drastic change (Fig. 1).

Organoleptic observations of the microemulsions were made every 2 weeks. Microemulsions F1, F2, and F3 that were stored at 28±2°C appeared clear; yellow (Pantone PMS 101), and homogeneous, and had a distinctive smell. Microemulsions F1, F2, and F3 that were stored for 12 weeks at 4±2°C appeared to be partially frozen and a slightly murky yellow color. If they were placed back at room temperature, the microemulsions became yellow (Pantone PMS 101) and were clear, with a distinctive smell, and no phase separation. Microemulsions F1, F2, and F3 stored at 40±2°C from week 0 to week 12 still looked clear.

RESULTS AND DISCUSSION

Microemulsion

The main experiment was carried out after obtaining the optimal microemulsion formula from the preliminary experimental results. The resulting microemulsion had a yellow color (Pantone PMS 101), clarity, and a distinctive smell. The microemulsions, F1, F2, and F3, had a distinctive smell (Fig. 1).
were yellow (Pantone PMS 101), had a distinctive smell, and no phase separation. Following a cycling test as many as six cycles, the microemulsions remained clear, stable, and yellow (Pantone PMS 101), with a distinctive smell. Microscopic observation showed that there were no crystals in the microemulsions.

W/O/W multiple emulsions

The main experiment was carried out after obtaining the optimal formula for multiple emulsions from preliminary experiments. The W/O/W multiple emulsions were white-yellowish in color (Pantone PMS 607), homogeneous, and odorless. When applied to the skin, the W/O/W multiple emulsions felt cool and comfortable.

At week 0, the pH values of the multiple emulsions F1, F2, and F3 were 3.31, 3.35, and 3.35, respectively. In general, the pH measurements of multiple emulsions preparations that were stored for 8 weeks at room temperature (28±2°C), low temperature (4±2°C), and high temperature (40±2°C) did not show a constant value, but the pH range did not drastically change. The pH values of the multiple emulsions ranged from 3.02 to 3.35; the presence of lactic acid in the formula resulted in acidic multiple emulsions. The pH measurements are shown in Fig. 2.

At week 0, multiple emulsions F1, F2, and F3 with spindle 3 at a speed of 2 rpm, had viscosities of 9500 cps, 10,000 cps and 10,500 cps, respectively. A tendency for a higher concentration of Tween 80 to result in a higher viscosity value was also shown. A high concentration of emulsifier would further reduce the size of globule diameter. A rheogram of multiple emulsions F1, F2, and F3 at week 0 indicates that those formulae had a pseudoplastic thixotropic flow. This showed decreasing viscosity, which was not immediately rectified when stress was not eliminated or reduced [12]. The viscosity measurements of multiple emulsions that were performed at weeks 4 and 8 showed a decline in viscosity grades. After storage for 12 weeks at room temperature (28±2°C), multiple emulsions F1, F2, and F3 still had a pseudoplastic thixotropic flow.

The average globule diameter of multiple emulsions were measured at 1000 times magnification, using an optical microscope equipped with a scale. At week 0, the globules of multiple emulsions F1, F2, and F3 had internal average diameters of 0.293, 0.292 and 0.284 µm, respectively, while the external globules had diameters of 0.605, 0.566, and 0.517 µm, respectively. The average globule diameter of F3 was smaller than those of F2 and F1, because of the concentration of Tween 80 that was most widely used in multiple emulsions F3; the higher the concentration of emulsifier, the smaller the globule size [13]. The microscopic measurements showed that multiple emulsions stored for 8 weeks at room temperature (28±2°C), low temperature (4±2°C), and high temperature (40±2°C) had changes in their globule size which tended to increase. It is because coalescence between the droplet phase in and phase out, which could be minimized using NaO to maintain the same osmotic pressure between the internal and external water phases [14]. The multiple emulsions globules are shown in Fig. 3. Measurements of the external globules of the multiple emulsions were also taken at week 8, using a PSA (Beckman Coulter®), but these measurements could only show the external diameter of the globules. It was found that multiple emulsion F1, with the lowest concentration of Tween 80 (2.5%), had globule size distribution profiles that tended to be stable after 8 weeks of storage at 28±2°C, 4±2°C, and 40±2°C. Multiple emulsion F3, with the highest concentration of Tween 80 (4.5%), showed different distribution profiles of globule sizes at the three storage temperatures. The average external globule diameters measured via PSA are shown in Table 1.

Multiple emulsions of F1, F2, and F3 that had been stored at low temperatures from week 0 to week 12 still looked white-yellowish in color (Pantone PMS 607), had no change in odor, and no visible phase separation. Multiple emulsions F1, F2, and F3 that had been stored at high temperatures from week 0 to week 8 still appeared to be yellowish-white, had no smell, and had no visible phase separation. However, in multiple emulsions F3 that had been stored at a temperature of 40±2°C, there was a significant yellow color on the upper surface of the preparation. This may have been the result of the hydrophilic surfactant in the continuous aqueous phase beyond the critical micelle concentration so that the lipophilic surfactant micelles solubilized lipophilic surfactant and removed it from the water continuous phase [14]. High temperatures could accelerate this phenomenon, resulting in decreased concentrations of lipophilic surfactant in the oil phase, and rupturing of the oil layer, thereby explaining the yellow color on the upper surface of the preparation. Cycling test results from six cycles of the three multiple emulsions formula showed that the preparation remained white-yellow (Pantone PMS 607) and there was no change in odor. Multiple emulsions preparations F1, F2, and F3 were stable without any phase separation. Microscopy showed that there were no crystals in the multiple emulsions preparations.

![Fig. 2: pH evaluation of the W/O/W multiple emulsions during 8 weeks at 28±2°C ( ), 4±2°C ( ), and 40±2°C ( )](image)

![Fig. 3: (a-c) Multiple emulsions globules of F1, F2, and F3 at week 0 under a microscope with ×1000 magnification](image)

| Formula            | Average globule diameter (µm) at week-8 |
|---------------------|----------------------------------------|
|                     | 28°C±2°C | 4°C±2°C | 40°C±2°C |
| Multiple emulsion F1| 2.425    | 1.830   | 2.345    |
| Multiple emulsion F2| 1.473    | 1.686   | 1.710    |
| Multiple emulsion F3| 1.751    | 1.824   | 1.808    |
CONCLUSION

Microemulsion with α arbutin, lactic acid, and niacinamide which was formulated with surfactant (Tween 80) at concentrations of 25%, 30%, and 35%, was clear and had a yellowish color, a pseudoplastic flow, an average globule size <100 nm, and was stable. Microemulsion F3, with a Tween 80 concentration of 35%, was the most stable formula because it had the smallest average globule size (2.397 nm) and the most stable distribution profile of globule size following 12 weeks of storage at 28±2°C, 4±2°C, and 40±2°C.

W/O/W multiple emulsions with α arbutin, lactic acid, and niacinamide could be formulated with 2.5%, 3.5%, and 4.5% of Tween 80 (external emulsifier), and also 3% of Span 80 (internal emulsifier) with a yellowish-white color, a pseudoplastic thixotropic flow, and internal and external globules, which could be clearly observed on microscopic observation. Multiple emulsions F1 with 2.5% Tween 80 was the most stable formula because it had a stable distribution profile of globule size during 12 weeks of storage at 28±2°C, 4±2°C, and 40±2°C.

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