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Formulation, Characterization, and In Vitro Evaluation of Sunscreen Cream Containing Kenikir (*Cosmos caudatus* Kunth.) Extract and Chicken Bone Collagen Hydrolysate

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Abstract

Background: *Sunlight is a major contributor to skin damage, including erythema, pigmentation, premature aging, and other related conditions. Kenikir extract contains flavonoid derivatives, which have the potential to act as sunscreen due to the presence of chromophore groups that can reduce the intensity of sun exposure on the skin. In this study, sunscreen was made from kenikir extract with the addition of collagen hydrolysate.* ***Objective:*** *This study aims to assess the effectiveness and characteristics of various sunscreen cream formulations produced.* ***Methods:*** *Kenikir extract was obtained through ethanol-based maceration, while collagen hydrolysate was prepared by isolating chicken bone collagen using acetic acid solvent, followed by enzymatic hydrolysis with bromelain. Four sunscreen cream formulations were developed using various concentrations of kenikir extract and collagen hydrolysate. The creams were characterized by evaluating sun protection factor (SPF), percent erythema, percent pigmentation, organoleptic properties, acceptance testing, homogeneity, pH, viscosity, centrifugation, and spreadability.* ***Results:*** *The cream containing kenikir extract was effective as sunscreen with an SPF of 45.59, erythema of 1.96%, and pigmentation of 1.27%. It also exhibited homogeneity, with a pH of 7.83 and a viscosity of 46,700 cps, which met the permissible range for sunscreen use. The stability test indicated that it was stable, with no separation observed.* ***Conclusion:*** *Sunscreen cream containing kenikir extract has the potential to protect the skin from excessive sun exposure due to the effectiveness and good characteristics according to the Indonesian National Standard (SNI).*

Keywords*: collagen hydrolysate, kenikir extract, sunscreen, SPF*

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INTRODUCTION

The primary cause of skin damage, including erythema, pigmentation, premature aging, and skin cancer, is ultraviolet (UV) radiation (Ahmad & Agus, 2013). Cosmetics are used to protect the skin both physically and chemically. Sunscreen is a component contained in topical formulations that has the ability to interact with UV radiation (Pasha, 2021). Synthetic chemicals are typically used in the cosmetic industry to produce sunscreens. However, the use of synthetic sunscreens sometimes causes irritation with a burning or stinging sensation and allergic photocontact reactions. As a result, natural ingredients are now being developed as sunscreens (Savira & Iskandar, 2020). To ensure effective photoprotection, natural sunscreens must contain at least one active ingredient with antioxidant properties (Ismail, 2013). Antioxidants in sunscreen formulations function to prevent or minimize UV-induced oxidative damage, increase the effectiveness of photoprotection, and reduce skin aging (Jesus *et al*., 2023).

Kenikir (*Cosmos caudatus* Kunth.) extract is rich in flavonoids derivatives such as quercetin, kaempferol, myricetin, catechin, luteolin, apigenin, quercetin 3-O-rhamnpside (quercitrin, quercetin 3-O-glucoside, quercetin 3-O-xyloside, quercetin 3-O-arabinofuranoside), and rutin that have the potential as sunscreen agents. Phenolic compounds, especially flavonoids, contain chromophore groups that can absorb UV radiation, thereby reducing the intensity of exposure to the skin *(*Lisnawati *et al*., 2019). Chromophore groups are unsaturated covalent structures that can absorb radiation in the ultraviolet and visible regions (Wardani *et al*., 2020).

Another ingredient that can be added to sunscreen formulations is collagen hydrolysate, which has been widely used as a substitute for synthetic antioxidants because it is safer, more nutritious, and therapeutically beneficial *(*Aguirre-Cruz *et al*., 2020). Collagen hydrolysate has the ability to inhibit the tyrosinase enzyme activity, which leads to skin pigmentation. In addition, when applied topically, it can hydrate the skin, increase skin elasticity, and eliminate wrinkles (Prokopova *et al*., 2021). Chicken bone waste is a potential and underutilized raw material source for collagen hydrolysate production.

A previous study has indicated that a nanocream prepared from kenikir leaves exhibited an SPF of 7.26 (Rahman & Herdaningsih, 2021). However, the study did not involve any viscosity tests, centrifugation tests, or determination of erythema and pigmentation values. In addition, a study by Wang *et al*. (2019) showed that collagen hydrolysate derived from chicken skin can increase the viability and production of pro-collagen 1, reduce the levels of reactive oxygen species (ROS), MMP-1, and MMP-9, induce phosphorylation of the discoidin domain receptor 2 (DDR2), and inhibit UV-induced phosphorylation of Akt and ERK1/2. Thus, kenikir extract and collagen hydrolysate derived from chicken bone are potential natural ingredients for sunscreen.

In this study, sunscreen creams were prepared using kenikir extract and collagen hydrolysate. Four cream formulations were developed, including a control cream, a kenikir extract cream, a collagen hydrolysate cream, and a combination cream. These formulations were evaluated in accordance with SNI 16-4399-1996 standards. The analyses carried out included tests for homogeneity, viscosity, pH, and SPF. In addition, the creams were subjected to quality assessments through organoleptic and acceptance tests, spreadability tests, centrifugation tests, and determination of erythema and pigmentation values.

MATERIALS AND METHODS

Materials

The materials used in this study were kenikir leaves obtained from Telagareja Market, Yogyakarta, chicken bones, 96% ethanol (technical grade), ethanol (Merck), glacial acetic acid (Merck), NaOH (Merck), aquades, bromelain enzyme (HIMEDIA), disodium hydrogen phosphate (Merck), sodium dihydrogen phosphate (Merck), stearic acid (technical grade), cetyl alcohol (technical grade), mineral oil (technical grade), glycerin (technical grade), dimethycone (technical grade), triethanolamine (TEA) (technical grade), and methyl paraben (technical grade).

Equipment

The tools used in this study were a set of glassware, an analytical balance, a grinder, a 30-mesh sieve, an oven, a basic equipment set consisting of a waterbath, condenser, and vacuum pump to replace a rotary vacuum evaporator, a shaker incubator (Witeg Wisd Shaking Incubator WIS-30), fume hood, mortar and pestle, magnetic stirrer, centrifuge (Labogene 406), Brookfield viscometer (DV-II+ Pro), freeze dryer, UV-Vis spectrophotometer (Hitachi U-1800), and FTIR spectrophotometer (Thermo Scientific Nicolet Is10).

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Methods

Maceration of kenikir leaves

Kenikir leaves were cleaned, dried, and ground using a grinder and sieved through a 30-mesh sieve. A total of 250 grams of kenikir leaf powder was macerated in 96% ethanol solvent at a ratio of 1:4 for 3 x 24 hours. The extract was then filtered using a filter paper. The filtrate obtained was then evaporated using a substitute apparatus for a rotary evaporator (Widiyantoro & Harlia, 2020).

Preparation of collagen hydrolysate

The preparation of collagen hydrolysate was adapted from the method described by Putri and Ningsih (2018). Cleaned chicken bones were ground using a blender and air-dried. A total of 100 grams of chicken bone powder was demineralized by soaking in 1N acetic acid solvent using a shaker incubator for five hours at room temperature. The demineralized bone was then neutralized with 10% NaOH and rinsed with distilled water. The mineral-free chicken bone powder was then extracted with acetic acid solvent at a ratio of 1:5 using a shaker incubator for five hours at 40°C. The filtrate and residue from the extraction were then filtered using a filter paper. Furthermore, the filtrate was neutralized with 10% NaOH. The filtrate was then decanted, and only the white precipitate was collected. The white precipitate was then centrifuged at a speed of 4,000 rpm and the wet solid was oven-dried at 40°C for three days to produce dry collagen. Chicken bone collagen was then hydrolyzed using bromelain enzyme at a concentration of 0.002 grams/ml in 600 ml phosphate buffer solution at 55°C for four hours in a shaker incubator. The enzyme was then inactivated by heating at 100°C for 10 minutes, then centrifuged. Finally, the resulting wet solid was dried using a freeze dryer.

Sunscreen cream formulation

The oil phase in beaker I contain stearic acid, cetyl alcohol, mineral oil, and dimethylcone, was heated at 75°C. In beaker II, the water phase containing TEA, glycerin, methyl paraben, and distilled water was prepared. The water phase was gradually added to the oil phase with continuous stirring until the mixture was homogeneous. Following the formation of the oil-in-water emulsion, the active ingredients were added to the mixture as specified in Table 1, and the mixture was stirred for 25 minutes until homogeneous (Elcistia & Zulkarnain, 2018).

Organoleptic and acceptance tests

Organoleptic tests were performed through direct observation of the shape, color, and odor of the sunscreen cream formulations (Prastya, 2019). Acceptance tests were conducted by 27 panelists by assessing the color, odor, and texture of the cream using a 7-point scale, where 7 indicated very much like, 6 much like, 5 like, 4 neutral, 3 dislike, 2 somewhat dislike, and 1 very much dislike (Guttifera *et al*., 2020).

Table 1. Sunscreen cream formulations in gram

Material	Formula			
	A	B	C	D
Kenikir Extract	-	-	0.3	0.3
Hydrolyzed Collagen	-	2	-	2
Stearic Acid	7.5	7.5	7.5	7.5
Cetyl Alcohol	2	2	2	2
Mineral Oil	2.2	2.2	2.2	2.2
Dimethylcone	4	4	4	4
TEA	1.5	1.5	1.5	1.5
Glycerin	1.8	1.8	1.8	1.8
Methyl Paraben	0.2	0.2	0.2	0.2
Aquadest	Add 100	Add 100	Add 100	Add 100

*A: basic cream; B: collagen hydrolysate cream; C: kenikir extract cream; D: collagen hydrolyzate-kenikir extract cream.

Homogeneity test

The cream was applied to a glass slide, spread, and observed visually by touch. A homogeneous cream formulation was indicated by the absence of coarse grains in the cream (Prastya, 2019).

pH test

A pH meter was used to measure the dissolved cream (1 g) in 100 ml of distilled water (Prastya, 2019).

Spreadability test

A 500 mg sample of the cream was placed at the center of a graduated round glass and another weighed round glass was placed on top of the cream for one minute. The diameter of the cream that spread after adding a load of 100 grams, 150 grams, or 200 grams was then recorded (Prastya, 2019).

Viscosity test

The cream was put into a container, and spindle No. 64 was installed. The rotor was operated at a speed of 12 rpm with a torque percentage of 11%. After the reading of the Brookfield viscometer stabilized, the value was recorded and multiplied by the spindle correction factor (Suhery *et al*., 2023).

Centrifugation test

The cream was put into a centrifuge tube and centrifuged at 3,700 rpm for five hours. This test aimed

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to detect the presence of phase separation in the cream formulation (Pratasik *et al.*, 2019).

Determination of spf value, % erythema, and % pigmentation

A total of 2 grams of cream was dissolved in 10 ml of ethanol PA and mixed until homogeneous and then filtered using a filter paper. Subsequently, the absorbance at a wavelength of 290-400 nm and its transmittance at a wavelength of 292.5-372.5 nm were measured at intervals of 5 nm. The results of absorbance and transmittance were used to calculate SPF, percentage of erythema, and percentage of pigmentation (Syahrani, 2015).

Data analysis

The pH, viscosity, spreadability, SPF, and percentage transmission for erythema and pigmentation were analyzed using IBM SPSS Statistics version 29.0.1.0. The data obtained were tested for normality (using the Saphiro-Wilk test) and homogenity (using the Levene's test). If the data were normally distributed, analysis was continued with one-way ANOVA. However, if the data were not normally distributed, it was continued with the Kruskal-Wallis test. Data from the homogeneity, organoleptic, and stability tests were analyzed descriptively. Acceptance test data (color, odor, and texture) were analyzed statistically using univariate analysis, followed by Duncan's post-hoc test if significant differences were found (Mayangsari *et al*., 2022).

RESULTS AND DISCUSSION

Kenikir extract

The maceration process of kenikir leaves used ethanol as a solvent due to its polarity and ability to dissolve flavonoid compounds. In addition, ethanol has good absorption capacity, neutrality, resistance to microbial growth, non-toxicity, and relatively low evaporation temperature. During maceration, the ethanol solvent penetrated the cell walls of kenikir leaves and entered the cell cavity containing active compounds. These compounds dissolved into the solvent and moved out of the cell due to the difference in concentration between the solution inside and outside. The process was repeated until a balanced concentration between the solution outside and inside the cell was reached (Najib, 2018). The resulting kenikir extract had a yield of 4.168% and a texture similar to blackish brown cotton candy, with a unique aroma.

Hydrolyzed chicken bone collagen

Chicken bone collagen was obtained through the process of reducing the size of chicken bones, drying, acid-based demineralization, and extraction (Nurjanah, 2020). To increase the bone surface during the extraction process, it is necessary to cut the chicken bones (Rahmawati & Nurjanah, 2020). The addition of acid in the demineralization process aims to remove minerals (impurities) and loosen the bone structure. The demineralization process results in a highly acidic pH, thereby necessitating neutralization with NaOH. During the extraction process with acid solvents, the triple helix structure of the collagen is broken down (Putri & Ningsih, 2018). Following re-neutralization and decantation, collagen was obtained with a yield of 4.802%. The collagen was then hydrolyzed using the bromelain enzyme to produce short peptides. The resulting collagen hydrolysate was then analyzed using an FTIR spectrophotometer to compare its functional group profile with that of collagen and commercially available collagen hydrolysates.

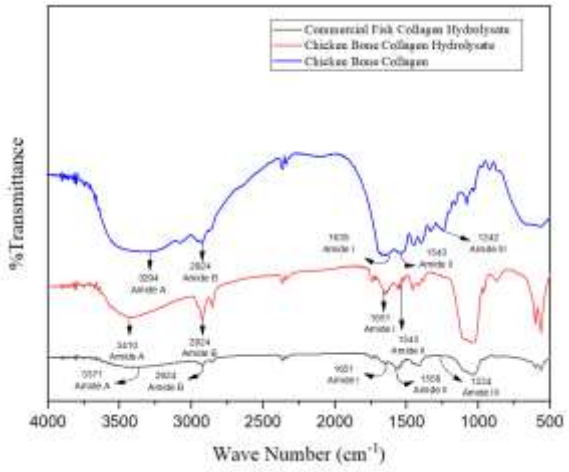

Figure 1. FTIR of chicken bone collagen, commercial collagen hydrolysate, and chicken bone collagen hydrolysate

Collagen has standard absorption peaks in the regions of the amide bands A and B as well as I, II and III. Figure 1 shows that chicken bone collagen had an absorption peak in the amide III region at 1234 cm^{-1}. After hydrolysis using bromelain, this peak was no longer observed within the amide III range (1229 cm^{-1}-1301 cm^{-1}), while commercial fish collagen hydrolysate showed a low-intensity admide III absorption. The absence or reduction in intensity of amide III confirmed that the triple helix structure of the collagen transformed into a random coil shape due to the dissociation of hydrogen bonds during hydrolysis. Schmidt *et al.* (2020)

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compared the FTIR spectra of collagen and collagen hydrolysate produced by treatment with the flavourzyme and alcalase enzyme. The spectrum of collagen hydrolyzed with the flavourzyme enzyme had a low-intensity amide III absorption peaked at 1450 cm^{-1}, and the absorption disappeared in hydrolysate produced using the alcalase enzyme. Enzymatic treatment can break the bonds in polypeptide chains, thereby generating a higher yield of peptides (León-López *et al* ., 2019).

SPF test

According to Figure 2, cream A containing no active ingredients was ineffective as a sunscreen because the SPF was below the minimum protection value. Cream B also lacked sunscreen efficacy, with an SPF of 1.539 ± 0.001, while cream C containing kenikir extract was a good sunscreen because the SPF value met the ultra-protection category. Cream D, comprised of active ingredients such as kenikir extract and collagen hydrolysate, was also effective as a sunscreen although it exhibited a lower SPF of 38.91 ± 0.799.

Table 2. SPF test results

Code	Formula	SPF Value	Standard Deviation
A	Base Cream	1.139	0.001
B	Hydrolyzed Collagen Cream	1.539	0.018
C	Kenikir Extract Cream	45.59	4.049
D	Combination Cream	38.91	0.799

After determining the SPF, the Saphiro-Wilk normality test and the homogeneity test were conducted using SPSS. The results indicated that the SPF data were not normally distributed ($p = 0.009 < 0.005$) and not homogeneous ($p < 0.001$). Therefore, the analysis was continued with a non-parametric test, namely the Kruskal-Wallis test, whose results showed no significant differences among the four sunscreen formulations ($p = 0.080 > 0.05$)

The hydrolyzed collagen cream was not effective as a sunscreen because of the presence of peptides with varying molecular weights resulting from the lack of a purification process. Song *et. al* (2017) reported that the molecular weight of collagen peptides affects the effectiveness in repairing photoaging on the skin. The administration of collagen peptides with low molecular weights (0.5 kDa < BM < 1 kDa) demonstrated greater efficacy in repairing photoaging on mouse skin than collagen peptides with high molecular weights (>1 kDa). This is attributed to the greater availability of electron donors and enhanced free radical scavenging capcacity of low molecular weight collagen peptides. The composition and sequence of their amino acids also play a role in the effectiveness of collagen peptides. A high composition of hydrophobic amino acids is present in collagen peptides with high antioxidant activity, including pro, his, tyr, trp, met, and cys amino acids. In addition, hydrophobic amino acids at the C and N terminals such as Ala, Phe, Leu, and Prp contribute significantly to antioxidant activity (Li *et al.*, 2021).

Compared to the base cream, adding kenikir extract alone significantly increased the SPF. This is attributed to the presence of flavonoid compounds in the kenikir extract. According to Rafi *et al*. (2023), kenikir contains isoquercitrin, quercetin-3-O-rutinoside, avicularin, rutin, quercitrin, vitexin compounds which are known for their antioxidant properties. These compounds can protect the skin from sun exposure due to the chromophore groups in the form of an aromatic ring that has the ability to absorb UV radiation and emit light with lower energy. This mechanism helps prevent the adverse effects of exposure to UV radiation.

The combination cream, which contains both kenikir extract and collagen hydrolysate, offered excellent protection against UV rays, but its SPF (38.9075) was lower than that of the cream containing only kenikir extract. The UV absorption capacity of the combination cream may be compromised possibly due to the antagonistic interactions between kenikir extract and collagen hydrolysate, resulting in lower SPF. Such interactions have been reported in previous studies, including between soy protein hydrolysate and polyphenolic flavonoid cyanidine-3-ortho-glucoside, which was found to reduce antioxidant activity against ABTS and DPPH free radicals (Wu et al., 2021).

Similarly, Peres *et al*. (2017) formulated a sunscreen with active ingredients, namely octocrylene, avobenzone, titanium dioxide, and collagen hydrolysate. The SPF was 41% lower than the formulation without the addition of collagen hydrolysate to the sunscreen cream. Furthermore, the addition of collagen hydrolysate to a combination of octocrylene, avobenzone, and titanium dioxide caused a decrease in SPF by 38%. These findings support the notion that negative interactions between natural ingredients, excipients, and sunscreen carriers can reduce the effectiveness of the sunscreen formulation.

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Percentage of erythema

Redness following UV exposure is a symptom of an acute inflammatory reaction known as erythema. When the skin is exposed to UVB radiation, the skin sensitizer absorbs the radiation and undergoes excitation to generate ROS. These ROS interact with mast cells to release mediators (such as histamine) which can induce vasodilation of blood vessels, causing skin redness (Pratama *et al*., 2020). The percentage of erythema transmission indicates the proportion of sunlight that penetrates the sunscreen formulation and can lead to erythema or redness on the skin.

As shown in Table 3, cream A had a high erythema percentage of 77.351% ± 0.001, indicating its ineffectiveness in protecting the skin from UVB-induced erythema. Cream B also lacked effectiveness in protecting the skin from UVB-induced erythema, but it transmitted less UVB radiation than the base cream, with a percentage of 39.98% ± 0.018. Cream C offered additional protection and was successful in preventing erythema, as it transmitted UVB radiation to the skin at 1.965% ± 4.049. Cream D could prevent erythema, as it transmitted less UV radiation to the skin than the kenikir extract cream. This is possible because the active compounds in cream C absorbed more light at the transmittance wavelength of 317,5-372.5 nm than at the erythema-inducing wavelength of 292.5-317.5 nm.

Table 3. Percentages of erythema transmittance

Code	Formula	Percentage of Erythema (%)	Standard Deviation
A	Base Cream Hydrolyzed	77.351	0.001
B	Collagen Cream Kenikir Extract	39.98	0.018
C	Cream Combination	1.965	4.049
D	Cream	1.648	0.799

The erythema data obtained were tested using the Shapiro-Wilk normality test and the homogeneity test using SPSS. The results indicated that the data were normally distributed (0.057 > 0.05), allowing for parametric testing using one-way ANOVA. The ANOVA test revealed a significant difference among the four formulations (p = 0.002 < 0.005), indicating the need for further analysis. The homogeneity test showed that the data were not homogeneously distributed (p = 0.001 < 0.005). Therefore, the Games-Howell test was employed. The additional examination revealed no difference between two formulas.

Percentage of pigmentation

Melanin production is directly affected by UV radiation, which causes skin pigmentation, through the stimulation of various keratinocyte cytokines such as interleukin-1, α-MSH, and ACTH. These cytokines bind to melanocortin-1 receptors, thereby stimulating tyrosinase activity. Increased tyrosinase activity leads to the proliferation of melanocytes and an increase in melanin production. Melanin that accumulates in keratinocytes causes skin to darken or tanning (Mamoto *et al*., 2013). Additionally, UV radiation can damage the sulfhydryl groups present in the epidermis. These groups function as inhibitors by binding to Cu ions essential for the tyrosinase enzyme. Damage to the sulfhydryl groups can lift the inhibition of the tyrosinase enzyme, allowing the enzyme to function optimally and triggering melanogenesis (Fajriah, 2021).

The percentage of pigmentation transmittance refers to the amount of UV radiation that penetrates the skin despite the application of sunscreen, which can result in skin pigmentation or darkening. As shown in Table 4, the base cream transmitted 94.04% ± 0.549 of UVA radiation at a wavelength of 320-375 nm, indicating that it was ineffective in preventing UVA-induced pigmentation. Cream B was able to protect the skin from the effects of pigmentation, placing it in the fast-tanning category, by transmitting 75.484% ± 2.52 of UVA radiation. Sunscreens in the fast-tanning category allow significant UVA penetration, which can rapidly darken the skin without causing erythema. Cream B had the ability to prevent pigmentation from UVA radiation, but its protective ability was minimal. In contrast, creams C and D had very good ability to protect the skin from UVA-induced pigmentation by only transmitting 1.274% ± 0.057 and 1.585% ± 0.042 of UVA radiation, respectively, classifying them in the sunblock category.

Table 4. Percentages of pigmentation transmittance

Code	Formula	Percentage of Pigmentation (%)	Standard Deviation
A	Base Cream Hydrolyzed Collagen	94.004	0.549
B	Cream Kenikir	75.484	2.52
C	Extract Cream Combination	1.274	0.057
D	Cream	1.585	0.042

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Based on the Saphiro-Wilk normality test, the percentage of pigmentation transmittance data were not normally distributed ($p = 0.007 < 0.05$). In addition, the homogeneity test showed that the data were not homogeneous ($p < 0.001$). Therefore, the Kruskal-Wallis test, a non-parametric test, was used for further analysis. The results showed no significant differences among the four sunscreen formulations ($p = 0.083 > 0.05$).

Organoleptic and acceptance tests

As shown in Table 6, the highest average preference score (5.11 ± 1.07) was recorded for cream A, which was classified as "like" by panelists due to its extra white color indicating a clean cream formulation. Cream B, with a color ranging from pure white to milky white, showed a decrease in brightness due to the addition of collagen hydrolysate. This change affected the panelists' preference, resulting in a score of 4.67 ± 1.02 within the neutral category. Cream D exhibited a pure bone white color with low brightness, which reduced its preference score (4.33 ± 1), falling under the neutral category. Cream C with kenikir extract displayed a creamy texture, which influenced its preference score, which was the lowest (3.74 ± 1.35), placing it in the "dislike" category. According to the univariate analysis, the preference scores for cream color showed a significant difference ($p = 0.01 < 0.05$). Therefore, further analysis was carried out using the Duncan's test. This test revealed that the preference score level for the kenikir extract cream was significantly different from the other three formulations.

Table 6 also illustrates that the panelists had nearly identical preferences for the aroma of the four creams. According to Table 5, all four creams exhibited an aroma similar to stearic acid because of its high concentration following aquadest in the formulation. The univariate test yielded a significance level greater than 0.05 ($p = 0.721$), suggesting no difference in the preference level for the aroma of the four creams.

As illustrated in Table 6, the panelists had similar preferences for the texture of the four creams. The texture of all four creams was semi-solid (Table 5). According to the univariate test, no significant differences in texture preferences were observed ($p = 0.117 > 0.005$).

Table 5. Organoleptic test results

Formula	Color	Aroma	Texture
Base Cream	Extra White	Stearic Acid	Semi Solid
Hydrolyzed Collagen Cream	Pure White	Stearic Acid	Semi Solid
Kenikir Extract Cream	Cream	Stearic Acid	Semi Solid
Combination Cream	Cottage White	Stearic Acid	Semi Solid

Table 6. Acceptance test results

Parameter	Code	Likeability Score	Standard Deviation
Color	A	5.11	1.07
	B	4.67	1.02
	C	3.74	1.35
	D	4.33	1
Aroma	A	4.37	1.04
	B	4.15	0.95
	C	4.3	1.03
	D	4.07	0.83
Teksture	A	4.93	0.96
	B	4.89	1.12
	C	4.41	1.47
	D	4.33	1

Homogeneity test

To determine the uniformity of ingredient dispersion in the cream formulations, a homogeneity test was conducted. A good cream is defined by the absence of coarse grains and a uniform color distribution. The cream formulation must be homogene to prevent irritation when applied to the skin surface, and so that each gram of cream contains ingredients with the same levels and effectiveness. According to the test results, the base cream and kenikir extract cream exhibited homogenous mixtures with evenly distributed color. This indicated the effective performance of the emulsifier agents used in the formulation. The emulsifier, a non-ionic surfactant, causes all ingredients to be evenly dispersed, resulting in a homogenous cream formulation (Hendrawan *et al.*, 2020). Non-ionic surfactants such as cetyl alcohol and stearic acid can be used as emulsifying agents and contribute to the physical stability of the formulation (Devi *et al.*, 2019).

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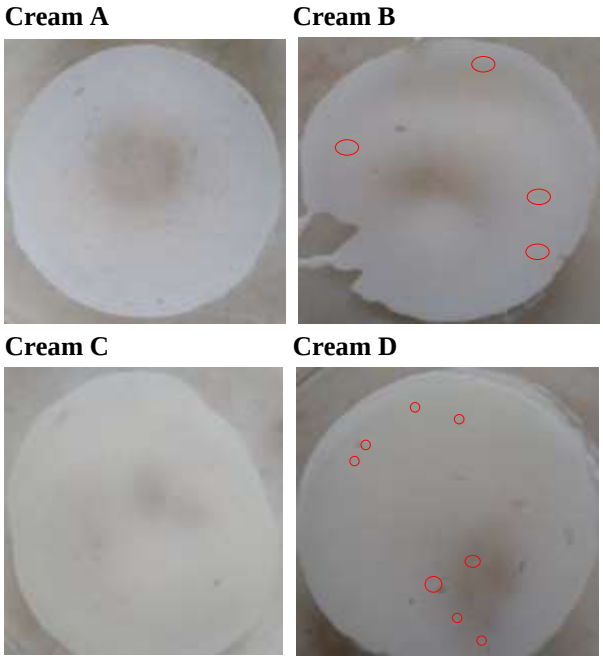

Figure 2. Homogeneity test results. Red circles indicate unmixed collagen hydrolysate granules

In contrast, the collagen hydrolysate cream and combination cream failed to achieve homogeneity because of the presence of coarse collagen hydrolysate grains (Figure 6). Proteins that contain more hydrophobic amino acids have less water solubility than those that contain more hydrophilic amino acids. Collagen consists of approximately 80% of non-polar amino acids such as glycine, alanine, valine, and proline. The composition of collagen chains is made up of thousands of amino acids that have repeating Gly-X-Y sequences, where X and Y sequences are mostly occupied by proline and hydroxyproline (Cherim *et al*., 2019).

pH Test

A cream that is too acidic may cause skin irritation, while a cream that is too alkaline may result in itching and flaking of the skin (Sari, 2014). All four cream formulations had pH values within the range permitted by SNI 16-4399-1996 (4.5-8), indicating they are safe for use.

Table 7. pH test results

Code	Formula	pH	Standard Deviation
A	Base Cream	7.874	0.059
B	Hydrolyzed Collagen Cream Kenikir Extract	7.03	0.014
C	Cream Combination	7.833	0.007
D	Cream	6.964	0.041

The Saphiro-Wilk normality test and homogeneity test showed that the pH data were not normally distributed ($p = 0.006 < 0.05$) and not homogeneous. Therefore, the analysis was continued with the non-parametric Kruskal-Wallis test. The results showed no significant differences among the four sunscreen formulas ($p = 0.083 > 0.05$).

Cream A had a pH of 7.874 ± 0.059, which was a weak base. Cream C had a lower pH than its base cream, which was 7.833 ± 0.007. Kenikir extract lowers the pH of the formulation because it contains phenolic compounds that can release H^+ ions from their hydroxyl groups (Tambun *et al*., 2016). Cream B had a lower pH than the base cream, which was 7.03 ± 0.014. This is because the added collagen hydrolysate is composed of amino acids. Amino acids contain both hydroxyl (-COOH) and amine (-NH), allowing them to behave as acids or bases depending on the surrounding environment (amphoteric). If the amino acid is in a strong acidic environment, the substance will be basic, but if the substance is in a basic environment, the substance will be acidic (Wahyudiati, 2017). When collagen hydrolysate is added to a weak base cream, the amino acid will act as an acid and release H^+ ions, resulting in a decrease in the pH of the formulation.

Cream D was prepared by adding kenikir extract first, followed by collagen hydrolysate. The addition of kenikir extract caused a slight decrease in the pH, although the cream remained weakly alkaline. When collagen hydrolysate was added, the amino acids released H^+ and further decrease the pH.

Viscosity Test

The viscosity test was conducted to assess the thickness of the cream formulation, which directly influences its ease of application to the skin. A cream is considered good when its viscosity is neither too thick nor too runny. According to SNI 16-4399-1996, a high-quality sunscreen cream should have a viscosity between 2,000 and 50,000 cps. As shown in Table 8, the four creams met this standard with viscosities above 2000 cps and below 50,000 cps.

The Shapiro-Wilk normality test and homogeneity test showed that the viscosity data were not normally distributed ($p = 0.003 < 0.05$) and not homogeneous. Therefore, further analysis was conducted using the non-parametric Kruskal-Wallis test, whose results showed no significant differences among the four sunscreen formulations (0.083>0.05).

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Table 8. Viscosity test results

Code	Formula	Viscosity (Cps)	Standard Deviation
A	Base Cream	43,500	25
B	Hydrolyzed Collagen Cream	19,100	100
C	Kenikir Extract Cream	46,700	50
D	Combination Cream	41,550	550

The addition of collagen hydrolysate resulted in decreased viscosity, while the addition of kenikir extract resulted in increased viscosity. The base cream had a viscosity of 43,525 ± 25 cps. The presence of hydroxyl groups in kenikir extract can increase viscosity because of the high number of hydroxyl groups present in its flavonoid compounds, particularly quercetin. These hydroxyl groups can interact with similar groups in the components of the cream formulations, such as stearic acid, TEA, cetyl alcohol, glycerol, methyl paraben, and aquadest by forming hydrogen bonds. These hydrogen bonds occur when hydrogen atoms in both kenikir extract and cream components bind to more electronegative atoms, specifically oxygen atoms. The increase in hydrogen bonds strengthens the interactions among components, leading to a higher viscosity. Kuncari and Praptiwi (2014), citing Contreras and Sanchez, noted that hydrogen bonds can increase cross-linking between chains, thereby increasing viscosity. In contrast, the addition of active ingredients such as collagen hydrolysate decreases the viscosity of the base cream. Collagen hydrolysate can act as a natural humectant (Sionkowska *et al*., 2020) which can reduce the viscosity of the cream and make its consistency more liquid. Humectants are utilized to minimize water loss from the formulation, helping to prevent dryness and increase spreadability.

Centrifugation Test

The stability of an emulsion is determined by its ability to resist changes in its properties over time. The more stable the emulsion, the slower its properties change (Del Río-Ortuño *et al*., 2022). The centrifugation technique, which operates based on Stokes' law, is used to determine cream stability. Under increased gravitational force, phase separation occurs more rapidly, thereby accelerating the creaming process. Creaming is a process where layers with different concentrations form within an emulsion. Gravitational force causes the raising of particles with lower densities to the surface, and vice versa. Centrifuging a cream formulation for five hours at a speed of 3,700 rpm is equivalent to the effect of gravitational force for one year (Dewi *et al*., 2014).

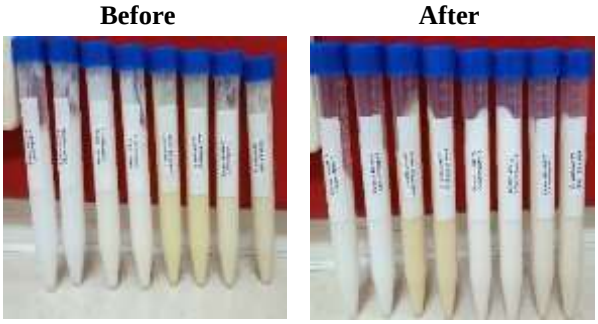

Figure 3. Centrifugation test. The four creams did not experience phase separation after centrifugation

Figure 4 shows that the four cream formulations did not separate, indicating that they were stable over a one-year storage period. By combining stearic acid and triethanolamine, the molecules on the surface will be packed more tightly, increasing the strength of the interfacial layer and enhancing the stability. The stability of the preparation can be improved with the use of cetyl alcohol as a cosurfactant, which can increase the density of the emulsifier molecules at the emulsion interface. Cetyl alcohol also functions to increase consistency and as a non-ionic surfactant (Sari *et al*., 2021).

Spreadability Test

The spreadability test was conducted to evaluate the ability of the cream formulation to spread when applied to the skin surface. Greater spreadability allows the active ingredients to be dispersed evenly, enhancing their therapeutic effects. A good spreadability range of the cream is approximtaley 5-7 cm (Syarif *et al*., 2015). As shown in Table 10, the four formulas demonstrated poor spreadability.

Table 9. Spreadability test results

Code	Formula	Spread Power Test (cm)	Standard Deviation
A	Base Cream	4.55	0.167
B	Hydrolyzed Collagen Cream	4.183	0.033
C	Kenikir Extract Cream	4.967	0
D	Combination Cream	4.042	0.075

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A normal distribution was observed in the spreadability test data, as shown by the Shapiro-Wilk normality and homogeneity test. Therefore, a one-way ANOVA parametric test was conducted. The ANOVA test revealed a significant difference ($p = 0.190 > 0.005$), indicating that further analysis was required. The homogenity test showed that the data were not homogeneous ($p < 0.001$), so the Games-Howell test was employed. The additional examination revealed a difference in spreadability between collagen hydrolysate cream and kenikir extract cream. This may be attributed to the high viscosity of the formulations. In addition, the poor spreadability of the collagen hydrolysate cream and the combination cream is likely due to the uneven mixing of hydrolyzed collagen during formulation. Greater pressure is required when applying creams with low spreadability to the skin so that it is evenly distributed (Sari & Susiloningrum, 2022).

CONCLUSION

The kenikir extract cream and combination cream were effective in protecting the skin from UV radiation with ultra protection indicated by the SPF and percentages of erythema and pigmentation. Meanwhile, the base cream and collagen hydrolysate cream were less effective as sunscreens. Based on the characterization tests, the four creams were stable with no phase separation during the centrifugation test and met the standards for viscosity and pH. However, the four creams had poor spreadability which can affect the ease of application. The panelists had similar preferences for the aroma and texture of the four creams and favored formulations with a clean white color.

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AUTHOR CONTRIBUTIONS

Conceptualization, A.Z., I.Q.A.; Methodology, A.Z.; Software, A.Z.; Validation, A.Z., I.Q.A.; Formal Analysis, A.Z.; Investigation, A.Z.; Resources, A.Z., I.Q.A.; Data Curration; A.Z.; Writing - Original Draft, A.Z., I.Q.A.; Writing - Review & Editing, A.Z., I.Q.A.; Visualization, A.Z.; Supervision, I.Q.A.; Project Administration, A.Z.; Funding Acquisition, A.Z.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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