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Optimization of oil-based liquid lipstick formula from sesame seed oil (*Sesamum indicum* L.) and antibacterial activity test against *Staphylococcus aureus*

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ABSTRACT: Liquid lipstick is a type of lip color cosmetic that consumers highly favor. However, repeated use of liquid lipstick can promote the growth of *Staphylococcus aureus* (*S. aureus*) bacteria. The formulation of liquid lipstick involves several components, including a mixture of wax, oil, and other additives. Sesame seed oil (*Sesamum indicum* L.), incorporated into the formulation, serves as both a base and preservative due to its fatty acid content, which is expected to inhibit the growth of *S. aureus* bacteria. Microcrystalline wax is added to achieve a good texture and enhance the adhesion properties of the formulation. This study aimed to determine the optimum formula and evaluate the antibacterial activity of the liquid lipstick formulation. The formula was developed using a 2^2 factorial design method, with sesame seed oil and microcrystalline wax as variables. The optimum formula was analyzed using Design Expert® 13 software based on parameters such as viscosity, spreadability, and adhesion. The final optimum formula consisted of 70% sesame seed oil and 5% microcrystalline wax. The formulation demonstrated good stability during the cycling test, maintaining red color, vanilla and sesame-like aroma, liquid texture, and pH 5. The viscosity was 11797.51 cP, the spreadability was 6.06 cm, and the adhesion time was 64 seconds. Antibacterial activity, assessed using the disk diffusion method, yielded an inhibition zone diameter of 15±1.322 mm, indicating a strong inhibition zone. Based on the research findings, it was concluded that the optimum sesame seed oil-based liquid lipstick formula exhibited good product characteristics, stability, and strong antibacterial activity.

KEYWORDS: Antibacterial; liquid lipstick; microcrystalline wax; sesame seed oil.

INTRODUCTION

Lipstick is a cosmetic product used as a lip colorant, designed to enhance aesthetics in facial makeup while protecting and moisturizing the lips. Among the various types of lipstick, liquid lipstick stands out for its ease of use and efficiency [1]. A study conducted by BPOM revealed that hundreds of brands available in the market are unsafe for use because they primarily consist of synthetic materials, which can cause harmful side effects to the skin. One such material is synthetic preservatives, such as parabens. Liquid lipstick formulations typically have a higher oil phase content and a lower pigment concentration, ranging from approximately 0% to 5% [2]. Oils constitute 50–70% of the formulation to provide a smooth and soft application effect [3].

Sesame seed oil (*Sesamum indicum* L.) is an ideal oil base for formulations, offering both antibacterial and antioxidant properties. The fatty acids present in sesame seed oil include oleic acid, linoleic acid, palmitic acid, and stearic acid. The antibacterial activity of sesame seed oil is primarily attributed to the presence of oleic and linoleic acids [4]. Studies have shown that sesame seed oil is most effective against *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), and *Streptococcus pyogenes* (*S. pyogenes*) [5]. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of sesame seed oil against *S. aureus*, as determined using the microdilution method, were reported to be 32 mg/mL and 128 mg/mL, respectively [6].

S. aureus is a gram-positive pathogenic bacterium that can infect the skin and is commonly found in liquid lipstick products. Repeated use and improper storage can lead to microbial contamination. A study found that liquid lipsticks used for approximately 12 months were contaminated with *S. aureus* and *E. coli* [7]. Therefore,

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enhancing antibacterial activity is crucial to inhibit microbial growth during manufacturing, storage, and consumer use [8]. Another critical component influencing the physical form and stability of liquid lipstick is the wax base. Microcrystalline wax, commonly used in skincare and cosmetic products, enhances texture and consistency in formulations. It not only improves the spreadability of a product but also aids in emulsifying oils and helps evenly distribute active ingredients across the skin. Microcrystalline wax at a concentration of 5–10% provides good viscosity and adhesion properties. Meanwhile, sesame seed oil is expected to enhance the spreadability of the liquid lipstick during application [9]. In this will optimization of utilized a combination of microcrystalline wax and sesame seed oil as the base of lipstick and the evaluation of its antibacterial activity as an innovation for safe cosmetic products. The experimental design employed a two-factor, two-level factorial approach to determine the optimum liquid lipstick formula, with sesame seed oil and microcrystalline wax as the two factors tested at low and high concentration levels. Based on the above, further research is necessary to optimize the liquid lipstick formulation using sesame seed oil as a base and a natural preservative. Evaluation tests, including viscosity, spreadability, and adhesion, are crucial for determining the optimal formula using Design Expert 13 software. This research highlights the significance of incorporating herbal ingredients into formulations to reduce the adverse effects associated with synthetic materials.

▪ MATERIALS AND METHODS

Materials

Sesame seed oil (PT. Darjeeling Sembrani Aroma, Indonesia), microcrystalline wax (Multiwax, USA), cetyl alcohol (Brataco, Indonesia), kaolin (Brataco, Indonesia), dimethicone (Brataco, Indonesia), tocopherol (Brataco, Indonesia), titanium oxide (Mitra Jaya Chemical, Indonesia), vanillin (Merck, USA), liquid paraffin (Rajol WT®, India), D&C red 36 (Brataco, Indonesia), *Staphylococcus aureus* culture (BioMerieux®, France), propyl paraben (Brataco, Indonesia), nutrient agar (Merck, USA), nutrient broth (Merck, USA), barium chloride (Merck, Germany), sulfuric acid (Merck, Germany), and distilled water.

Factorial design formula

The formula was designed using two key factors in the preparation of liquid lipstick: sesame oil and microcrystalline wax. The concentration of sesame seed oil used in the formulation ranged from 50% to 70% [3]. A factorial design was employed to determine the optimal formula or composition as a base and antibacterial agent. The formula was created using a 2^2 factorial design, where 2^k represents two concentration levels (high and low) and two factors (independent variables). Based on this formula, a total of $2^k = 2^2 = 4$ formulas were created.

Table 1. Formula using 2^2 factorial designs.

Factor	Low level (%)	High level (%)	F1	F2	F3	F4
Sesame seed oil (A)	50	70	-	-	+	+
Microcrystalline wax (B)	5	10	-	+	-	+

A = Factor A (sesame seed oil); B= Factor B (microcrystalline wax); (-) = Low level; (+) = High level; F= Formula

Preparation of liquid lipstick

The preparation process begins with melting the microcrystalline wax and cetyl alcohol in a porcelain dish. Once melted, these components are combined with sesame seed oil, dimethicone, and liquid paraffin in a beaker placed on a magnetic stirrer at 85 °C. Next, kaolin is dissolved in liquid paraffin and added to the beaker. The mixture is then homogenized using a stirring rod. The temperature is then allowed to drop to 65 °C before slowly adding tocopherol, titanium dioxide, vanillin, and D&C red 36. Liquid paraffin is added until the total preparation reaches 100 mL. The mixture is stirred continuously until a homogeneous preparation is achieved.

Table 2. Formula of liquid lipstick.

Materials	Formula (%)			
	F1	F2	F3	F4
Sesame seed oil	50	50	70	70
Microcrystalline wax	5	10	5	10
Cetyl alcohol	2	2	2	2
Kaolin	3	3	3	3
Dimethicone	10	10	10	10
Tocopherol	0.05	0.05	0.05	0.05
Titanium oxide	0.50	0.50	0.50	0.50
Vanillin	0.30	0.30	0.30	0.30
D&C red 36	0.10	0.10	0.10	0.10
Ad liquid paraffin	100	100	100	100

Evaluation of liquid lipstick

Organoleptic

The organoleptic test evaluates the color, odor, and texture of each formulation. Observations are performed, and the results are recorded for all formulations [9].

pH

The pH of the formulation is determined using a pH meter. The electrode is immersed in the sample, and once the reading stabilizes, the pH value is recorded [10].

Homogeneity

To assess homogeneity, the liquid lipstick is applied to a transparent microscope slide. The preparation is considered homogeneous if no visible coarse particles are observed during examination [10].

Washability

In the washability test, 1 g of the sample is applied to the back of the hand and rinsed under running water. The time required to remove the sample completely is recorded [11].

Viscosity

The viscosity of the liquid lipstick is measured using a viscometer. A 30 mL sample is poured into a beaker, and the rotor is activated on a cup-and-bob viscometer set to 30 rpm. Once the needle stabilizes, the viscosity value is recorded [12].

Spreadability

The spreadability test involves placing 1 g of the sample between two transparent slides. A 125 g weight is applied for 1 minute, and the resulting spread area is measured and recorded [9].

Adhesion

To test adhesion, the sample is placed between two microscope slides, and a 1 kg weight is applied for 5 minutes. An additional 80 g weight is then added, and the time required for the sample to detach from the slides is recorded [9].

Determination of the optimum formula for liquid lipstick

Data from the formulation evaluations are analyzed using factorial design methods with Design Expert® 12 software to assess the main effects and interactions of the factors used. The software identifies the optimum formula based on the desired response criteria. The desirability value, which ranges from 0 to 1, is used to determine the best formula, with a value closer to 1 indicating a more optimal formulation [13].

Evaluation of the optimum liquid lipstick formula

The organoleptic, viscosity, spreadability, and adhesion tests follow the same procedures as previously described.

Stability

The stability of the liquid lipstick is evaluated using the cycling test method. The preparation is stored at 4 °C for 24 hours, followed by 40 °C for another 24 hours, constituting one cycle. Physical observations of the preparation are made after each cycle. The test is conducted over 12 days, completing a total of six cycles [13].

Hedonic

The hedonic test involves a visual inspection and application of the product to the back of the hand by panelists. The panelist consisted of 30 females aged 17-22 years. Each panelist evaluates the product and records their preferences on a scoring sheet. Parameters assessed include color, odor, and application. Preferences are rated on a five-point scale: (5) strongly like, (4) like, (3) neutral, (2) dislike, and (1) strongly dislike [13]. The test requires 30 panelists who meet specific inclusion and exclusion criteria, as outlined in national standards [14].

Inclusion criteria include panelists who are willing to participate, interested in the hedonic test, consistent in their evaluations, free from allergies to the liquid lipstick components, healthy, free from ENT (ear, nose, throat) diseases, not color-blind, and not experiencing psychological disorders. Exclusion criteria apply to panelists who voluntarily withdraw from the test.

Antibacterial activity

The antibacterial activity was tested using the disk diffusion method [15]. To prepare the 0.5 McFarland turbidity standard, mix 9.95 mL of 1% H_2SO_4 with 0.05 mL of 1% $BaCl_2$. This solution corresponds to a bacterial density of 10^7–10^8 CFU/mL. A bacterial suspension was prepared by transferring a loopful of a rejuvenated bacterial culture grown on nutrient agar (NA) medium into a tube containing 5 mL of nutrient broth (NB) medium. The tube was incubated at 37 °C for 24 hours. To perform the test, add 1 mL of the bacterial suspension to a petri dish and pour 20 mL of NA media. Mix thoroughly to homogenize and allow it to solidify. Place paper discs (0.5 cm in diameter) soaked in the sample solution on the surface of the media. Incubate the petri dishes upside down at 37 °C for 24 hours. Measure the clear zones (inhibition zones) around the discs using a caliper. The inhibition zone was calculated using the formula:

$$\text{İnhibition zone (mm)} = \frac{\text{Longest diameter of inhibition zone (mm)} + \text{Shortest diameter of inhibition zone (mm)}}{2}$$

Data analysis

The data obtained from the formulation evaluation were analyzed using factorial design methods with Design Expert® 13 software to identify the main effects, factor interactions, and the optimum formula based on the software's analysis. Hedonic test data were processed using SPSS for Windows, version 25.0. Normality was assessed using the Shapiro-Wilk test at a significance level of 0.05. If the data were normally distributed ($p>0.05$), one-way ANOVA was used. Meanwhile, if the data were not normally distributed ($p<0.05$), the Kruskal-Wallis test was applied. Data from the disk diffusion antibacterial activity test, comparing the formula with 70% sesame oil and the optimum formula, were analyzed using SPSS 16 for Windows. Statistical correlations of the response results were evaluated using ANOVA with a 95% confidence level.

▪ RESULTS

Physical quality testing of liquid lipstick

The four liquid lipstick formulas, with varying concentrations of sesame seed oil and microcrystalline wax, were analyzed using Design Expert® 13 software. These formulas were evaluated for organoleptic, pH, homogeneity, washability, viscosity, spreadability, and adhesion. The test results for the four formulas are summarized in Table 3, while visual representations of the formulations (F1, F2, F3, and F4) are shown in Figure 1.

(a) (b) (c) (d)

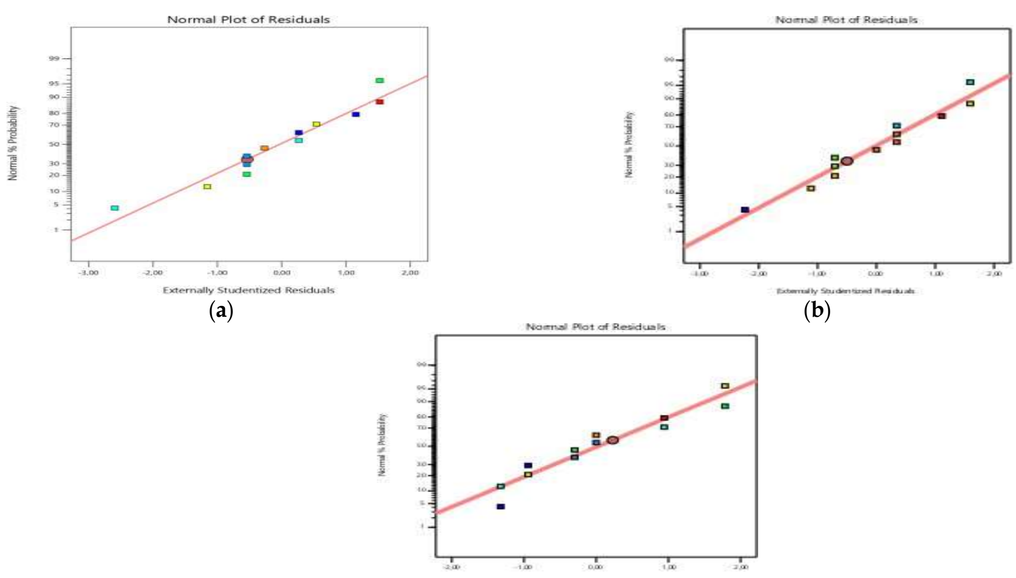
Figure 1. The results of liquid lipstick preparation for each formula (a) F1; (b) F2; (c) F3; (d) F4.

Table 3. Test results for liquid lipstick preparations.

Parameters	Test result				Requirements
	F1	F2	F3	F4	
Texture	Liquid	Liquid	Liquid	Liquid	Liquid
Odor	Vanilla and specific sesame seed oil	Vanilla and specific sesame seed oil	Vanilla and specific sesame seed oil	Vanilla and specific sesame seed oil	Specific odor
Color	Mahogany (#CA2400)	Mahogany (#CA2400)	Boston university (#C30000)	Boston university (#C30000)	Red
pH	5. ±0.05	5.5±0.11	5.3±0.15	5.3±0.05	4.5-6.5
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
Washability (s)	>60	>60	>60	>60	>60
Viscosity (cPs)	11827.23±84.05	12689.02±185.58	11738.23±111.18	12778.17±111.18	10000-20000
Spreadability (cm)	6.30±0.08	6.27±0.04	6.06±0.09	5.57±0.12	5-7
Adhesion (s)	64.33±1.24	63.33±1.25	64.00±0.81	69.00±0.82	>60

Table 4. Viscosity model analysis.

Responses	Parameters						
	Mean	Standard Deviation	CV (%)	R^2	Adjusted R^2	Predicted R^2	Adequate Precision
Viscosity	12258.13	128.68	1.05	0.9538	0.9365	0.8961	14.00
Spreadability	6.05	0.11	1.85	0.9115	0.8783	0.8009	11.36
Adhesion	65.92	1.29	1.96	0.7811	0.6990	0.5075	6.71



(c)

Figure 2. Response model results as a normal plot of residuals (a) viscosity; (b) spreadability; (c) adhesion.

Table 5. Coefficients for viscosity, spreadability, and adhesion.

Response	Parameters	Intercept	A (Sesame seed oil)	B (Microcrystalline wax)	AB (Interaction)
	Coefficient		397.58	475.47	-85.77
Viscosity	p-values	12258.13	<0.0001*	<0.0001*	0.2645
	Contribution (%)		35.1393%	62.5522%	0.8310%
	Coefficient		-0.233	-0.133	-0.116
Spreadability	p-values	6.05	<0.0001*	0.0033*	0.0068*
	Contribution (%)		57.8171%	18.8791%	14.4543%
	Coefficient		0.5833	1.75	0.75
Adhesion	p-values	65.91	0.1562	0.0015*	0.0790
	Contribution (%)		6.7031%	60.3283%	11.0807%

Note: * Significant factors (p < 0.05)

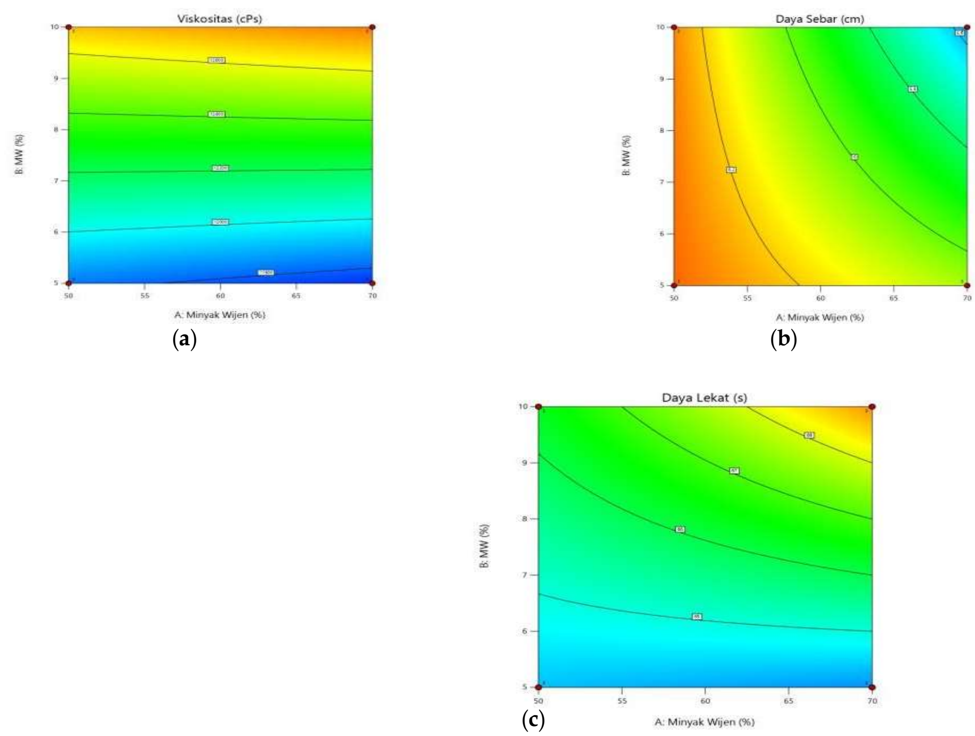


Figure 3. Two-dimensional (2D) contour plots for model responses: (a) viscosity, (b) spreadability, (c) adhesion.

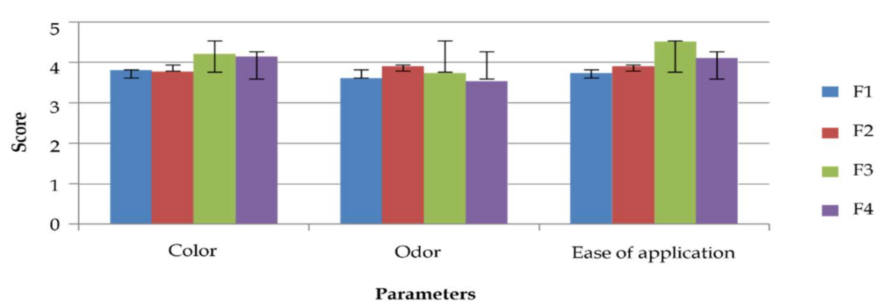


Figure 4. The hedonic test scores for the liquid lipstick.

Table 6. Evaluation data for the optimum liquid lipstick formula.

Response	Prediction	Observation	95% Confidence Interval (CI)		95% Prediction Interval (PI)	
			Low	High	Low	High
Viscosity (cPs)	12689	11797.1	125.17.7	12860.3	11983.8	13394.3
Spreadability (cm)	6.2667	6.06	6.1178	6.4155	5.6538	6.8794
Adhesion (s)	66.3333	64.00	64.6145	68.0521	59.6538	73.4091

Table 7. Stability data for the optimum liquid lipstick formula.

Parameters	Stability test	
	Before	After
Color	Boston university (#C30000)	Boston university (#C30000)
Odor	Vanilla and specific sesame seed oil	Vanilla and specific sesame seed oil
Texture	Liquid	Liquid
Viscosity (cPs)	11797.51±111.18	10994.16±52.35
pH	5.30±0.15	5.10±0.05
Homogeneity	Homogeneous	Homogeneous

Table 8. Antibacterial test results against *Staphylococcus aureus.*

Test Group	Treatment	Inhibition Diameter (mm) ± SD	Inhibition Category
Positive control	Sesame seed oil	17.83±0.76^{d}	Strong
Negative control	Liquid paraffin	0.00±0.00^{a}	None
Comparison control 1	Optimum liquid lipstick formula without sesame seed oil	0.00±0.00^{a}	None
Comparison control 1	Optimum liquid lipstick formula without sesame seed oil + propyl paraben	14.00±1.00^{b}	Strong
Test control	Optimum formula	15.33±0.76^{c}	Strong

Note: Values followed by different superscript letters indicate significant differences according to Duncan's Post Hoc test ($p < 0.05$).

▪ DISCUSSION

The organoleptic test assessed the physical properties of the liquid lipstick, including texture, odor, and color. All four formulas exhibited a liquid texture, influenced by the higher concentration of sesame seed oil compared to microcrystalline wax. A characteristic sesame seed oil odor was present in all formulas, even with the addition of vanillin as a masking agent. The color consistency varied with the oil concentration. Formulas F1 and F2, containing 50% sesame seed oil, produced the same color, while F3 and F4, with 70% sesame seed oil, resulted in a deeper hue. The darker color in higher oil concentrations is due to the natural pigmentation of sesame seed oil, which intensifies when combined with the cosmetic pigment D&C Red 36.

The pH values of all four formulas met the acceptable skin pH range of 4.5–6.5 [16]. The results showed a slight decrease in pH with higher sesame seed oil concentrations, attributed to the weak acidic properties of the fatty acids present in the oil. The homogeneity test confirmed that all formulas were homogeneous, with no visible coarse particles or clumps. The even distribution of the preparation during use indicates that the ingredients were well-dissolved and mixed. Proper and consistent stirring played a significant role in achieving uniformity [17]. Homogeneous formulations are essential for user acceptability and ease of application, ensuring even color distribution without clumping [13]. The washability test evaluated the resistance of the liquid lipstick under running water. Longer washing times indicate higher resistance. The results showed that all four formulas exhibited high resistance, with washability times exceeding 60 seconds. This resistance is attributed to the oil phase in the lipstick, which reduces its solubility in water. Higher viscosity further contributed to longer washability times, as more viscous formulations are less likely to be removed quickly.

The viscosity, spreadability, and adhesion data were analyzed to determine the optimum formula using Design Expert® 13 modeling, as shown in Table 4. The viscosity test results for the four formulas fell within the acceptable range for good liquid lipstick viscosity. The ideal viscosity for liquid lipstick ranges between 10,000 and 20,000 cPs [9]. Viscosity significantly influences the spreadability, adhesion, and washability parameters of liquid lipstick. Viscosity is inversely proportional to spreadability; higher viscosity leads to lower spreadability. Conversely, viscosity is directly proportional to adhesion and washability, where higher viscosity enhances these properties. The recommended spreadability for liquid lipstick ranges between 5–7 cm [9]. Test results confirmed that all formulas met the criteria for good spreadability. Wax plays a crucial role in spreadability, as increasing wax concentration reduces spreadability. Adhesion testing evaluates the product's ability to adhere to application surfaces, such as the lips. Cosmetic products are considered to meet adhesion standards if they adhere for more than 60 seconds [9]. The adhesion test results for all four liquid lipstick formulas exceeded this threshold, indicating strong adhesion upon application.

The adjusted R^2 values indicate that the model explains 93.65%, 87.83%, and 69.90% of the variability in the viscosity, spreadability, and adhesion responses, respectively. Similarly, the predicted R^2 values show that the model accurately predicts 89.61%, 80.09%, and 50.75% of the responses. The slight difference between these values (<0.2) supports the validity of the model. Adequate precision values, which exceed the threshold of 4, suggest a strong signal-to-noise ratio. This indicates that the model is reliable and capable of providing accurate predictions with a strong correlation between predicted and actual values [18]. The results of the viscosity, spreadability, and adhesion response model are shown in Figure 2. The normal plot of residuals confirms a normal distribution of data, as the points align closely with the normal line. This allowed ANOVA to be performed. The residual plot also indicates that the differences between actual and predicted responses follow a straight line. ANOVA test results for viscosity, spreadability, and adhesion responses confirmed that all requirements were met.

The coefficients for viscosity, spreadability, and adhesion parameters are shown in Table 5. The ANOVA results indicate statistical significance ($p<0.0001$), confirming that factors A (sesame seed oil) and B (microcrystalline wax) significantly influence viscosity, as both have p-values <0.05. Viscosity increases with higher concentrations of sesame seed oil or microcrystalline wax, as indicated by positive coefficients. However, the interaction between A and B (AB) reduces viscosity, as reflected by the negative coefficient. Factor B contributes more significantly than factor A, demonstrating that microcrystalline wax has a greater impact on viscosity values. Conversely, the results for spreadability indicate that factors A, B, and AB significantly affect this parameter, with p-values <0.05. Spreadability decreases as the levels of A, B, and AB increase, as indicated by negative coefficients. Factor A has a greater influence on spreadability compared to Factor B, as indicated by its higher contribution percentage. For adhesion, only factor B significantly influences this parameter, with a p-value <0.05. Adhesion increases with higher levels of A, B, and AB, as indicated by positive coefficients. Factor B shows a more substantial contribution than factor A. Additionally, adhesion is influenced by viscosity, as higher viscosity generally correlates with higher adhesion. However, adhesion values are lower compared to spreadability values under similar conditions.

The relationships between factors and responses are visually represented in Figure 3 using color gradients to illustrate different response levels for viscosity, spreadability, and adhesion. Orange represents the highest response values, while blue indicates the lowest values. The lowest response levels modeled for viscosity, spreadability, and adhesion were 11,678.6 cPs, 5.4 cm, and 60.0 seconds, respectively. Contour plots depict combinations of component values that yield identical responses, with lower regions representing lower response levels and higher regions representing higher levels. The ANOVA results confirm the significance and reliability of the recommended model, as visually illustrated through two-dimensional (2D) contour plots.

Hedonic test

The hedonic test scores for the liquid lipstick were evaluated based on panelists' preferences regarding color, scent, and ease of application. The results, as shown in Figure 4, highlight the panelists' overall impressions of the different formulas. Formula 3 (F3) received the highest scores for color and ease of application, although the differences among formulas were not statistically significant. Panelists favored F3, which contained 70% sesame seed oil and 5% microcrystalline wax, due to its excellent spreadability and vibrant color. The deeper hue produced by the higher concentration of sesame seed oil was particularly appealing to the panelists. Scent received mixed feedback. While some panelists appreciated the distinctive sesame aroma, others rated it lower due to personal preference. Statistical analysis revealed that color and ease-of-application scores had non-normal distributions ($p<0.05$), as indicated by normality tests. However, the Kruskal-Wallis test results ($p>0.05$) confirmed no significant differences among the formulas for these parameters. For scent, the Kruskal-Wallis test yielded $p<0.05$, indicating significant differences likely caused by varying scent preferences among the panelists.

Optimum liquid lipstick formula

Optimization was conducted to achieve the desired responses (desirability) and determine the optimal concentration ratio of sesame seed oil and microcrystalline wax. The optimum formula, identified using Design Expert® 13, consisted of 70% sesame seed oil and 5% microcrystalline wax. The factorial design method helped identify dominant factors and significant interactions influencing viscosity, spreadability, and adhesion. The desirability value of the optimum formula was 0.982, indicating excellent suitability. A desirability value closer to 1 reflects better alignment with desired response parameters [12]. The optimum formula was evaluated for viscosity, spreadability, and adhesion, with results shown in Table 6. The observed values for these parameters fell within the 95% confidence interval (CI) and prediction interval (PI) ranges. The CI indicates the confidence in the observed data, while the PI reflects the range of predicted values based on the highest and lowest observations [19].

Stability testing ensures the preparation retains its properties and meets quality criteria during storage. Parameters observed included organoleptic properties, pH, viscosity, and homogeneity. The organoleptic and homogeneity results showed no changes before and after the cycling test, indicating stability, as shown in Table 7. The pH remained within the skin's acceptable range of 4.5–6.5 [16], with values of 5.3 before and 5.1 after the test. Statistical analysis ($p>0.05$) showed no significant difference in pH values, further confirming stability. The viscosity decreased slightly after the test, consistent with temperature-related behavior, but remained within acceptable limits. This decrease in viscosity occurs because viscosity is affected by changes in temperature, where viscosity is inversely proportional to the temperature factor, whereby if the temperature increases, the viscosity will decrease. These results demonstrate that the optimum liquid lipstick formula maintained its properties and met stability requirements for extended use.

Antibacterial activity

The antibacterial activity test for the optimum liquid lipstick formula was performed using the disk diffusion method. The test aimed to evaluate antibacterial activity against pathogenic bacteria by measuring the diameters of inhibition zones. These bacteria are commonly found on the skin, respiratory tract, and digestive tract. The results are summarized in Table 8. The inhibition zones observed for various treatments indicate the efficacy of each against *S. aureus*.

The inhibition zone diameters varied across the test groups. The positive control (pure sesame seed oil) exhibited the largest inhibition zone, measuring 17.83 mm±0.76 mm, categorized as strong inhibition (11–20 mm) [20]. The test control (optimum formula) produced an inhibition zone diameter of 15.33 mm±0.76 mm, also indicating strong antibacterial activity. This activity is primarily attributed to the 70% sesame seed oil in the formula, which contains antibacterial agents such as oleic acid and linoleic acid [4]. The comparison control 2 (formula containing propyl paraben) demonstrated an inhibition zone of 14 mm±1 mm, reflecting strong antibacterial activity. Parabens are widely used as preservatives in cosmetic preparations to prevent microbiological contamination, with a maximum concentration limit of 0.4% in single form [23]. This is attributed to propyl paraben, a common cosmetic preservative that inhibits the growth of microorganisms. Conversely, the negative control (liquid paraffin) and comparison control 1 (formula without sesame seed oil) exhibited no inhibition zones, as neither liquid paraffin nor the formula without sesame oil contains antibacterial agents.

The mechanism of action of sesame seed oil is primarily due to the fatty acids linoleic acid and oleic acid, which disrupt bacterial cell membranes and interfere with cellular functions, effectively inhibiting *S. aureus* and other gram-positive bacteria such as *Basillus subtilis* and *Micrococcus kristinae* [21], [22]. Additionally, arachidonic acid in sesame oil enhances antibacterial activity by generating free radicals through peroxide reactions catalyzed by Fe^{2+} and H_2O_2, which damage bacterial macromolecules. Propyl paraben acts by altering cell membrane permeability, leading to partial lysis and inhibiting cellular metabolism and protein synthesis [23]. Statistical analysis was conducted using SPSS version 16. Normality and homogeneity tests were followed by one-way ANOVA. Post Hoc Duncan test results revealed significant differences among the positive control, test control, and comparison control 2 ($p<0.05$). However, the negative control and comparison control 1 showed no significant differences in antibacterial activity. These findings confirm significant variations in antibacterial activity among the tested treatments.

▪ CONCLUSION

Liquid lipstick preparation with variations of sesame seed oil (*Sesamum indicum* L.) and microcrystalline wax based on the results of physical evaluation tests produces a significant effect on viscosity, spreadability, and adhesive test. The antibacterial activity test demonstrated that the optimum liquid lipstick formula containing 70% sesame seed oil and microcrystalline wax 5% effectively inhibited *S. aureus* growth, producing a strong inhibition zone of 15.33 mm. The antibacterial activity of sesame seed oil is attributed to its fatty acid composition, highlighting its effectiveness as a natural antibacterial agent. In contrast, the absence of sesame oil or other antibacterial agents in certain formulations resulted in no inhibition activity, underscoring the critical role of sesame seed oil in enhancing the antibacterial properties of the lipstick formula.

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