



STABILITY TEST OF FACIAL MOISTURIZING CREAM FROM SNAIL SLIME (*Achatina fulica*) WITH VARIATIONS IN STEARIC ACID

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ABSTRACT

The skin is exposed to free radicals from the environment every day which can cause premature aging. The aging process is characterized by decreased sweat gland production, followed by decreased skin moisture due to reduced skin elasticity and the skin's ability to retain water, so the skin needs moisturizer. Snail slime (*Achatina fulica*) contains active substances glycosaminoglycan and allantoin with a concentration of 3% functioning as a tightener, softener and moisturizer for facial skin and contains achasin protein which has antibacterial activity against *Propionibacterium acnes* with an inhibitory power of $16.0 \text{ mm} \pm 2.6$ at a concentration of 11%. This study aims to determine the effect of achasin protein on *Propionibacterium acnes* on skin elasticity. to know the variation of stearic acid concentration and stability of facial moisturizing cream from snail slime. This study used an experimental method with 3% snail slime as the active ingredient and stability testing was carried out using 2 methods, namely cycling test and centrifugation. Snail slime was formulated into a cream preparation with varying stearic acid concentrations of 5%, 7%, 9% and 11%. Based on the results of the stability test using the cycling test method, it was found that all cream formulas were physically stable because they did not experience phase separation, while based on the results of the stability test using the centrifugation method, it was found that the cream in formula 4 was more physically stable when compared to formulas 1, 2 and 3 because it experienced phase separation at the 150th minute. Conclusion: The resulting facial moisturizer cream was stable in the cycling test method but not stable in the centrifugation method.

ABSTRAK

Kulit setiap hari mengalami paparan radikal bebas dari lingkungan yang dapat mengakibatkan penuaan dini. Proses penuaan ditandai dengan menurunnya produksi kelenjar keringat, lalu diikuti dengan kelembaban kulit yang menurun karena adanya daya elastisitas kulit dan kemampuan kulit untuk menahan air sudah berkurang, sehingga kulit memerlukan pelembab. Lendir bekicot (*Achatina fulica*) memiliki kandungan zat aktif *glycosaminoglycan* dan *allantoin* dengan penggunaan konsentrasi 3% berfungsi sebagai pengencang, pelembut dan pelembab kulit wajah serta mengandung protein *achasin* yang mempunyai aktivitas antibakteri terhadap *Propionibacterium acnes* dengan daya hambat sebesar $16,0 \text{ mm} \pm 2,6$ pada konsentrasi 11%. Penelitian ini bertujuan untuk mengetahui variasi konsentrasi asam stearat dan stabilitas krim pelembab wajah dari lendir bekicot. Penelitian ini menggunakan metode eksperimental dengan zat aktif lendir bekicot 3% dan uji stabilitas dilakukan dengan 2 metode yaitu *cycling test* dan *centrifugasi*. Lendir bekicot diformulasikan menjadi sediaan krim dengan

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variasi konsentrasi asam stearat sebesar 5%, 7%, 9% dan 11%. Berdasarkan hasil uji stabilitas menggunakan metode *cycling test* diperoleh bahwa semua formula krim stabil secara fisik karena tidak mengalami pemisahan fase sedangkan berdasarkan hasil uji stabilitas menggunakan metode *centrifugasi* diperoleh krim pada formula 4 lebih stabil secara fisik jika dibandingkan dengan formula 1, 2 dan 3 karena mengalami pemisahan fase pada menit ke-150. Krim pelembab wajah yang dihasilkan stabil pada metode *cycling test* tetapi tidak stabil pada metode *centrifugasi*.

INTRODUCTION

Skin is exposed to free radicals from the environment every day, which can lead to premature aging (Mulyawan, D and Suriana, N., 2013). Various environmental factors such as weather, smoking, diet, stress, alcohol, and fatigue can cause facial skin health problems. The main cause of premature aging experienced by Indonesians is excessive activity in the sun (Bogadenta, A., 2012). Aging is a natural process in human life closely related to various degenerative processes. The aging process is characterized by a decrease in sweat gland production, followed by decreased skin moisture due to reduced skin elasticity and the skin's ability to retain water. The process of skin pigmentation increases on the face, usually visible wrinkles/creases, dry and rough skin, dark spots, and decreased skin elasticity (Ardhie, MA., 2011). Snail slime creams can improve skin elasticity due to active compounds (Santika & Suhesti, 2022).

Snails are said to have many benefits from their meat to their mucus. Snails are a source of high-quality animal protein because they contain complete essential amino acids in addition to having a high iron content (Dewi, SP., 2010). Snail slime (*Achatina fulica*) contains active substances glycosaminoglycan and allantoin with moisturizing and skin regeneration properties (Choi & Kim, 2019; Lee & Park, 2020). The active substance glycosaminoglycan and allantoin content in snail slime (*Achatina fulica*) with the use of a concentration of 3% functions as a tightener, softener and moisturizer for facial skin (Putriawan, AL., 2012) and contains achasin protein which has antibacterial activity against *Propionibacterium acnes* with an inhibitory power of 16.0 mm \pm 2.6 at a concentration of 11% (Mardiana, ZH et al., 2015). Achasin protein has antibacterial activity against *Propionibacterium acnes* with inhibitory power (Kim et al., 2018).

Skin whitening/brightening is a cosmetic product used to lighten or remove unwanted skin discoloration. Skin lightening cosmetics are generally made in cream form. Creams have advantages including being easy to apply to the skin, easy to wash off after application, can be used on skin with wet wounds, and evenly distributed. A good cream preparation must be physically stable. Physical stability can be seen by the occurrence of creaming, flocculation, and clumping which can also be observed visually by the presence of phase separation, changes in emulsion viscosity and the occurrence of phase inversion (Lahman L, et al.,1994).

Cream is a topical preparation commonly used for local therapy (Nugroho dan Akhmad K. (2013). Creams are preferred by the public because they are easy to clean and spread (Ansel HC,et. el., 2012). The use of creams can also provide a cooling, shiny, and moisturizing effect on the skin. O/W type creams are made by dispersing oil and water. The advantage of O/W type creams is that they provide an optimum effect because they are able to increase the concentration gradient of active substances that penetrate the skin, thereby increasing percutaneous absorption (Engelin, 2013).

The use of stearic acid as an emulsifier in O/W type cream preparations can make the cream softer so that its viscosity value becomes low. A base with a high viscosity value will cause the drug diffusion coefficient value in the base to have a low value, so that the drug released from the base will be small (Lahman L, et al.,1994). In cream preparations, thickeners are used to regulate the viscosity and stability of the product. Cetyl alcohol is an

alcohol with a high molecular weight that functions as a thickening agent and stabilizer for oil-in-water preparations (Ansel HC, et. el., 2012).

Stability testing is one of the requirements before raw materials or final products can be sold to the general public. Stability testing uses accelerated stability testing with the cycling test method for 6 cycles (1 cycle of the formula stored at a temperature of $\pm 4^{\circ}\text{C}$ for 24 hours and $\pm 40^{\circ}\text{C}$ for 24 hours) observations every 1 cycle with the observed parameters namely organoleptic tests, homogeneity, pH, spreadability, adhesive power (Indriaty, et. al., 2019) as well as stability testing using the centrifugation method (Handali, S, et. al., 2011).

METHOD

1. Preparation of Ingredients

The materials used were obtained from the Pharmacy Laboratory of the Indonusa Polytechnic of Surakarta, and some chemicals were obtained from distributors or chemical stores. The snail slime was obtained from the Semanggi area of Surakarta City.

2. Snail Slime Collection

The snails obtained were not fed for 1 day, then their mucus was collected by scraping the surface of the snail's stomach and stored in a refrigerator. After the mucus was collected, they were fed for 1 day, then fasted again for 1 day, then the mucus was collected again in the same way and stored in the same way. Then, when it was to be used for the formula, it was filtered first (Indriaty, et. al., 2019).

3. Preliminary Test

Snail slime was observed organoleptically for shape, color, odor, and pH. Observations were conducted at the Pharmaceutical Technology Laboratory of the Indonusa Polytechnic of Surakarta and Muhammadiyah University of Surakarta.

4. Making Facial Brightening Cream

The design of the cream formula can be seen in Table 1.

Table 1. Facial lightening cream formula from snail slime

Material	FI (%)	F.II (%)	F.III (%)	F.IV (%)
Snail Slime	3	3	3	3
Stearic acid	5	7	9	11
Cetyl alcohol	2	2	2	2
Liquid paraffin	10	10	10	10
Yellow Vaseline	10	10	10	10
TEA	2	2	2	2
Propylene glycol	15	15	15	15
Glycerin	2	2	2	2
Methyl paraben	0.18	0.18	0.18	0.18
Propyl paraben	0.02	0.02	0.02	0.02
Alpha tocopherol	0.5	0.5	0.5	0.5
Aquadest	Ad 100	Ad 100	Ad 100	Ad 100

The cream preparation is made by melting the oil phase (stearic acid, cetyl alcohol, propyl paraben, yellow vaseline, liquid paraffin) while stirring and maintaining the temperature at 70°C (mixture I) and heating the water phase (aquadest, methyl paraben, glycerin, propylene glycol, TEA) while stirring and maintaining the temperature up to 70°C (mixture II). Then the emulsion is made by adding the water

phase into the oil phase little by little while stirring using a homogenizer until a cream phase is formed (mixture III). Then the active ingredient snail slime is added little by little into mixture III and stirred until homogeneous. Finally, alpha tocopherol is added to each formula and stirred until homogeneous. The concentration of snail slime used is 3% with variations in stearic acid concentration of 5%, 7%, 9% and 11%.

5. Evaluation of Preparations

Stability testing using the cycling test method was conducted by storing the cream preparation at a temperature of $\pm 4^{\circ}\text{C}$ for 24 hours and then transferred to a temperature of $\pm 40^{\circ}\text{C}$ for 24 hours (one cycle). Testing was carried out on cycles 1 to 6 (except for the cream type, syneresis, and viscosity tests) including:

a. Organoleptic test

Organoleptic testing was carried out visually, starting with observing the shape, color, and smell of the four cream preparations that had been made.

b. Homogeneity test

The homogeneity test is carried out by applying the cream preparation to a glass slide and then observing whether the preparation is homogeneous or not.

c. pH test

This test is performed using a pH meter. An electrode previously calibrated with acetate buffer (pH 4.0) and phosphate buffer (pH 7.0) is dipped into the cream preparation. The resulting pH is recorded. A good pH is one that matches the skin's pH (4.5-6.5).

d. Spreadability test

The spreadability test was carried out by weighing 0.5 g of cream and placing it right in the middle of a glass plate with a diameter scale underneath, then covering it with another glass plate that had been weighed and adding a load of 50, 100, 150, 200 and 250 g every 1 minute. The spreadability of semisolid preparations is divided into 2, namely semistiff (<5 cm) and semifluid (5-7 cm).

e. Adhesion test

A 0.5g weight of cream was placed on a glass slide whose area had been determined. Another glass slide was then pressed on top of the cream and held down with a 1kg load for 5 minutes. The glass slide was then placed on the test equipment. A load of 80g was released and the time it took for the two glass slides to separate was recorded. A good adhesive strength requirement is no less than 4 seconds.

f. Cream type test

This cream type test uses a staining method. The cream is smeared on a glass slide, then dripped with methylene blue and observed under a microscope. If the methylene blue spreads evenly, the cream is O/W; if the methylene blue separates, the cream is W/O.

g. Synergy test

A 10 g cream preparation was put into a cream pot and then stored at a temperature of $\pm 10^{\circ}\text{C}$, observations were made at 24, 48, and 72 hours. A good gel did not show any syneresis.

h. Viscosity test

The viscosity test was performed using a Brookfield LV viscometer by observing the numbers on the viscometer scale. The test was carried out on the 0th cycle after the preparation was made and the 6th cycle. A 300 g cream preparation was placed in a container, then the appropriate spindle was lowered into the preparation to the specified limit, running the spindle at a certain speed and observing the fixed scale. The multiplication factor can be seen in the table according to the speed and spindle used.

i. Stability Test

- 1) Cycling test method
This test uses the cycling test method, which is an accelerated stability test for the preparation where one cycle of the cream preparation is stored at a temperature of 4°C for 24 hours, then removed and placed at a temperature of 40°C for 24 hours. The experiment was repeated for 6 cycles, each cycle was observed with several parameters to see whether or not there were changes in the physical properties of the cream preparation (Indriaty, et. al., 2019).
 - 2) Centrifugation
Cream Stability Testing by Centrifugation 5 g of cream sample was placed in a centrifuge tube and centrifuged at 3,750 rpm for 5 hours or 5,000-10,000 rpm for 30 minutes. This test was conducted to determine the presence of phase separation in the cream preparation (Handali,S, et. al., 2011).
6. Data Collection and Data Analysis Techniques
- a. Data collection technique
Due to the experimental nature of this research, data collection was conducted in the laboratory. The data obtained from this study will include stability testing of the snail slime facial lightening cream preparation using organoleptic, homogeneity, pH, spreadability, adhesion, viscosity, flow properties, cream type, and synergy tests. This data will then be further analyzed.
 - b. Data analysis
Data analysis from the results of the physical test of snail slime cream was carried out qualitatively with a descriptive approach.

RESULTS AND DISCUSSION

1. Preliminary Test

In this study, the samples used were snails obtained from the Semanggi area, Surakarta City. The snails to be used were not fed for 1 day, then their mucus was taken by scraping the surface of the snail's stomach and stored in a refrigerator. After the mucus was taken, they were fed for 1 day, then fasted again for 1 day, then the mucus was taken again in the same way and the same storage. Then, when it was to be used for the formula, it was filtered first (Indriaty, et. al., 2019). The snail slime was observed organoleptically, covering color, odor and shape as well as a pH test, the results of which were as shown in Table 2.

Based on Table 2, it is stated that snail slime has basic properties with a pH of 8.08 because snail slime contains active substances glycosaminoglycan and allantoin which function as tighteners, softeners and moisturizers for facial skin (Putriawan, AL., 2012) and achasin protein which has antibacterial activity against *Propionibacterium acnes* (Mardiana, ZH et. al., 2015).

Table 2. Results of preliminary snail slime tests

Color	White
Smell	Odorless
Form	Liquid
pH	8.08

2. Physical Evaluation

Natural ingredient-based creams require thorough physical and chemical stability testing (Rahmawati et al., 2021). The physical stability tests performed are as follows:

a. Cream Type Test

Determination of the emulsion type is carried out to determine the type of W/O or O/W in a cream preparation. The results of the cream emulsion type test indicate that the cream has an O/W emulsion type through the methylene blue solution dye dispersion test. This is because the volume of the dispersed phase (oil phase) used in the cream is smaller than the dispersing phase (water phase), so that the oil globules will be dispersed into the water phase and form an O/W type emulsion. O/W cream formulations show better spreadability and moisturizing effects (Putri & Santoso, 2018). The results can be seen in Figure 1.

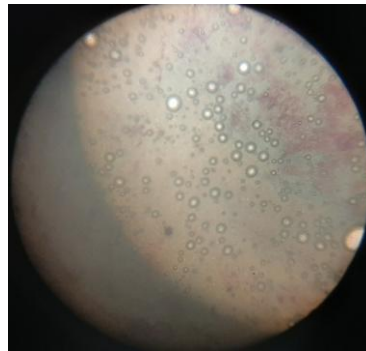


Figure 1. Type of O/W Emulsion

b. Synergy Test

Syneresis is the process by which water escapes from a cream, where the cream contracts, squeezing water out of the cells, resulting in a smaller, denser appearance. A high syneresis rate indicates a cream that is physically unstable when stored at temperatures below 10°C. During the syneresis measurements, the cream was stored in a refrigerator at ±10°C for 24, 48, and 72 hours. The results showed that the cream in formula 4 had the highest syneresis value (10.007%) at the 72nd hour (Figure 2) This means that the water that comes out of the formula 4 cream is the most compared to formulas 1, 2, and 3, so it is the least physically stable (Ansel HC, et. al., 2012). Factors that influence syneresis include acidity and water binding capacity (Sawitri ME, et. al., Manap A & Palupi TWL. (200)

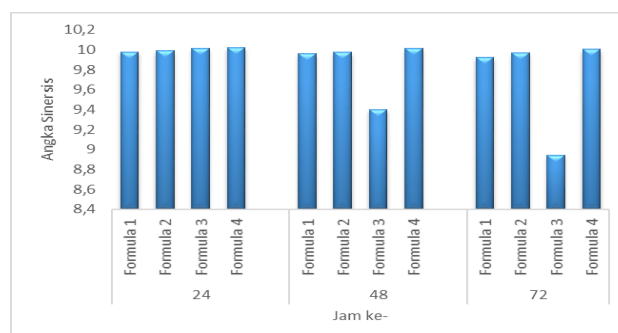


Figure 2. Synergy value of cream at 24, 48 and 72 hours

c. Viscosity Test

Viscosity is an expression of the resistance of a liquid to flow. The higher the flow viscosity, the greater the resistance. Viscosity affects the rate of drug absorption from the digestive tract (Sawitri ME, et. al., 2010), the thicker the longer the drug absorption. The cream preparation was measured for its initial viscosity at week 0 and in a cycling test for 6 cycles at a temperature of $\pm 4^{\circ}\text{C}$ for 24 hours then transferred to a temperature of $\pm 40^{\circ}\text{C}$ for 24 hours (one cycle) (Figure 3). The results showed that all cream formulas experienced fluctuations in viscosity. In accordance with Newton's flow, the greater the viscosity of a liquid means the greater the unit area force (shearing stress) required to produce a certain rate of shear (Ansel HC, et. al., 2012).



Figure 3. Results of viscosity measurements during 6 cycles

d. Cream Stability Test by Cycling Test Method

The cream was examined for organoleptic parameters, homogeneity, pH, spreadability and adhesiveness for 6 cycles by storing the cream preparation at a temperature of $\pm 4^{\circ}\text{C}$ for 24 hours then transferred to a temperature of $\pm 40^{\circ}\text{C}$ for 24 hours (one cycle). The organoleptic results can be seen in Table 3. Based on Table 3 organoleptically, the resulting cream is stable in color and odor and does not experience phase separation during 6 storage cycles and the resulting cream is homogeneous. The results of the observations can be seen in Table 4.

Table 3. Results of organoleptic stability tests

The cycle	Parameter	Formula			
		I	II	III	IV
0	Phase separation	There isn't any	There isn't any	There isn't any	There isn't any
	Color	White	White	White	White
	Smell	Odorless	Odorless	Odorless	Odorless
1	Phase separation	There isn't any	There isn't any	There isn't any	There isn't any
	Color	White	White	White	White
	Smell	Odorless	Odorless	Odorless	Odorless
2	Phase separation	There isn't any	There isn't any	There isn't any	There isn't any
	Color	White	White	White	White
	Smell	Odorless	Odorless	Odorless	Odorless
3	Phase separation	There isn't any	There isn't any	There isn't any	There isn't any

	Color	White	White	White	White
	Smell	Odorless	Odorless	Odorless	Odorless
4	Phase separation	There isn't any	There isn't any	There isn't any	There isn't any
	Color	White	White	White	White
	Smell	Odorless	Odorless	Odorless	Odorless
5	Phase separation	There isn't any	There isn't any	There isn't any	There isn't any
	Color	White	White	White	White
	Smell	Odorless	Odorless	Odorless	Odorless
6	Phase separation	There isn't any	There isn't any	There isn't any	There isn't any
	Color	White	White	White	White
	Smell	Odorless	Odorless	Odorless	Odorless

Table 4. Results of homogeneity stability test

The cycle	Formula			
	I	II	III	IV
0	Homogeneous	Homogeneous	Homogeneous	Homogeneous
1	Homogeneous	Homogeneous	Homogeneous	Homogeneous
2	Homogeneous	Homogeneous	Homogeneous	Homogeneous
3	Homogeneous	Homogeneous	Homogeneous	Homogeneous
4	Homogeneous	Homogeneous	Homogeneous	Homogeneous
5	Homogeneous	Homogeneous	Homogeneous	Homogeneous
6	Homogeneous	Homogeneous	Homogeneous	Homogeneous

This pH measurement aims to determine whether the cream made is safe and does not irritate the skin when used. According to (Tranggono and Latifah, 2007) the pH requirements for a good topical preparation are in accordance with the natural pH of the skin, namely 4.5-6.5. The results of the pH test showed that all the resulting cream formulas exceeded the pH range of a good topical preparation. pH formula cream affects skin irritation potential and should be optimized (Fitria & Handayani, 2019). This was because the active substance used (snail slime) had a basic pH, namely (8.08) and several compositions of ingredients used in the formula also had a basic pH, so that the resulting cream preparation had a neutral pH and tended to be basic (Budiman A., 2012). The results of the pH measurement can be seen in Figure 4.

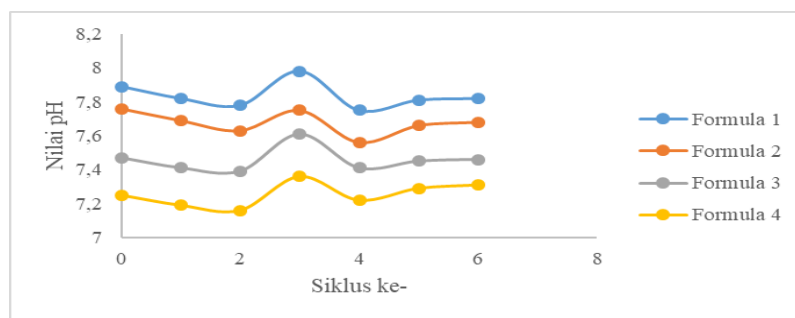


Figure 4. Results of pH measurements during 6 cycles

The spreadability test aims to determine the ability of the cream base to spread so that it can be seen how easily the preparation is applied to the skin. Good spreadability causes contact between the drug and the skin to be broad, so that absorption into the skin occurs quickly. According to Garg (2002), a comfortable spreadability diameter for use for semisolid preparations is 5-7 cm. The results of the spreadability test indicate that all formulas meet the standards for good spreadability of preparations. The results can be seen in Figure 5.

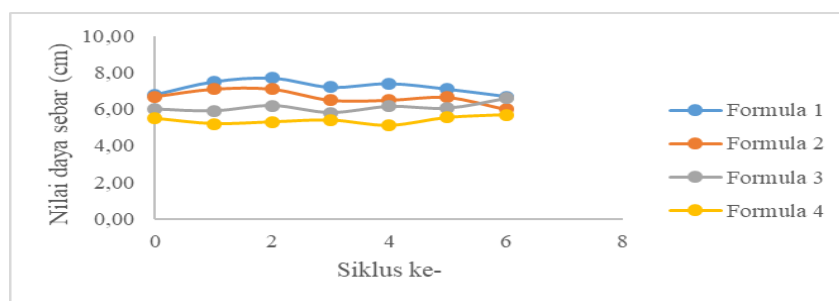


Figure 5. Results of measuring the spreading power during 6 cycles

The adhesion test aims to determine the time required for the cream to adhere to the skin. Good adhesion allows the cream to not easily come off and adheres to the skin longer, thus producing the desired effect. Variations in stearic acid concentration affect cream stability and viscosity (Sukmawati & Wulandari, 2017; Nuraini & Hidayat, 2020). According to (Rachmalia et al., 2016) the requirement for good adhesion for topical preparations is more than 4 seconds. The results of the adhesion test showed that all formulas did not meet the requirements for good topical preparations. The results of the adhesion test can be seen in Figure 6.

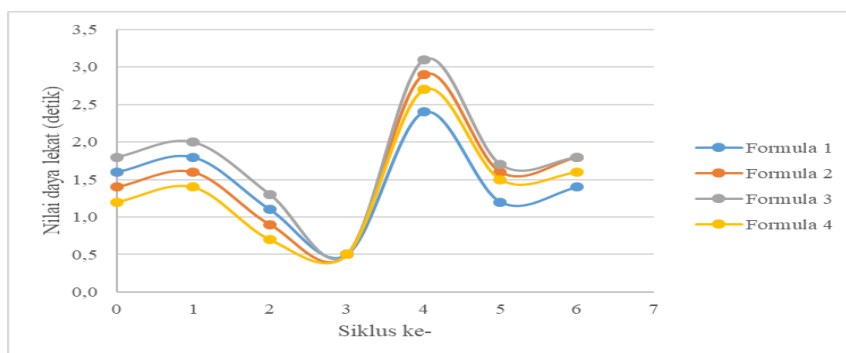


Figure 6. Results of measuring the spreading power during 6 cycles

e. Cream Stability Test by Centrifugation Method

Table 5. Results of cell cream centrifugationama 5 hours

Minute to-	Formula ke-	Whether separation occurs or not
30	F1	There is no separation
	F2	There is no separation
	F3	There is no separation
	F4	There is no separation
60	F1	There is foam
	F2	There is foam
	F3	There is foam
	F4	There is foam
90	F1	There is still foam (not increasing)
	F2	There is still foam (less foam)
	F3	No foam
	F4	No foam
120	F1	There is still foam
	F2	There is still foam
	F3	There is thin foam
	F4	No foam
150	F1	There is a separation
	F2	There is a separation
	F3	There is a separation
	F4	There is no separation
180	F1	There is a separation
	F2	There is a separation
	F3	There is a separation
	F4	There is no separation
210	F1	There is a separation
	F2	There is a separation
	F3	There is a separation
	F4	There is no separation
240	F1	There is a separation
	F2	There is a separation
	F3	There is a separation
	F4	There is no separation
270	F1	There is a separation

	F2	There is a separation
	F3	There is a separation
	F4	There is no separation
300	F1	There is a separation
	F2	There is a separation
	F3	There is a separation
	F4	There is no separation

This test was conducted to determine the stability of the cream after high-speed shaking using a centrifuge. The cream was placed in an Eppendorf tube at 3,750 rpm for 5 hours, equivalent to the effect of gravity for 1 year. The centrifugation test results showed that F4 cream did not experience phase separation and was physically stable, while creams in F1, F2, and F3 experienced separation at 150 minutes. The centrifugation method is widely used to predict physical stability of creams (Wijaya & Sari, 2016).

CONCLUSION

Facial moisturizing creams from snail slime with stearic acid concentrations of 5%, 7%, 9%, and 11% were successfully formulated. Stability tests using the cycling test method showed all formulas were physically stable without phase separation over 6 temperature cycles. However, centrifugation tests revealed that the formula with 11% stearic acid (formula 4) was more stable than the others. The cream's pH tended to be basic due to the snail slime and other ingredients, indicating a need for adjustment to better match skin pH. All formulas exhibited good spreadability, but adhesion needs improvement. Overall, formula 4 demonstrated the best stability and potential as an effective facial moisturizer. Further research is recommended to optimize pH, adhesion, and conduct clinical safety and efficacy tests.

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