

Physical Stability, Photostability, and Sunscreen Effectiveness of Combination Cream of Arabica Green Coffee Bean Extract (*Coffea arabica*) and Octyl Methoxycinnamate

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ABSTRACT

Arabica green coffee beans (AGCB) have antioxidant compounds as photo protectors. It's used to stabilize and increase the SPF value of octyl methoxycinnamate in sunscreen because its effectiveness will be decreased when exposed to UV light. The purpose of this study was to determine the effect of a combination of various concentrations of arabica green coffee bean (AGCB) extract and octyl methoxycinnamate (OMC) on the physical properties and SPF value of sunscreen cream. AGCB extract with concentrations (F1) 0%; (F2) 2.5%; (F3) 5%; (F4) 7.5% (with the addition of OMC concentration of 7.5%), and (F5) 7.5% (without the addition of OMC). The formulas were tested for physical properties to determine stability in 4 weeks of storage, cycling test for 4 cycles, and photostability to determine the SPF value and irritation test on the best formula. The results of the physical stability test on the five formulas showed a sig value > 0.05 which means the preparation is stable. Formulas F1, F2, F3, F4, and F5 with exposure of 1 hour, 5 hours, and 10 hours obtained a significance value > 0.05, which means that the presence of UV light exposure in the AGCB extract combination cream can stabilize the decrease in SPF value. Formula 4, which is stable without separation during the cycling test and the stability of the SPF value of 41, then in the irritation test does not cause irritation so it can be concluded that formula 4 is the optimum formula.

Keywords: Arabica green coffee bean extract; Octyl methoxycinnamate; Physical stability; Photostability, SPF

INTRODUCTION

The skin is a part of the body that is in direct contact with foreign objects, including sun exposure which, when it hits the skin, will have positive and negative impacts on human health. Excessive sunlight will cause several problems, including sunburn, erythema, hyperpigmentation, premature aging, and skin cancer (Khafifa et al., 2022). Efforts to prevent skin damage due to sunlight can use sunscreen preparations because can to provide protection against UV radiation and broad spectrum effect, namely protecting the skin from UVA and UV B rays (Rosita and Murrukmihadi, 2014).

OMC is one of the most commonly used UVB filters in sunscreen preparations, due to its high absorption ability in the 290-320 nm wavelength region. It has a little allergic risk to the skin. The FDA approved OMC concentration limit is 7.5% - 8.5%, which will work by absorbing UV B rays and turning them into heat (Arianto et al., 2019). Exposure to UV light will trigger OMC

degradation due to photo-oxidation reactions so its effectiveness as a sunscreen will decrease. It is needed a photoprotector that be able to increase the effectiveness of sunscreens by providing a good SPF value (Sutarna et al., 2015).

Antioxidants as photoprotective agents, can against free radicals in sunscreens due to sunlight. Antioxidant active ingredients derived from plants are preferred because they have a broad UV absorption spectrum, photostabilit, and have the ability to protect against oxidative stress (Lia Ningsih et al., 2022). Green coffee beans (*Coffea arabica*) contain rich polyphenols such as chlorogenic acid, caffeic acid, coumarin and ferulic acid that prevent the skin from oxidative damage. Green coffee contains more chlorogenic acid as the main polyphenolic compound than roasted coffee (Desai and Mallya, 2021). These secondary metabolite compounds have potential sunscreen activity due to the presence of chromophore groups (conjugated single double bonds) which are able to absorb UV rays, both UV A and UV B, thereby reducing their intensity on the skin (Sari, 2021). The combination of active ingredients arabica green coffee beans (AGCB) extract and

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Table I. Formula of AGCB extract and OMC Combination Sunscreen Cream

Material	Concentrations (%)				
	F1	F2	F3	F4	F5
Arabica Green Coffee Extract	0	2,5	5,5	7,5	7,5
Octyl methoxycinnamate	7,5	7,5	7,5	7,5	0
Dimethicone	4	4	4	4	4
Mineral oil	2,2	2,2	2,2	2,2	2,2
Stearic acid	12,37	12,37	12,37	12,37	12,37
TEA	2,63	2,63	2,63	2,63	2,63
Cetyl alcohol	2,07	2,07	2,07	2,07	2,07
Glycerin	1,8	1,8	1,8	1,8	1,8
Citric acid	0,5	0,5	0,5	0,5	0,5
Methyl Paraben	0,2	0,2	0,2	0,2	0,2
Coffea fragrance	qs	qs	qs	qs	qs
Aquades	ad 100	ad 100	ad 100	ad 100	ad 100

octyl methoxycinnamate (OMC) is one way to obtain a broad-spectrum effect on sunscreen preparations that maintains the effectiveness of sun protection factors.

Thus, this study aims to determine the physical stability and effectiveness of sunscreens containing a mixture of AGCB extract and OMC physical UV filters by in vitro methods and irritation testing on test animals.

METHODOLOGY

Materials and Tools

The active ingredients used were arabica green coffee bean extract (*Coffea arabica*) purchased from PT Phytochemindo Reksa and Octyl Methoxycinnamate (Chemspec Chemical, India). The cream base was composed by stearic acid (Brataco; Indonesia), triethanolamine (Sigma-aldrich; USA), liquid paraffin (Brataco; Indonesia), dimethicone (Brataco; Indonesia), citric acid (Brataco; Indonesia), cetyl alcohol (Brataco; Indonesia), glycerin (Brataco; Indonesia), methylparaben (Sumber Berlian; Indonesia), distilled water (General labora; Indonesia), coffee fragrance oil (Van Aroma; Indonesia), ethanol p.a (Merck; Jerman), dichloromethane (Merck; Jerman CaCO₃ (Merck; Jerman). The equipment used in this study were glassware (pyrex), spreadability test, UV-Visible spectrophotometer (Shimadzu), viscometer (Brookfield), pH meter (Hanna), refrigerator, UV lamp 366 nm, waterbath (Memmert), oven (Memmert), rabbit fur scissors, rabbit cage.

The test animals used were healthy albino rabbits weighing \pm 2 kg as many as three animals for the primary irritation test. This test has been

approved by the Research Ethics Committee of the Faculty of Veterinary Medicine, Gadjah Mada University, with Number: 031/EC-FKH/Eks/2023.

Methods

Analysis of Caffeine and Chlorogenic Acid Content of Extracts

Weigh as much as 500 mg of arabica green coffee bean extract powder dissolved in 100ml of distilled water then stirred using a magnetic stirrer 500 rpm for 1 hour at a temperature of 70°C. Add 250 mg of CaCO₃ then filter with filter paper. Do the liquid-liquid extraction using 25 ml dichloromethane for four times. The water phase was collected, and the dichloromethane phase was evaporated to obtain pure caffeine, then analyzed using a UV-Visible spectrophotometer with a concentration range of 5 mg/ml-15 mg/ml for chlorogenic acid and standard caffeine solutions. Samples were read at concentrations of 5 μ g/mL - 15 μ g/mL (Hamdani and Nurman, 2020).

Formulation of AGCB Extract and OMC Combination Sunscreen Cream

Sunscreen cream was prepared according to the formula in Table I. It was melting the oil and water phase separately. The oil phase (**a**) of dimethicone, mineral oil, stearic acid, and cetyl alcohol were heated in a porcelain cup on a water bath at approximately 75°C while stirring, the active substance of OMC was added to the oil phase. The water phase (**b**) is placed in a different cup made by mixing TEA, glycerin, and methylparaben. Afterthat, add some distilled water and heat on a waterbath at 75°C. If it has been mixed, the water

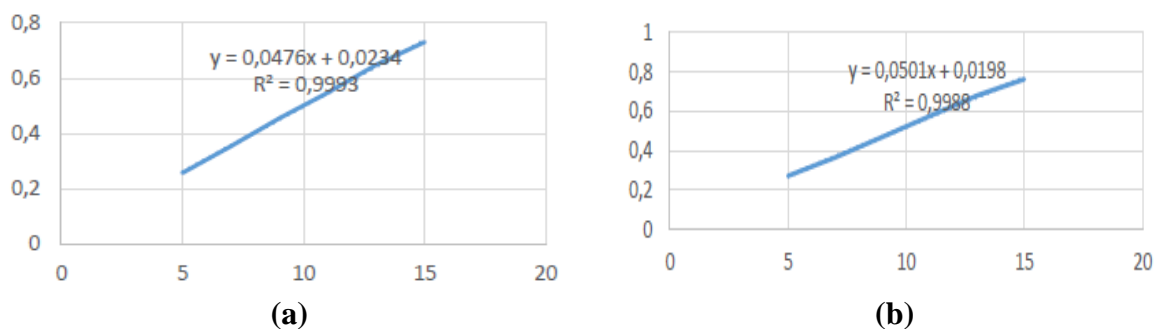


Figure 1. Calibration curve of standard solution (a) caffeine standard solution; (b) chlorogenic acid standard solution

phase is slowly added to the oil phase, while constant stirring is done until a homogeneous cream mass is formed, add citric acid. The formed cream mass is then placed in a clean container and protected from light.

Evaluation of Physical Stability of AGCB Extract and OMC Combination Sunscreen Cream

Evaluation of the physical properties of the cream includes organoleptic test, pH measurement, spreadability, and viscosity at storage of week 0 to week 4.

Spreadability test

There is a number of 0.5 grams of cream was placed in the center of the round glass balance and then covered with the weighed glass cover and observed for 1 minute. Subsequently, 50 grams of weight was added every minute until the total weight was 250 grams. Diameter measurements were taken every minute (Zulkarnain et al., 2022).

Testing the pH value of cream preparations

Tests were carried out using the HANNA H1 5211 pH meter which had previously been calibrated with pH 4, 7 and 10. Measurements were made by dipping the pH meter in the cream preparation.

Viscosity Test

Viscosity test was carried out using Brookfield DV-I PRIME Viscosimeter with spindle No.6 and rpm 100. Read the Ps (Poise) value and % torque listed on the tool screen (Zulkarnain et al., 2022).

Besides room temperature stability, an accelerated testing was carried out to determine the effect of extreme temperatures and the effect of gravity on the storage stability of the cream preparation.

Thermal cycling test

This test is carried out to observe the stability of the preparation under different conditions. This test was carried out on samples stored at temperatures at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and then observed physical changes including cream color and phase separation (Smaoui et al., 2017). The test was carried out for 4 cycles (1 cycle equals 24 hours) (Nurfita et al., 2021).

Mechanical test (centrifugation)

The cream preparation was placed in a conical tube and put it into a centrifuge at 5000 rpm for 30 minutes, and observed the physical changes that occurred in the cream (Nurfita et al., 2021).

In Vitro Sunscreen Photostability Testing

This test was conducted in vitro based on the research of Rosita et al., 2017 with modifications to determine the SPF value of the AGCB extract and OMC combination sunscreen cream before irradiation and after UV light irradiation. A total of 1.5 grams of sunscreen cream preparation was applied to a glass plate and then irradiated with a UV B lamp with a wavelength of 366 nm with a variation of irradiation duration of 0, 1, 5 and 10 hours in a closed cabinet. Then the cream before and after irradiation was taken as much as 0.1 gram in 10 ml of 96% ethanol and

measured the absorbance at a wavelength of 290-320 nm with an interval of 5 nm using UV-Vis spectroscopy. The absorbance results are determined based on Mansur equation method.

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

Description: EE = Erythema effect spectrum; I = Solar intensity spectrum; Abs = Sample absorbance; CF = Correction factor.

Irritation Test of Sunscreen Cream Preparations

This test was conducted in accordance with BPOM RI Regulation No. 7 of 2014 concerning guidelines for non-clinical toxicity testing in vivo. This test was conducted to evaluate the combination of AGCB extract and OMC sunscreen cream on the effect of irritation on the skin of adult albino rabbits weighing 1.5-2 kg (3 animals). The preparation was applied to rabbits at a dose of 0.5 g and left for 4 hours. Then the rabbits were observed at 1, 24, 48, and 72 hours, and up to 14 days. Observations were made on the incidence of erythema and edema in the test animals (BPOM, 2014).

Data analysis

The results of the evaluation of physical stability and photostability of AGCB extract and OMC combination sunscreen creams including viscosity, spreadability, and pH value as well as photostability of each formula were analyzed with SPSS version 26 starting with homogeneity test, One-Way ANOVA followed by Post-Hoc test using Tukey with 95% confidence level.

RESULT AND DISCUSSION

Caffeine and Chlorogenic Acid Content of Extracts

For the preliminary test, the levels of chlorogenic acid and caffeine in AGCB extract were analyzed using a UV-Vis spectrophotometer at a wavelength of 322.4 nm for chlorogenic acid and 273 nm for caffeine in AGCB extract. The absorbance values of chlorogenic acid and caffeine were then entered into a linear regression equation as shown in Figure 1.

The analysis resulted in a chlorogenic acid concentration value of 4.341% with concentration 0,25 mg/ml, which is in accordance with the literature that chlorogenic acid levels in arabica green coffee (*Coffea arabica*) are 4.1-7.9% (Hamdani and Nurman, 2020). The caffeine content in arabica green coffee beans ranges from

1.45%-2.38% but it depends on each type of bean and place of growth (Babova et al., 2016) the test results for caffeine concentration showed a result of 2.219%. Chlorogenic acid belongs to caffeic acid and quinic acid compounds that have antioxidant potential. Caffeine is often used as an active ingredient in cosmetics due to its strong antioxidant properties, which protect cells from UV radiation and effect the photoaging process of the skin. It has a purpose as a combination in sunscreen.

Physical Stability Evaluation Test of Sunscreen Cream

Organoleptic and homogeneity

Organoleptic and homogeneity observations were carried out with test parameters: odour, colour, consistency and homogeneity of sunscreen cream preparations. At week 0, after the cream formulation, each formula shows a different colour of the preparation. It is caused by the differences in extract concentration. The higher concentration of the extract, the darker the preparation, as seen in Figure 2.

Based on the results observed that from week 1 to week 4, the sunscreen cream preparation did not experience changes in odor, color, consistency and homogeneity Each formula stored at room temperature for four weeks did not show significant organoleptic changes and homogeneity as presented in Figure 2. It is categorized as organoleptically stable. The stored cream preparation was homogenous for four weeks, and there were no coarse grains. The purpose of testing cream homogeneity is to see color distribution, even mixing and the absence of coarse grains (Mansauda et al., 2022). In F2, F3, and F4 at the time of week four storage, the color tends to be slightly darker than the previous storage but the consistency remains thick and homogeneous without coarse grains. This occurs due to differences in the amount of AGCB extract concentration which is different in each formula, the addition of AGCB extract variations will give a color that tends to be darker.

A good cream preparation is to have a physical consistency that does not change during its storage period and is stable under extreme conditions or shocks, and physical stability is an important parameter that must be met. In this study, cream with o/w emulsion type was chosen because it has advantages over the w/o type, namely its ability to spread more easily on the skin surface, does not cause a sticky impression and is acceptable (Kusuma et al., 2021).

This spreadability test is carried out determine the ability of the cream to spread on the

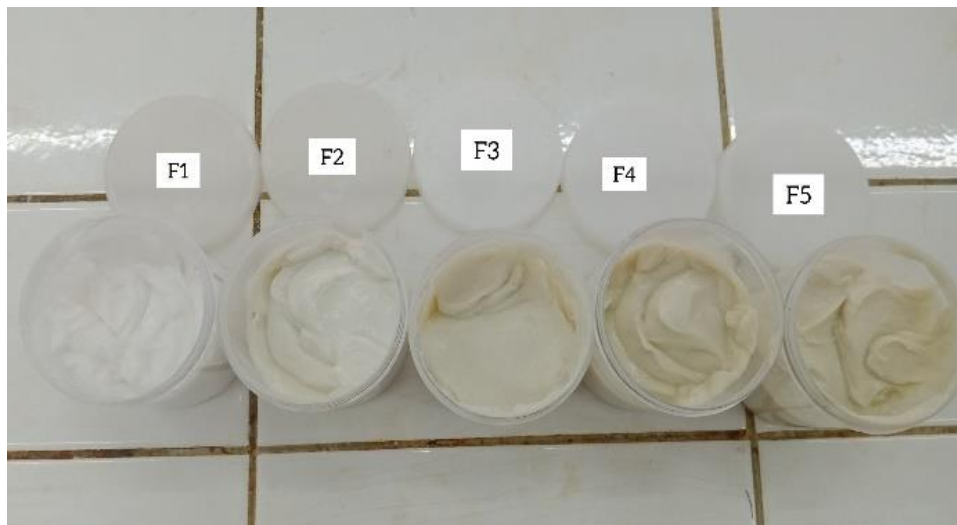
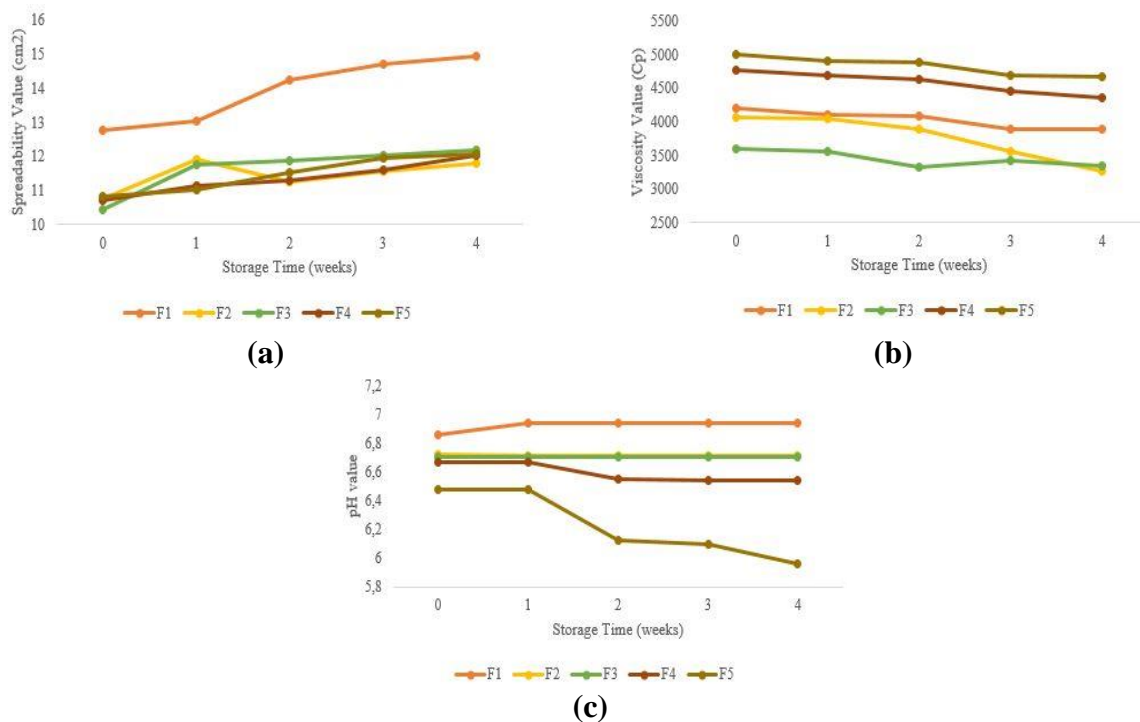


Figure 2. Preparation of AGCB Extract and OMC Combination Sunscreen Cream



skin surface when applied. Good cream preparation has a large have great spreadability so it can be used on the surface of the skin without excessive pressure and show a semisolid consistency that is very pleasant to use with a good spread of cream with a 5-7 cm diameter (Kusuma et al., 2021). Based on Figure 3. The test results show the more concentration of AGCB extract, the smaller the spreadability. The result indicates that adding the extract will give a thick cream

consistency. In the physical stability test, the spreadability from week 0 to week 4 shows an increase in the spreading value or gets thinner. The analysis results show a sig value > 0.05, meaning that the spreadability in each week has increased, which is not significant. It means that it is stable because it is still in the specified range.

pH testing is carried out because it plays an important role in the irritating effect of cream preparations that will be used on the skin surface.

Table II. Accelerated stability testing results of thermal cycling test and centrifugation

	Formula				
	F1	F2	F3	F4	F5
Thermal cycling test	+	+	+	-	+
Centrifugation test	-	-	-	-	-

Description: (-) : not separating; (+) : separating

As required by SNI that cream sunscreen preparations have a pH range of 4,5-8. pH values that are too acidic will irritate, and pH values that are too alkaline will cause scaly and itchy skin (Mansauda et al., 2022). In Figure 3. it can be observed that as the addition of AGCB extract will decrease the pH value, and F1 without the addition of AGCB extract tends to be more alkaline. This is due to the presence of secondary metabolite compounds contained in AGCB extract such as polyphenol groups, and flavonoid groups which are acidic (Sugihartini et al., 2021), while F1 with OMC active substances have a pH value that tends to be alkaline. In the physical stability test, the pH value from week 0 to week 4 shows a decrease in pH value, but the analysis results show a sig value > 0.05 , which means that the decrease in pH value every week is not significant or it can be said that there is no significant difference and tends to be stable.

The viscosity test is carried out to see the flowability or viscosity of a preparation. The viscosity value is inversely proportional to the spreadability value, the higher the viscosity value, the lower the ability to spread. The thickening agent influences the viscosity of the preparation; if the preparation is too thin, it will reduce its ability to stick and spread on the surface of the skin. However, if it is too thick, it will affect its flowability when removed from the container (Sugihartini et al., 2021). Based on Figure 3. it can be observed that the more AGCB extract increases, the thicker the sunscreen cream ideally has a viscosity value of 2,000-50,000 cps in accordance with SNI 16-43990-1996 (Sugihartini et al., 2021). In the physical stability test, the viscosity decreased from week 0 to week 4, this is proportional to the decrease in pH value, but the analysis showed a sig value > 0.05 , which means that the decrease in viscosity every week is not significant or it can be said that there is no significant difference and tends to be stable.

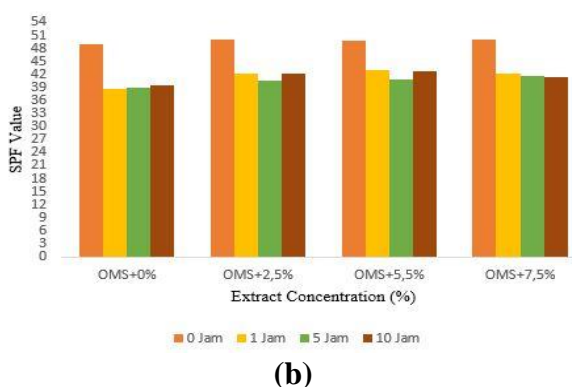
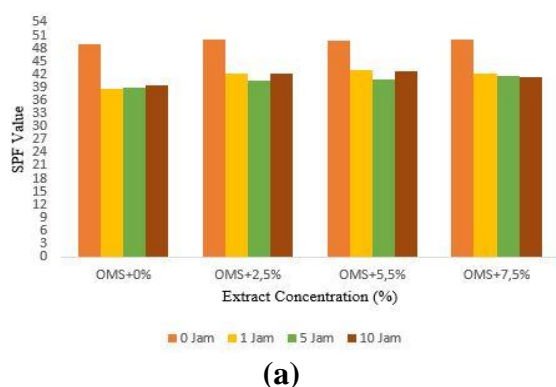
In addition to the storage period to ensure the stability of the formulation is carried out using

an accelerated test with heating and cooling for 4 cycles in the thermal cycling test, the cream formulation has good stability if there is no sign of separation between the oil phase and the water phase.

In accelerated testing using two different temperatures, unstable results were obtained in F1, F2, F3 and F5 as found in table 2. This can be caused by the separation of water and oil in the cream because they have different melting point and freezing point temperatures, and emulgators are needed that are able to bind lipophile and hydrophile groups. F4 with the same combination of AGCB extract and OMC concentrations has a more stable ability at extreme temperatures. F1, F2, F3 and F5, after heating and cooling tests experienced organoleptic changes and the cream became inhomogeneous because high temperatures will cause a decrease in cream consistency due to an increase in the kinetic energy of atom. In the cold temperature process, water crystals will form which have a more regular and tight structure so that the cream cannot flow. At 4°C the water phase freezes and tends to shrink, resulting in narrowing. This causes separation of the water and oil phases (Hamsinah et al., 2016). The next physical stability test was the evaluation of cream separation by the centrifugation method at 5000 rpm for 30 minutes. This test was conducted to determine the effect of gravity during storage. In Table II. F1, F2, F3, F4 and F5 cream preparations did not experience separation due to shaking or shaking with centrifugation. This indicates that the AGCB extract and OMC combination cream has physical stability and is not affected by gravity.

Determination of SPF (Sun Protector Factor) Value and Photostability

The SPF value indicates the activity of the sunscreen to protect the skin from sunlight. With the variation of AGCB extract concentration combined with OMC, the SPF value was increased but not significantly. At a concentration of 10.000



ppm, the sunscreen showed ultraprotection. In Figure 3, it can be seen that the combination of AGCB extract and OMC 7.5% has the potential to increase the SPF value of sunscreens without irradiation and with irradiation still in the ultracategory. OMC has photostability problems due to the influence of UV exposure which causes a decrease in OMC levels in sunscreen preparations (Sutarna et al., 2015). Arabica green coffee extract as a photoprotector because it has secondary metabolite compounds, namely caffeine and chlorogenic acid as antioxidants that can bind to free radicals. The requirement for a good sunscreen is to be able to maintain the ability to protect against UV rays and experience a decrease in SPF value so that to get maximum results, and repeated application is carried out every 2 hours (Kockler et al., 2012). Based on Figure 5, it can be seen that in F1 (without AGCB extract combination) the SPF value before and after UV exposure decreased by 21.3% on UV exposure for 1 hour, in F2 (2.5% AGCB extract combination) decreased by 15.8%, F3 (5% AGCB extract combination) decreased by 13.6% but on 5-hour exposure decreased by 18.0%. Formula 4 with a combination of AGCB extract 7.5% decreased by 15.3% but the SPF value was stable at 5 to 10 hours exposure, formula 5 (without OMC combination) had the smallest decrease of 9.4% but had a smaller SPF value compared to formulas with OMC combination.

Based on the analysis using SPSS one way anova Tukey method on the SPF value of the 0 hour exposure group compared to the 1 hour, 5 hour and 10 hour exposure groups in each formula obtained a significance value of $0.00 < 0.05$, this indicates a difference between

the groups before exposure and after exposure. In groups F2, F3, and F4 with exposure of 1 hour, 5 hours and 10 hours obtained a

significance value > 0.05 which means that exposure of 1 hour, 5 hours and 10 hours does not experience significant differences or it can be said that the combination of AGCB extract is can stabilize the SPF value due to UV light.

From the results of physical stability and photostability testing of SPF values, the most optimum and qualified result is F4 with the ratio of AGCB extract and OMC (7.5%: 7.5%), then F4 is carried out irritation testing.

Irritation Test

The irritation test is carried out to ensure that the cream preparation has safety in use after being made and to ensure that it does not have an effect in long-term use. Based on data on rabbit skin smeared with formula 4 and gauze control, the irritation score (primary irritation index) is 0 or no signs of redness irritation appear on rabbit skin and is included in the negligible response category (very mild) so that formula 4 which has the best physical stability and SPF value can be used safely and comfortably.

CONCLUSION

The difference in concentration variations of AGCB extract combined with OMC as an active substance does not affect the physical stability of the cream during storage for 4 weeks and the centrifugation test does not experience separation so it can be said to be stable. The higher the concentration of AGCB extract, the higher the viscosity and the lower the spreadability and pH value. Based on the results of the accelerated thermal cycling test stability test, the cream experienced a separation phase between the water and oil phases and obtained the optimum formula of cream preparation formula 4 because it has the most stable physical stability and good SPF value and does not cause irritation.

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