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INTRODUCTION Skin is the largest part of the human body located at the outermost extent, protecting the body from external environments1. One of the possible environmental factors for skin damaged is ultraviolet radiation (UVR). In continuous exposure, UVR leads to some molecular damage (DNA photodamage) and clinical damage (erythema, tanning, skin cancer, photoaging) to the skin. UVR activates matrix metalloproteinases (MMPs), which are implicated in photoaging and collagen breakdown2,3.

The topical formulation that contains filtering or scattering UVR is called sunscreen, and the efficacy can examine by measuring the sun protection factor (SPF)4,5. One of the body's photoprotective parts, which role as a broadband UV-absorbing agent, is melanin. Melanin is a human skin pigment that acts as a protection from UVA, UVB, and visible blue light, which has radical scavenging and antioxidant properties6. However, endogenous melanin does not adequately protect the skin, especially in tropical climate areas like Indonesia. Thus, it is necessary to use sunscreens containing exogenous melanin or melanin-related compounds or mimic endogenous melanin7.

One of the melanins from natural sources known to have potential as free radical scavengers are squid (Loligo sp.) ink melanin8. Squid ink plays a significant role in eliminating intracellular excessive reactive oxygen species (ROS) and improve its antioxidant ability9,10. It also has anti-retroviral, anti-inflammatory, antimicrobial activity, and other traditional uses11. Antioxidants can protect against photo-induced radical reactions, thereby helping sunscreens in inorganic and organic UV filters12. Also, there was a positive relation and linear correlation between sunscreen and antioxidants13.

There is currently no research on using squid ink as an active ingredient in sunscreen products because of its limitation in the low solubility of organic solvent and water8. UV-absorbing and antioxidant activity within squid ink may be used as the active compound in sunscreen lotion to increase its benefit and utilization. Lotions are emulsion dosage forms for external application to the skin, which has many characteristics like creams. Lotions consist of an oil-in-water emulsion, water washable, and widely acceptable cosmetically14.

Therefore, this study investigates the physicochemical characteristics, stability, antioxidant activity, and UV protection effectivity of squid ink powder in lotion preparations. MATERIALS AND METHODS Materials Squid from Sendang Biru beach was purchased from the local market in Malang city, East Java, then dried to a dry squid ink powder. The squid used was determined in the Department of Biology, Faculty of Science and Technology, Universitas Airlangga. Squid is different from cuttlefish (Sepia

sp.) morphologically.

The color of the ink produced by squid is blue-black, while cuttlefish produces a brownish ink color. Other materials including virgin coconut oil (VCO), cetyl alcohol, triethanolamine (TEA), stearic acid, glycerin, propylene glycol, natrium edetate, butylated hydroxytoluene (BHT), methylparaben, propylparaben, fragrance, and distilled water in technical grade for lotion preparation. The DPPH (2,2-diphenyl-1-picrylhydrazil), ascorbic acid and methanol pro analysis for the antioxidant test, and Rattus norvegicus strain Wistar for SPF determination test's subject.

Methods Squid ink powder preparations Freshly obtained squids were dissected, and ink glands were manually removed from the viscera. The 50 g of ink squid was added with 100 mL HCl 0.5 M in a tight light condition. The solution was then stirred using a magnetic stirrer for 30 minutes then stored for 24 hours at 10°C9. After 24 hours, the solution was centrifuged for 15 minutes and dried and stored in the climatic chamber at 60°C15. Squid ink powder lotion preparation The oil-in-water lotion was prepared with the composition as shown in Table I. The water-soluble components (part A) and the oil-soluble components (part B) were mixed at 70°C, separately.

The water phase was added to the oil phase with continuous stirring. The squid ink powder was mixed to the lotion base and added with fragrance homogeneously16. Table I. Squid ink powder lotion formulation Ingredients _Formula I _Formula II _ Formula III _ Squid ink powder _1 g _2 g _3 g _ _VCO _5 g _5 g _5 g _ _Cetyl alcohol _2 g _2 g _2 g _ _Stearic acid _10 g _10 g _10 g _ _TEA _2 g _2 g _2 g _ _Propylene glycol _5 g _5 g _5 g _ _Glycerin _8.5 g _8.5 g _8.5 g _ Methylparaben _0.1 g _0.1 g _0.1 g _0.1 g _0.1 g _0.1 g _0.1

g _ Citrus fragrance _qs _qs _qs _ Distilled water _Until 100% _Until 100% _Until 100% _ Physicochemical evaluation of squid ink powder lotion The physicochemical evaluation involved was organoleptic, determination of pH value, homogeneity, viscosity, and gel spreadability. The organoleptic test acts as a factor in the physicochemical change parameters and the acceptability of the preparation17. The organoleptic test was observed as its color, scent, and texture visually. pH value was measured using a digital pH meter, and homogeneity was analyzed by visual inspection for any coarse particle's existence.

Viscosity was measured by Brookfield Viscometer, and gel spreadability was determined by applying gel in between two glass slides, then added with some weights16. Stability testing of squid ink powder lotion Real-time method Real-time stability studies of the different formulations were carried out under different temperature conditions (4°±2°C;

30°±2°C; 40°±2°C) and checked the effect on its organoleptic, homogeneity, and pH value. All formulations were stored in vial glass for 30 days18.

Freeze-thaw cycling method Freeze-thaw cycling stability studies was determined by storing the preparation in a refrigerator at 4°±2°C for 24 hours, then moved into a climatic chamber at 40°±2°C for 24 hours and counted as one cycle. This test was held in six cycles (12 days)19. Antioxidant DPPH scavenging activity test This method was adapted and modified from Fatimah Zaharah and Rabeta20 as well as Saputri et al.21. The samples with different concentrations of squid ink powder lotions and ascorbic acid as a positive control were reacted with the DPPH radical in methanol solution. About 5 mL of the sample (in methanol) was mixed with 1 mL of 0.4 mM DPPH solution.

UV protection effectivity test This test was determined by observing the erythema on the animal's skin test exposed to UV light. This test was using the male R. norvegicus strain Wistar as an experimental animal. The test was carried out by shaving the rats' back hair about 2 cm x 2 cm, then applying the samples and putting them on an Exo Terra lamp for six hours. Rats were divided into five groups (n = 6), a positive control group (using ParasolTM), a negative control group (using lotion without active), and three sample test groups (using a lotion with 1%, 2%, and 3% of squid ink powders) 17.

Parasol lotion had been clinically tested and contains active ingredients such as ethyl p-methoxycinnamate, benzophenone-3, and titanium dioxide, widely used in sunscreen preparations. This lotion had good protection against sunlight by reflecting and scattering UV radiation. The erythema score used was 0-4 which showed no erythema = 0; very little erythema (diameter <25 mm) = 1; erythema was clearly visible (diameter 25-30 mm) = 2; moderate erythema (diameter 30-35 mm) = 3; and severe erythema (diameter >35 mm) = 422.

Research ethics approval This research was approved by the Health Research Ethics Committee of Universitas Muhammadiyah Malang with Approval Code E.5.a/266/KEPK-UMM/XII/2019. RESULTS AND DISCUSSION Physicochemical evaluation of squid ink powder lotion The squid ink powder lotions had a soft texture, black in color, and citrus scent. The black color of these lotions follows the squid ink powder's color, and formula III had sharper color than formula II and I, as shown in Figure 1.

Visually, the squid ink powder lotions had no coarse particle, which indicated that the preparations were homogenous. The measurement of pH value, viscosity, and lotion spreadability was shown in Table II. / a / b / c Figure 1. Physical appearance of squid ink powder lotions in three replications of Formula I (a); Formula II (b); and Formula III (c) Table II. Physical and chemical characteristics of squid ink powder lotions Formula _pH _Viscosity (cps) _Spreadability (g/cm) _ _I _7.38 \pm 0.04 _3,083 \pm 629 _0.0037 \pm 0.0000 _ _III _7.30 \pm 0.02 _3,583 \pm 382 _0.0036 \pm 0.0000 _ _III _7.04 \pm 0.01 _4,667 \pm 289 _0.0033 \pm 0.0002 _ _ The different squid ink powder concentrations (1%, 2%, and 3%) had different pH, viscosity, and spreadability values. As shown in Table II, the higher concentration of squid ink powder resulted in lotion preparation with a lower pH value.

This phenomenon occurred when the squid ink powder was made, the solvent (HCl 0.5M) remains; hence the pH value was lower due to high powder content. However, the pH value results still qualify the pH range requirements of the skin tolerance (4–7)23 and the pH value requirement for sunscreen preparation (4.5–8)24. The viscosity value was significantly different and higher with the addition of squid ink powder. The spreadability value of Formula I was the highest and decreased with the addition of the active ingredient.

This phenomenon proves that the higher viscosity, the lower the spreadability value25. Stability testing of squid ink powder lotion Real-time method The results of real-time stability testing of squid ink powder lotions showed no changes in color, odor, and phase separation after storage at 4°, 30°, and 40°C. Significantly, the pH values were affected by the addition of squid ink powder and the storage temperature. Table III showed that the pH value of squid ink powder lotions was decreased over time, but the preparations were most stable at 30°C storage.

Table III. The pH value in real-time stability of squid ink powder lotion preparations Formula _1st day _30th day _ _ _ _4(C _30(C _40(C _ _I _7.38 \pm 0.04 _7.22 \pm 0.04 _7.20 \pm 0.07 _6.98 \pm 0.14 _ _II _7.30 \pm 0.02 _7.05 \pm 0.01 _7.15 \pm 0.02 _6.87 \pm 0.03 _ _III _7.04 \pm 0.01 _6.85 \pm 0.02 _6.92 \pm 0.09 _6.58 \pm 0.08 _ _Freeze-thaw cycling method The pH value results of the freeze-thaw cycling test of squid ink powder lotions showed that all formulas did not change organoleptically and showed no phase separation. The pH

value measurement results of this evaluation were shown in Table IV. The three formulas' pH values were different, but formula II was most stable than formula I or III. Table IV.

The pH value in freeze-thaw stability of squid ink powder lotion preparations Formula $_1$ st day $_1$ th day $_1$ $_1$ 7.38 \pm 0.04 $_1$ 7.26 \pm 0.02 $_1$ 11 $_1$ 7.30 \pm 0.02 $_1$ 7.25 \pm 0,02 $_1$ 111 $_1$ 7.04 \pm 0.01 $_1$ 7.10 \pm 0,01 $_1$ 7.40 Antioxidant DPPH scavenging activity test Antioxidant activity was examined by the DPPH scavenging method. The antioxidant will react with DPPH by electron donate mechanism, which stabilizes DPPH by decreasing the intensity of DPPH's violet color and turns into yellow21.

Ascorbic acid was a positive control, which was well known as a potent antioxidant for DPPH scavenging activity. Squid ink powder showed DPPH scavenging activity, which had an IC50 value of about 46.24 ppm (Table V). These results indicate that squid ink powder had a potent antioxidant activity, which categorized as a very powerful antioxidant (<50 ppm)26. Table V. IC50 value of ascorbic acid and squid ink powder Sample _Concentration (ppm) _DPPH scavenging activity (%) _IC50 (ppm) _ _Ascorbic acid _1 2 3 4 5 _46.92 \pm 0.73 53.35 \pm 0.81 58.94 \pm 0.80 66.76 \pm 1.02 68.99 \pm 1.12 _1.44 _ _Squid ink powder _5 10 20 50 100 _17.75 \pm 0.83 18.28 \pm 1.25 26.07 \pm 1.16 26.97 \pm 1.02 95.73 \pm 0.92 _46.24 _ _ Table VI showed the scavenging activity of squid ink powder when formulated into lotion preparations.

Formula III (squid ink powder 3%) gave out the highest value in antioxidant scavenging DPPH activity compared with Formula I and II. The higher of squid ink powder concentration, the higher scavenging activity obtained. These results indicate that squid ink powder lotions were categorized as weak antioxidants (250-500ppm)26. The freeze-thaw cycling test implies that the antioxidant DPPH scavenging activity of squid ink powder lotions was decreased but not significantly. Table VI. Antioxidant DPPH scavenging activity (%) of squid ink powder lotions Formula _Before freeze-thaw (%) _ _I _15.83 ± 0.013 _15.18 ± 0.012 _ _II _20.95 ± 0.019 _21.13 ± 0.002 _ _III _29.12 ± 0.023 _27.08 ± 0.024 _ _ UV protection effectivity test Erythema is induced by UV-B radiation causes cellular immunologic changes that lead to blood vessel dilation22.

As shown in Table VII, this study showed that the squid ink powder lotions significantly decrease in the erythema area compared to the negative control group. These lotions were able to inhibit the effects of acute UV exposure. The presence of antioxidant activity could explain this finding. Antioxidant activity had been shown to enhance protection against UV-induced DNA damage by reducing oxidative stress and inhibiting NF-kB. It also neutralizes the UV-induced free radicals. It made this agent could play a

role as potential "non-sunscreen" agents27. Table VII. UV protection effectivity value Samples _Erythema area (mm2) _Erythema Score _ _Positive control _0 \pm 0 _0 _ _Negative control _159.27 \pm 48.84 _4 _ _Formula | Formula | Formula

It showed antioxidant activity with DPPH scavenging activity of 29.12 ± 0.023% and also inhibited UV exposure. It indicates that these squid ink powder lotions had the potential as a sunscreen product. REFERENCES Boer M, Duchnik E, Maleszka R, Marchlewicz M. Structural and biophysical characteristics of human skin in maintaining proper epidermal barrier function. Postepy Dermatol Alergol. 2016;33(1):1-5. doi:10.5114/pdia.2015.48037 Rittié L, Fisher GJ. Natural and Sun-Induced Aging of Human Skin. Cold Spring Harb Perspect Med. 2015;5(1):a015370. doi:10.1101/cshperspect.a015370 Pandel R, Poljšak B, Godic A, Dahmane R. Skin Photoaging and the Role of Antioxidants in Its Prevention.

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