



## Optimization of Sunscreen Cream Formulation Containing Ethanolic Extract of Avocado Leaves (*Persea americana* Mill.) or Mangosteen Rind (*Garcinia mangostana* L.) Combined with Octyl Methoxycinnamate

(Optimasi Formula Krim Tabir Surya Mengandung Ekstrak Etanolik Daun Alpukat (*Persea americana* Mill.) atau Kulit Manggis (*Garcinia mangostana* L.) Kombinasi dengan Oktil Metoksisinamat)

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### ABSTRACT

**Background:** Most sunscreens contain chemicals that can cause health and environmental problems. Natural actives can be an alternative to minimize the use of chemicals. Avocado leaves (*Persea americana* Mill.) and mangosteen peel (*Garcinia mangostana* L.) contain active compounds that have UV absorption. **Objective:** In this study, the optimal composition of a mixture of ethanol extracts of avocado leaves or mangosteen peel with octyl methoxycinnamate (OMC) was determined based on the Sun Protection Factor (SPF) and adhesive properties using Response Surface Methodology (RSM). **Methods:** The optimal formula calculated with RSM was then made and underwent a series of tests, including a hedonic test with a scale of 0-9 on 30 respondents, a freeze-thaw stability test, and an accelerated stability test with the Arrhenius equation. **Results:** The RSM analysis showed that the optimal concentration of extract and OMC was 10% and 0.75% (avocado leaves) & 0.65% (mangosteen peel). The optimized formula resulted in adhesive properties and SPF of  $7.41 \pm 0.03$  and  $16.52 \pm 0.16$  (avocado leaves) &  $7.43 \pm 0.04$  and  $15.84 \pm 0.15$  (mangosteen peel) respectively. Both optimized formulations were physically stable against freeze-thaw and had a  $t_{90}$  of 29 minutes (avocado leaves) and 17 minutes (mangosteen peel). The average hedonic scale obtained was 7.8 (avocado leaves) and 7.9 (mangosteen peel), indicating that both optimized formulations were preferred by the respondents. **Conclusions:** The sunscreen formulation with an optimized combination of active ingredients from avocado leaf extract or mangosteen peel extract with OMC using RSM results in preparation with SPF value and physical properties that meet the requirements.



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## INTRODUCTION

Please Sunlight plays a role in the formation of vitamin D3. However, the excessive exposure of ultraviolet (UV) rays that are contained in sunlight can cause health problems such as sunburn, eye damage, and skin cancer (Wacker *et al.*, 2013). The use of sunscreen is recommended to protect the skin from excessive exposure to UV radiation. However, most sunscreens on the market contain chemical active ingredients that cause human and environmental health problems (EPA, 2021). Some of chemical ingredients are restricted in their use. These restrictions reduce the value of the Sun Protection Factor (SPF) produced. Alternatively, natural active substances can be used in combination to preserve the desired SPF value.

Previously, numerous studies have been conducted to create various types of sunscreen formulations. Certain studies have utilized active ingredients, such as metal oxides, as reflectors. These include combinations of oxybenzone, octyl methoxycinnamate (OMC), ZnO, and TiO<sub>2</sub> (Al-Saeedi & Dahmash, 2022). Other formulations have solely relied on active inorganic ingredients such as ZnO, TiO<sub>2</sub>, and Ca<sub>2</sub>SiO<sub>4</sub> (Abbas et al, 2021). Some natural ingredients, such as spinach powder, green tea extract, and showy Asian grapes fruit extract, have also been utilized as active ingredients in sunscreens (Taniyadukkam et al., 2023; Chen et al, 2022; Geraldine & Hastuti, 2018). Nevertheless, the combination of natural and chemical ingredients in sunscreens has not been thoroughly examined, particularly concerning materials that are considered waste, such as avocado leaves and mangosteen fruit peels.

The natural active compounds that we proposed in this study are derived from avocado leaves (*Persea americana* Mill.) and mangosteen rind (*Garcinia mangostana* L.). Avocado leaves contain a flavonoid compound, namely quercetin (Kemenkes RI, 2017). Mangosteen rind contains polyphenol compounds in the form of xanthenes (Yatman, 2012). Both possess UV absorption at a wavelength around 200-400 nm (Kumar *et al.*, 2016; Ahmad *et al.*, 2013). Based on the previous research, the SPF value of the ethanolic extracts of avocado leaves and mangosteen peel is still below 15 (14.45 and 8.94) which is the minimum SPF value recommended by the FDA (Pontoon, 2016; Iryani *et al.*, 2021). Therefore, in this study, we combined the natural active substances with OMC. Octyl methoxycinnamate is an UV active substance commonly used in sunscreens that works by absorbing the UVB rays (Serpone *et al.*, 2007).

In this study, the optimization of avocado leaves' ethanolic extract combination and OMC as well as ethanolic extract of mangosteen rind and OMC were carried out based on the SPF value and adhesion. The optimization method employed Response Surface Methodology (RSM) using RStudio software. The working principle of this method is to carry out the experiments using a very minimal amount of sample but more sample is to be added if needed. The optimization stage of RSM started with a first-

order experimental design to determine the relationship between factors and responses which the relationship is assumed to be linear. If the relationship did not match, the second order was continued, until a fit relationship is found. One of the designs in the second-order RSM is the Central Composite Design (CCD). The advantage of the RSM method, especially with the CCD design, is that the number of samples used is less with the two variables included in the form of independent variables (Myers, 2009). After that, the optimal combination obtained was tested for physical and chemical stability, as well as hedonic test to investigate whether the sunscreen cream is acceptable to the public.

## **MATERIAL AND METHODS**

### **Materials**

The tools used in this research are precision balance (Ohaus), analytical balance (Mettler Toledo), hotplate (HEIDOLPH), UV-Vis spectrophotometer (Shimadzu UV-1800), magnetic stirrer, adhesive test equipment, petri dish, universal pH indicator (Merck), viscometer (Haake), trinocular microscope (Olympus CX33), thermometer, sonicator (Brandson), water bath (Mettler), refrigerator, and glassware (Iwaki).

The materials used in this study are ethanolic extract of avocado leaves, ethanolic extract of mangosteen rind (both plants were obtained in Surakarta area and then extracted by BRIN (Badan Riset dan Inovasi Nasional), OMC (Merck), ethanol 96% (Merck), stearic acid (Sigma-Aldrich), triethanolamine (Sigma-Aldrich), tween 80 (Sigma-Aldrich), cetyl alcohol (Sigma-Aldrich), methylparaben (Sigma-Aldrich), propylparaben (Sigma-Aldrich), glycerin (Sigma-Aldrich), carbomer (Sigma-Aldrich), isopropyl myristate (Sigma-Aldrich), rose oil (PT. Pasifik Kimia Indonesia), liquid paraffin (Sigma-Aldrich), distilled water (CV. Megatama Mandiri), methylene blue (Merck), cream container, parchment paper, and a gap load of 1 kg.

### **Methods**

#### **Preparation of Cream Base**

The preparation of the cream base was divided into two phases, the oil and the water phase. The oil phase consists of stearic acid, cetyl alcohol, propylparaben, isopropyl myristate, and paraffin which were heated on a hotplate at 70 °C and stirred constantly using a magnetic stirrer until homogeneous. The aqueous phase consists of tween 80, triethanolamine mixed with carbomer (already developed with water), glycerin, methylparaben, and distilled water which were heated on a hotplate at 70 °C and stirred constantly using a magnetic stirrer until homogeneous. The oil phase was then added to the water phase slowly while being stirred constantly until the temperature cooled down to 30-40°C. After the base was prepared, the ethanolic extract of avocado leaves or mangosteen rind which had been mixed with OMC was added to the base while being stirred constantly until homogeneous. Rose oil was added to create

the fragrance (approximately 3 drops) (Wikantyasning *et al.*, 2021; Mailana *et al.*, 2016). The cream base formula is presented in Table 1.

Table 1. Cream base formulation.

No.	Material	Formula (g)	Function
1.	Stearic acid	1	Solvent (oil phase)
2.	Triethanolamine	2	pH regulator
3.	Tween 80	8	Emulsifying agent
4.	Cetyl alcohol	3	Stiffening agent
5.	Propylparaben	0.05	Preservative
6.	Methylparaben	0.2	Preservative
7.	Glycerin	10	Humectants
8.	Carbomer	0.8	Gelling agent
9.	Isopropyl myristate	1	Emollient
10.	Liquid paraffin	5	Solvent (oil phase)
11.	Rose oil	3 drops	Fragrance
12.	Aquadest	69.95	Solvent (aqueous phase)

### Optimization of the Formula: Composition of Ethanolic Extract and OMC

The composition of the ethanolic extract, OMC, and the base in the sunscreen preparations was determined by RStudio using RSM. Formulas with various possible comparisons of the two active components and the bases are presented in Table 2. Furthermore, the SPF test was carried out using UV-Vis Spectrophotometry at a wavelength between 290-320 nm with an interval of 5 nm. The adhesion test was performed using an object glass and the time was recorded. The calculation of the SPF value employed the equation of Mansur (1986) and the table of constants EE X I Sayre (1979) (Dutra *et al.*, 2004).

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

Information:

CF = Correction factor (=10)

EE = Erythema spectrum of effect

I = Intensity of sunlight spectrum

Abs = Absorption of sunscreen products

Table 2. Ratio of Avocado Leaves extracts, OMC, and the base compositions.

Formula	Avocado Leaves (g)	OMC (g)	Base (g)
F1	1.0	0.06	8.94
F2	1.4	0.05	8.55
F3	1.0	0.04	8.96
F4	0.6	0.05	9.35
F5	1.5	0.06	8.44
F6	1.0	0.06	8.94
F7	0.6	0.07	9.33
F8	1.4	0.07	8.53
F9	1.0	0.08	8.92
F10	0.5	0.08	9.42

Table 2. Ratio of Mangosteen Rind Ethanolic Extract, OMC, and the base compositions.

Formula	Mangosteen Rind Ethanolic Extract (g)	OMC (g)	Base (g)
F1	1.0	0.06	8.94
F2	1.4	0.05	8.55
F3	1.0	0.04	8.96
F4	0.6	0.05	9.35
F5	1.5	0.06	8.44
F6	1.0	0.06	8.94
F7	0.6	0.07	9.33
F8	1.4	0.07	8.53
F9	1.0	0.08	8.92
F10	0.5	0.08	9.42

### Physical Stability Test of the Cream

Physical stability test of the optimal formula was carried out using the freeze-thaw method for 2 cycles (-20 °C for 24 hours and 25 °C for 24 hours) (Lachman *et al.*, 2008). After that, the physical parameters such as organoleptic & homogeneity, pH, and particle size were evaluated. Emulsion type was determined by means of methylene blue dye mixed on a glass plate containing sunscreen preparations and placed under a microscope. Viscosity test was carried out using a spindle viscometer no. 6 at a rotational speed of 20 rpm. Adhesion test was performed by applying 0.5 g of cream preparation on an object glass. Then, another glass was attached to it. The two fused glasses were then pressed with a 1 kg load for 5 minutes and the load was taken back. Spreadability test was carried out using a petri dish. As much as 0.5 g of cream preparation was smeared in the middle of the petri dish. Then another petri dish was attached and the diameter of the spread cream was calculated.

### **Chemical Stability Test of the Cream Preparation**

The chemical stability test of the optimal formula was carried out by an accelerated testing of the Arrhenius model. 20 mg of the sunscreen cream was placed in a test tube and then heated at a temperature of 60 °C, 70 °C, and 80 °C. Every 10 minutes, the test tubes were taken and immersed in an ice bath to stop the reaction. Then, the test tubes containing the sample were measured for absorbance to obtain 7 SPF points from 0 to 60 minutes. After that, the data was processed and the  $t_{1/2}$  and  $t_{90}$  were calculated (Lachman *et al.*, 2008; Chow, 2007).

### **Hedonic Test**

The hedonic test was carried out involving 30 respondents. The panelists' criteria were 15-35 years old. The sampling was conducted using convenience sampling, providing the respondents with a commercially available product and the sunscreen preparation. The respondents were not notified beforehand that they were given two different products to avoid any bias. Then, the respondents filled out a questionnaire containing enquiries about both products' color, aroma, and texture. The rating of the hedonic scale ranges from 1-9, where: (1) very, very dislike; (2) very dislike; (3) dislike; (4) somewhat dislike; (5) neutral; (6) somewhat like; (7) like; (8) really like; (9) very much like (Stone *et al.*, 2012).

### **Technical Data Analysis**

The results of the SPF test and the adhesion test were analyzed employing RStudio 1.3.1073 using Response Surface Methodology (RSM) to determine the optimal composition of the mixture. The data from the physical stability test of the optimal formula were analyzed using statistical analysis of the paired t-test and the Shapiro-Wilk test. The data from the chemical stability test were processed using simple linear regression. The hedonic test data were analyzed using RCBD ANOVA (Randomized Complete Block Design).

## **RESULTS AND DISCUSSION**

### **Formula optimization**

The data of the formula optimization was processed using the second-order RSM method to obtain an equation that models the relationship between the variable X (active substances concentration) and Y (response). The equations for the ethanolic extract of avocado leaves are  $Y1$  (SPF) =  $2,509X1 + 3,765X2 + 13,169$  and  $Y2$  (Adhesiveness) =  $2,725X1 - 2,212X1^2 + 7,268$ ; while for the ethanolic extract of mangosteen rind,  $Y1 = 2.444X1 + 2.075X2 + 15,457$  and  $Y2 = 2.744X1 - 2,230X1^2 + 7.256$ . The equations show that the concentration of ethanolic extract ( $X1=EXT$ ) and the concentration of OMC ( $X2=OMC$ ) have a positive effect on the SPF value ( $Y1$ ) significantly ( $p<0.05$ ), which indicates that the greater the concentration of EXT and OMC, the greater the SPF value. Meanwhile, the adhesion ( $Y2$ )

is only affected by the EXT ( $p < 0.05$ ) quadratic which indicates that there is a stationary value at a certain point marked by the resulting curve that tends to rise (Figure 1).

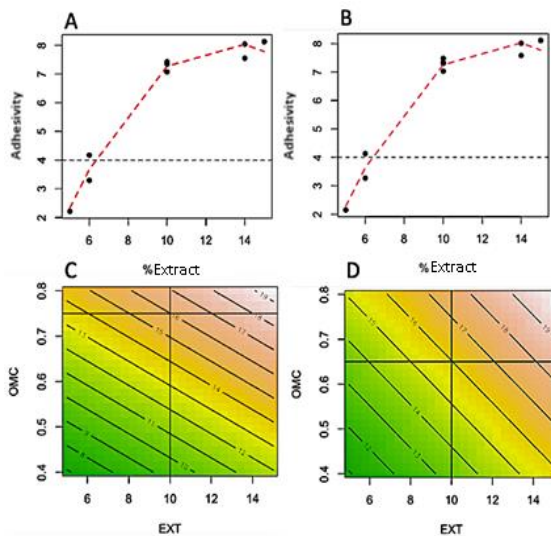


Figure 1. Contour plots of adhesion (A-B) and SPF (C-D) F1-F10. A,C = ethanolic extract of avocado leaves; B,D = ethanolic extract of mangosteen rind.

According to the literature, the requirement for a good adhesion is above 4 seconds (Fahr, 2018), meaning that in Figure 1, a stationary value (adhesion  $> 4$  seconds) is reached at the EXT concentration point of 10-14%. A good SPF value, according to the literature is above 15 which possesses a moderate protection (Wikantyasning *et al.*, 2021; Schalka *et al.*, 2011). The predetermined EXT concentration was used as the selected point on the X axis on the SPF contour plot (bottom image). Then, from the selected point, a line is drawn until it reaches the cut-off point on the SPF of 16 to meet the criteria of a good SPF value. After that, the cut-off point connected to the Y axis to find the OMC concentration which is 0.75% (combination with avocado leaves extract) and 0.65% (combination with mangosteen rind extract). The result showed that the desired SPF value and adhesion can be obtained with a fairly low concentration of EXT and OMC.

The concentration of EXT has a positive linear effect because the ethanolic extract of avocado leaves contains quercetin which absorbs the UV at around 200-400 nm, meaning that it provides SPF value (Kumar *et al.*, 2016; Pontoan, 2016). The ethanolic extract of mangosteen rind also contains xanthone compounds which have a similar wavelength range for absorbance (Ahmad *et al.*, 2013). OMC is a common substance used in a chemical or organic sunscreen that provides protection against UVB waves (290-320) (Serpone *et al.*, 2007). The optimal formulas in this study give an SPF value of  $16.52 \pm 0.16$  (ethanolic extract of avocado leaves + OMC) and  $15.84 \pm 0.15$  (ethanolic extract of mangosteen rind + OMC).



In the adhesion test, only the EXT variable displays a positive effect. The reason that the OMC does not play a role in the adhesion is because the OMC is a slightly viscous liquid which is quite slippery when exposed to the skin (Pubchem, 2021). Meanwhile, the EXT is a sticky viscous liquid and leaves an impression when exposed to the skin, therefore it significantly affects the adhesion. Adhesiveness is the ability of a semisolid preparation to adhere for a long time when used. The longer the adhesion of a preparation, the longer the contact time of the preparation with the skin (Allen *et al.*, 2014). The results of the optimal formula in terms of adhesion in this study are  $7.41 \pm 0.03$  seconds (ethanolic extract of avocado leaves + OMC) and  $7.43 \pm 0.04$  seconds (ethanolic extract of mangosteen rind + OMC).

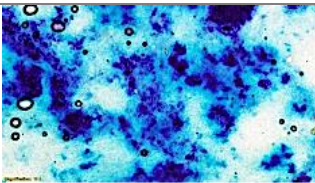
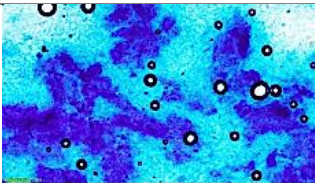
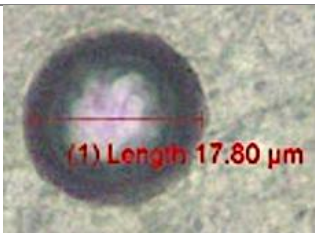
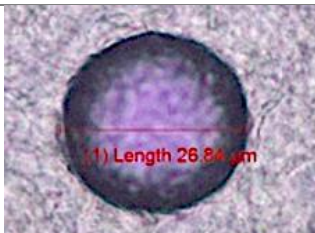
### Physical stability test

The data from the physical stability test were processed using statistical analysis of paired t-test and Shapiro-Wilk test. According to the literature, the p-value in the Shapiro-Wilk test above 0.05 indicates that both data are normally distributed and the p-value in the paired t-test above 0.05 indicates an insignificant difference (Gleichmann, 2022; Cahyono, 2015). Based on Table 3 and Table 4, the data generated before and after the freeze-thaw did not have deviations or said to be normally distributed. However, the p value of the t-test on the tested parameters showed a significant difference between before and after the freeze-thaw. In the organoleptic test, the preparations before and after the freeze-thaw did not show any difference in color, aroma, and texture. In the homogeneity test, the inhomogeneity in both treatments was indicated by green and brown spots on the surface. This can be attributed to the fact that the extract is crude (Malik *et al.*, 2017). However, these spots may disappear once they have been spread to the skin. In the pH test, both treatments showed the pH values ranging from 4.5 to 6.5, which is an ideal pH range for the preparation (Elmitra *et al.*, 2018).

Table 3. Physical stability test results (ethanolic extract of avocado leaves).

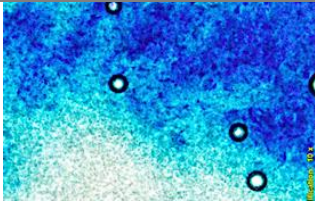
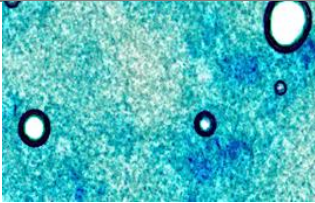
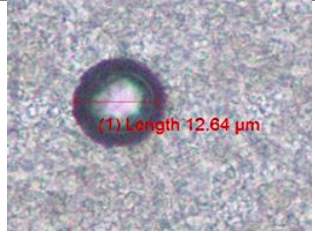
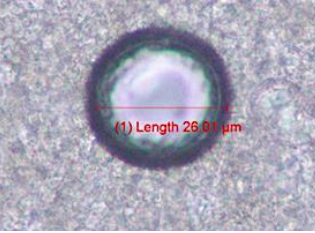
Parameter	Before Freeze-Thaw	After Freeze-Thaw	p value (t-test)	p value (Shapiro wilk test)
Organoleptic and homogeneity	The preparation is dark green and leaves-like with a distinctive rose aroma (from fragrance) and has a soft texture. The preparation is not homogeneous because there are green spots, but they disappear when applied to the skin.	The preparation is dark green and leaves-like with a distinctive rose aroma (from fragrance) and has a soft texture but is slightly denser. The preparation is not homogeneous because there are green spots, but they disappear when applied to the skin.	N/A	N/A
pH	$6 \pm 0$	$6 \pm 0$	N/A	N/A



Emulsion type	 O/W	 O/W	N/A	N/A
Particle size	 18.14 ± 0.374 μm	 24.69 ± 2.21 μm	0.025*	0.833
Viscosity	22896 ± 378.99 cps	36691 ± 87.797 cps	0.000*	0.686
Adhesion	7.407 ± 0.025 second	7.967 ± 0.085 second	0.013	1
Spreadability	6.6 ± 0.1 cm	5.7 ± 0.1 cm	0.004*	1

\*, p<0.05 showed that the data before and after freeze-thaw were significantly different based on the paired t-test.

Table 4. Results of physical stability test (ethanolic extract of mangosteen rind).

Parameter	Before Freeze-Thaw	After Freeze-Thaw	p value (t-test)	p value (Shapiro wilk test)
Organoleptic and homogeneity	The preparation is brown in color with a distinctive rose aroma (from fragrance) and has a soft texture. The preparation is not homogeneous because there are dark brown spots, but they disappear when applied to the skin.	The preparation is brown in color with a distinctive rose aroma (from fragrance) and has a soft texture but is slightly denser. The preparation is not homogeneous because there are dark brown spots, but they disappear when applied to the skin.	N/A	N/A
pH	5 ± 0	5 ± 0	N/A	N/A
Emulsion type	 O/W	 O/W	N/A	N/A
Particle size	 13.13 ± 1.80 μm	 24.66 ± 3.70 μm	0.013*	0.410
Viscosity	28175 ± 246.22 cps	38391 ± 430.36 cps	0.000*	0.800
Adhesion	7.43 ± 0.04 second	8.07 ± 0.12 second	0.004*	0,497

Spreadability	6.3 ± 0.1 cm	5.1 ± 0.1 cm	0.002*	1
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\*,  $p < 0.05$  showed that the data before and after freeze thaw were significantly different based on the paired t-test.

In the emulsion type test, both treatments resulted in an O/W system characterized by a blue external phase surrounding transparent colored droplets (Sinko, 2011). In the particle size test, the particle size of the preparation after the freeze-thaw was larger than that of the preparation before the freeze-thaw. However, both still meet the criteria, which is above 1  $\mu\text{m}$  (Fahr, 2018). The increase in particle size might be attributed to the temperature factor, where a lower temperature of the preparation will increase the particle size. This is due to the decrease in the surface tension of the oil and water phases of the cream, resulting in an increase in particle size (Yulianti *et al.*, 2015). In the viscosity test, the cream preparation also showed a statistically significant difference between before and after the freeze-thaw. The viscosity of the preparation after the freeze-thaw is greater than before the freeze-thaw. The increase is mainly due to the use of carbomer in the form of a polymer that forms a three-dimensional matrix with cross linking structure. After the freeze-thaw, it is suspected that the carbomer will further establish the matrix to absorb water, resulting in no phase separation (Forestryana *et al.*, 2020). In the adhesion test, the cream preparation after the freeze-thaw had a longer adhesion. The increase in the adhesion is directly proportional to the increase in viscosity (Istiqomah *et al.*, 2021). In the spreadability test, the preparation before the freeze-thaw had a greater spreadability. This can be attributed to the greater viscosity after the freeze-thaw, resulting in the decrease in the spreadability (Lumentut *et al.*, 2020). Although there were statistically significant differences in particle size, viscosity, adhesion, and spreadability of the formulation before and after freeze-thaw, these parameters were within acceptable limits and did not exhibit any physical instability such as emulsion type change, creaming, or cracking. The particles size increased significant but not more than 1  $\mu\text{m}$  and there was no change in emulsion form. The adhesion was still above the required value of 4 seconds, indicating that the formulation could remain on the skin for the required amount of time. Viscosity increased but remained within the acceptable range of 2000-50000 cPs. An increase in viscosity decreased the rate of sedimentation and increased emulsion stability according to Stokes' law. Spreadability decreased but was still within the accepted range of 5-7 cm (Fahr, 2018). Therefore, the formulation was considered stable after the freeze-thaw test.

### Chemical stability test

Based on the results of data processing, a plot of time vs  $\ln \text{SPF}$  was obtained by following the first-order reaction kinetics with a decreasing linear form (Figure 2). This is because the active substances in the cream preparation work by absorbing light, depending on their chemical structure. If the active substance is degraded, the SPF value decreases. The plot is then processed and a plot of  $1/T$

(temperature) vs  $\ln k$  is obtained, which follows the kinetics of the Arrhenius equation to form a linear plot with a negative slope (Figure 2). After an extrapolation, the estimated  $t_{1/2}$  (half-life) are 190 minutes for avocado leaves ethanolic extract with OMC and 111 minutes for ethanolic extract of mangosteen rind with OMC. The calculated  $t_{90}$  are 29 minutes for ethanolic extract of avocado leaves with OMC and 17 minutes for ethanolic extract of mangosteen rind with OMC. Further research is needed to validate the stability of the cream preparation at room temperature. This is due to the fact that the accelerated test of the Arrhenius model has a limitation, which is only valid for degradation reactions with an activation energy of 10-30 kcal/mol, meaning that if the product being tested undergoes photochemical reactions, decomposition due to freezing, contamination by microorganisms, and excessive agitation during distribution, the  $t_{90}$  cannot be determined accurately. It is not commonly used in accelerated stability tests in the pharmaceutical industry because it cannot predict a product's stability in real-time (Sinko, 2011). However, this method can be employed to acquire a preliminary data before a real-time stability test is carried out.

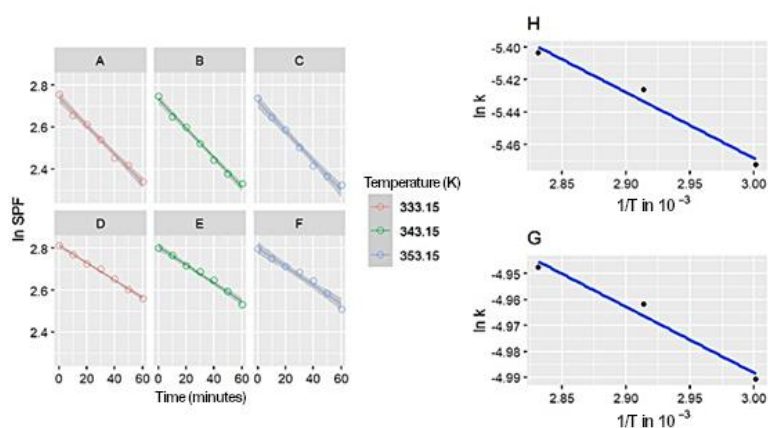


Figure 2. The accelerated stability test curve for sunscreen preparations is seen from changes in the SPF value (A-F) and the Arrhenius plot in the accelerated stability study (H-G). A-C, H = ethanolic extract of mangosteen rind; D-F, G = ethanolic extract of avocado leaves.

### Hedonic test

The hedonic test data in the form of assessment scores are presented in a histogram graph as a visualization of the frequency distribution (Figure 3). The mean, median, and mode of commercially available product and cream preparation are obtained for the color, aroma, and texture aspects. Then, it was analyzed using RCBD ANOVA to observe how significant the difference in hedonic scores is between the commercially available product and the test sample cream preparations. The result showed that the color and texture of the commercially available product and the test sample cream preparations are not significantly different in the hedonic scores, as indicated by the  $p$  value which is more than 0.05. However, the hedonic scores of the commercially available product and the test sample cream

preparations are significantly different with a p value of less than 0.05. Then, a further test was carried out using the Tukey HSD test and the average difference in the scores of the test sample cream preparations with the commercially available product is 0.833 and 1.1. This proves that the hedonic score for aroma of the test sample cream preparation is higher than the commercially available product (p values of 0.008 and 0.0006).

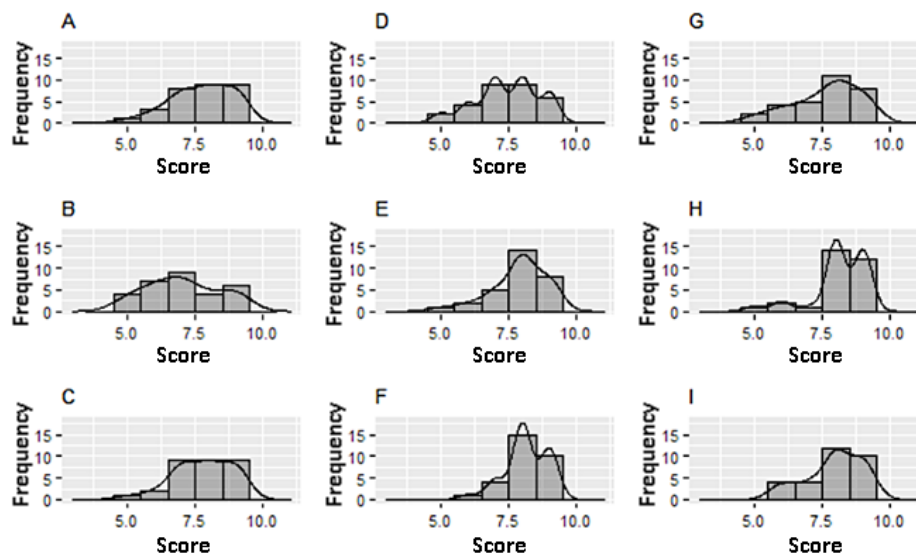


Figure 3. Histogram distribution of the commercially available product's hedonic test scores (A = color, B = aroma, C = texture), avocado leaves ethanolic extract (D = color, E = aroma, F = texture), and mangosteen rind ethanolic extract (G = color, H = aroma, I = texture). Based on the ANOVA test, there is no difference in the score of preference for color and texture among the sunscreen groups from the ethanolic extract of mangosteen rind, avocado leaves, and commercially available product ( $p < 0.05$ ). When viewed from the perception of aroma, the score on the commercial product is lower than that of the ethanolic extract of mangosteen rind and avocado leaves ( $p < 0.05$ ). However, there is no significant difference in the score of aroma assessment of the ethanolic extract of mangosteen rind and avocado leaves preparations ( $p > 0.05$ ).

### LIMITATION

The extract used in this study is crude, in a sense that unwanted substances such as chlorophyll, anthocyanin pigments, and other substances might interfere UV absorption which results in the decreasing SPF lower SPF values. The mechanism of the stabilization of the preparation after the freeze-thaw treatment has not been determined and explained. The chemical stability test using the Arrhenius model might not reflect the stability in a real time study. Hence, a real time stability test is necessary. The hedonic test is a preliminary test, a minimum of 100 respondents is needed to acquire a more valid data (Stone *et al.*, 2012).

### CONCLUSION

In this study, we successfully developed sunscreen cream preparations containing natural active substances using a combination of ethanolic extract of avocado leaves or mangosteen rind and OMC

with the optimal composition based on the SPF values and adhesion test is 10% extract, 0.75% and 0.65% OMC or avocado leaves and mangosteen rind extract respectively. Both formulas showed a decent physical stability using the freeze-thaw method and 30 subjects like the sunscreen. The  $t_{90}$  values for avocado leaves-containing sunscreen and mangosteen rind-containing sunscreen are 29 and 17 minutes respectively, employing an Arrhenius model accelerated stability test.

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### CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this article

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