

PAPER 123 – DEVELOPMENT OF A KERATIN-BASED HAIR CREAM FORMULATION FOR REPLENISHING DAMAGED HAIR: A RESPONSE SURFACE METHODOLOGY APPROACH

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ABSTRACT

This study aimed to develop a hair cream formulation that can replenish damaged keratin in hair caused by various hair treatments and activities. The active ingredient used in the hair cream was keratin extracted from chicken feathers. The hair cream formulations were designed and optimized using simplex lattice response surface methodology. Ten samples were produced by varying the percentage of distilled water, keratin protein, and emulsifier, while keeping all other ingredients constant, and the responses were pH and viscosity. The optimal percentage of the varied ingredients was determined as 54.54% distilled water, 2.59% keratin protein, and 7.87% emulsifying wax, with a pH of 6.0 and viscosity of 21,222cps. The selected optimal solution had a desirability value of 0.92. Stability assays were conducted to study the organoleptic properties, pH, phase separation, and spreadability of the hair cream formulations. The formulations exhibited great stability at room temperature (25°C) and -5°C, but were unstable at 50°C. The pH range of the formulations was adjusted to be within the isoelectric pH range of the hair. Therefore, the use of keratin extracted from chicken feathers as the active ingredient has a high potential to replenish damaged hair and provides a sustainable approach to hair care.

KEYWORDS: Hair, Keratine, Isoelectric pH, Viscosity, Chicken feathers, response surface methodology

18. INTRODUCTION

Hair is a unique pigmented filament of keratin that grows from follicles located in the dermis of the skin. It serves as a protective layer against mechanical stress and aids in regulating body temperature (Buffoli *et al.*, 2013). Hair is made up of two distinct parts; the follicle, which is the internal part, and the hair shaft, which is the external part and comprises of the cortex, cuticle, and medulla (Leeson *et al.*, 1985). The duration of hair growth is coordinated by various hormones and cytokines and is influenced by factors such as location of hair growth on the body, age, nutritional habits, and environmental factors (Wolfram, 2003; Mathew, 2016). Hair growth occurs in three phases; anagen, catagen, and telogen (Wolfram, 2003; Mathew, 2016). The keratin proteins, which are responsible for the strength and stability of hair, are produced during the hair growth process and are controlled by over 30 growth factors and cytokines (Erdogan, 2017; Hill *et al.*, 2010). These proteins are categorized into two families; the acidic type I keratins and the basic-neutral type II keratins (Erdogan, 2017; Hill *et al.*, 2010).

Keratin proteins in hair are classified into three broad groups: Alpha (α) keratin, which is found in the hair fiber cortex and has low sulfur content with an average molecular weight of 60-80 kDa; β -keratin, which forms the majority of the hair cuticle and is difficult to extract; and gamma (γ) keratin, which has a molecular weight of 15 kDa, is usually globular and is high in sulfur content (Hill *et al.*, 2010). The major proteins synthesized in the hair shaft include alpha-keratin, intermediate filaments, and keratin-associated proteins (KAP). There are approximately 54 functional alpha-keratin genes, of which 28 are acidic type I keratins, and 26 are basic-neutral type II keratins (Wu *et al.*, 2008; Swain *et al.*, 2008).

Activities such as styling and exposure to environmental factors can weaken the keratin disulfide bonds in hair, leading to hair damage such as breakage, porosity, and frizziness (Pressly *et al.*, 2016). Incorporating hydrolyzed keratin into hair care products can repair and restore the texture, luster, and manageability of hair. Keratin creams can be formulated as hair straightening creams or keratin treatment creams. Hydrolyzed keratin is usually added to the cool down phase of the formulation, in proportions ranging from 0.5wt% to 15wt% of the formulation, with a preference of 0.5wt% to 15wt% of the formulation (Michael & Marie, 2016). Keratin treatment creams aid in repairing disulfide bond damages resulting from various hair beautification activities, and may also contain other active ingredients along with cosmetically approved excipients (Ursula *et al.*, 2002).

19. THEORETICAL ANALYSIS

The formulation and composition of hair creams containing keratin proteins. Two types of hair creams, namely hair straightening creams and keratin treatment creams, are formulated using hydrolyzed keratin. Hair straightening creams break the disulfide bonds in hair keratin, whereas keratin treatment creams repair the

damage caused to the disulfide bonds. The composition of a hair cream formulation includes diluents, active agents, preservatives, and emollients. The ingredients should be safe for use on human hair and scalp and must not cause any adverse reactions or damage to hair. The preferred diluent is water, whereas the preferred preservatives include glycerin compounds, parabens, and essential oils. The amount of each ingredient ranges from 0.1wt% to 50wt% of the formulation, depending on the ingredient. Emollients are added in an amount ranging from 0.5wt% to 15wt% of the formulation. Suitable emollients include silicone compounds, polyols, triglycerides, and fatty acid esters (Santos *et al.*, 2017).

20. MATERIALS AND METHODS/METHODOLOGY/EXPERIMENTAL PROCEDURE

The hydrolyzed keratin (Table 1) was extracted from chicken feathers in the research laboratory of chemical engineering Ahmadu Bello University Zaria, Kaduna Nigeria. The reagents and other ingredients used in the formulation were gotten from Steve Moore Chemicals and local stores from Sabon Gari and Samaru market. The ingredients are shown in Table 1. Design expert version 13 was used to vary the solvent (distilled water), active ingredient (keratin protein) and emulsifier (emulsifying wax NF), using simplex lattice mixture design. The equipment and instruments used were gotten from the research laboratory and the PTDF laboratory in the department of Chemical Engineering Ahmadu Bello University Zaria Kaduna, Nigeria.

Table 1: Ingredients in the Formulations

Ingredients	Quantity (g)	Quantity wt%	Functions
1. Glycerin	6	6	Emollient
2. Sweet almond oil	6	6	Conditioning agents
3. Shea butter	15	15	Plant extracts
4. Coconut oil	6	6	
5. Vitamin E	0.5	0.5	Anti-oxidant
6. Sodium benzoate	1	1	Preservative
7. Citric acid	q. s	q. s	pH modifier

3.1 Experimental design

Design Expert version 13 was used to design the experiment varying the amount (percentage) of diluent (distilled water), active ingredient (keratin protein) and emulsifier (emulsifying wax) in each formulation using simplex lattice mixture design. Ten samples of 100 wt% hair cream were generated and formulated. The pH and viscosity of each sample were the responses analyzed. All other ingredients were kept constant in the 10 formulations.

3.2 Preparation of Ingredient

The ingredients were measured using beakers and weighing scale, and it was ensured all the ingredients sum up to 100 wt% of each formulation. They were further separated into three phases which are the water phase (phase A), oil phase (phase B) and cool down phase (phase C) as shown in Table 2. Sodium benzoate (preservative) solution was prepared by dissolving 30g of sodium benzoate powder in 47.8ml of distilled water at 15°C. Citric acid solution (pH modifier) was also prepared by dissolving 50g of citric acid powder in 50ml of distilled water.

Table 2: Phases in the Formulation

Phase A (water phase)	Phase B (oil phase)	Phase C (cool down phase)
Water	Shea butter	Hydrolyzed keratin
Glycerin	Coconut oil	Vitamin E
	Emulsifying wax NF	Sodium benzoate
	Sweet almond oil	Fragrance

3.3 Formulation of the Hair Cream

Phase B was heated using a hot plate and stirred with a spatula till it completely melted. Phase A was then prepared by adding glycerin to distilled water and heated to 75°C using a hot plate. Using a thermometer, it was ensured that phase A and B were at the same temperature (75°C) before proceeding with the formulation, this is to enable easy mixing of the two phases.

Phase B was added to phase A and stirred thoroughly with a magnetic stirrer. Once the mixture cooled down to 40°C the ingredients in phase C (cool down phase) were added individually to the mixture and stirred continuously. A laboratory emulsion mixer was then used to mix the formulation till all the ingredients were well blended as. The hair cream was transferred into jars and sealed properly.

3.4 Physicochemical Analysis

Before testing the formulation, the appearance, color, odor and homogeneity of the hair cream were observed and noted after preparation. Afterwards each sample was subjected to a centrifugation cycle of 3000 rpm for 30 minutes using a centrifuge and were observed for phase separation according to the ANVISA guide. The organoleptic parameters were observed.

The samples were further subjected to pH analysis using a pH meter at room temperature. The pH meter was calibrated with pH buffer 7 solution, it was then inserted into the formulation for 1 minute, the reading on the device was noted and recorded.

An NFV-E1 intelligent viscometer was used to measure viscosity of the formulations at room temperature using spindle number 4 at full scale rotation of 12 rpm for 30 seconds.

The spreadability of each formulation was determined using glass slides. 0.5 g of the sample was placed on a glass slide and a second glass slide was placed on it. A 250 g weight glass load was placed on the upper slide for 5 minutes. The load was removed and the spreading diameter was measured and recorded. The spreadability of the samples was calculated using Equation 1;

$$S = d^2 \frac{\pi}{4} \dots\dots\dots 1 \text{ (Viviane et al., 2009).}$$

3.5 Preliminary Stability Test

The stability test was carried out at varying temperatures, each sample was placed in transparent glass containers and were properly sealed with a lid. 10 g of each sample was subjected to stability test at 50 °C in an oven for 7 days, changes were observed at day 7. 20 g of each sample were also subjected to stability test at -5 °C for 15 days and the remaining bulk samples were stored at room temperature (25 °C) for 15 days. The changes were observed at an interval of 5 days. Changes in appearance, color, odor, phase separation, pH and spreadability were observed and noted at 5 days intervals (5, 10 and 15 days).

3.6 Adjusting the pH of the Samples

The pH of hair care formulations should be around the isoelectric pH of hair (Michael and Marie, 2016). The pH of the formulations was adjusted with citric acid at room temperature (25°C) on completion of the stability test. This is done by adding quantum sufficit (q.s) amount of citric acid to each formulation and stirred properly with spatula, a pH meter was used to check the pH of the formulation till it reaches the isoelectric pH.

21. RESULTS AND DISCUSSION

On formulation, the hair cream appeared to have a smooth texture with no sedimentation or coalesces. The color of the cream is slightly off white, this is because the Keratin protein used was ash color. The formulations had a characteristic pleasant odor of the fragrance used. There was no phase separation which means that the ingredients were well mixed and compatible.

4.1 Physico Chemical Properties of the Formulations

On preparation of the hair cream the following organoleptic properties were observed and stated in Table 3;

- Appearance: Formulations all had smooth texture, and there were no sedimentation or coalescence.
- Color: The color is off white
- Odor: Formulation had a pleasant odor
- Phase Separation: No phase separation, the two phases were well blended and the hair cream formulation was homogeneous.

Table 3: Physico-Chemical Properties of Hair Cream Formulations

Sample	pH	Viscosity (cp)	Spreading Diameter (cm)	Spreadability (cm)	Mass (g)
1	6.0	8390	6	28.28	76.81
2	5.9	24300	5.7	25.52	78.29

3	5.8	13940	5.9	27.34	85.53
4	6.0	24250	6.0	28.28	95.86
5	5.9	28100	5.7	25.52	108.61
6	6.0	21600	5.1	20.43	85.13
7	5.9	14180	5.4	22.90	88.08
8	5.9	24800	5.9	27.34	83.14
9	5.9	26400	6	28.28	89.57
10	5.9	26260	5.5	23.76	84.74

4.2 Design of Experiment Results

The responses of the experimental design are shown in Table 4. Other results obtained from design expert include the model summary statistics, the analysis of variance, fits statistics. These results are given for the pH and viscosity respectively. The numerical optimization solution and the graphical representation of the design were also obtained.

Table 4: Experimental Design Matrix

Std	Run	Component 1 A: Distilled water %	Component 2 B: Keratin protein %	Component 3 C: Emulsifying wax %	Response 1 pH	Response 2 Viscosity cps
7	1	55	3.5	6.5	6.0	8390
3	2	53.5	3.5	8	5.9	24300
4	3	55	5	5	5.8	13940
1	4	55	3.5	6.5	6.0	24250
6	5	52	5	8	5.9	28100
5	6	55	2	8	6.0	21600
10	7	55	5	5	5.9	14180
9	8	53.5	3.5	8	5.9	24800
8	9	53.5	5	6.5	5.9	26400
2	10	53.5	5	6.5	5.9	26260

It is evident that the variation of water, keratin protein and emulsifying wax have substantial effect on the viscosity of the formulations but not on the pH of the formulations. Sample 1 have the lowest viscosity this is because it has maximum amount of water and intermediate amount of keratin and emulsifying wax. Sample 5 have the highest viscosity which is as a result of it minimum amount of water content and maximum amount of keratin protein. It also gave the highest yield in terms of mass. Sample 3 and 7 have lesser viscosity compared to the remaining samples, this is because they have maximum amount of water and keratin protein but minimum amount of emulsifying wax.

4.2 pH analysis results

Table 5, 6 and 7 gives the model summary statistics, analysis of variance and the fit statistics for the pH response. A 3D graphical representation of the response is shown in Figure 4.1. Figure 4.2 shows the graph relating the predicted and the actual value.

Table 5: Model Summary Statistics

Source	Std. Dev.	R ²	Adjusted R ²	Predicted R ²	PRESS	
Linear	0.0422	0.7201	0.6401	0.4025	0.0267	Suggested
Quadratic	0.0255	0.9418	0.8690			
Special Cubic						Aliased

Linear model was suggested for the pH analysis which gave the difference between **Adjusted R² and the Predicted R² as 0.24** \approx 0.2 which is the acceptable value for **R²**.

Table 6: ANOVA for Linear Model

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	0.0321	2	0.0161	9.00	0.0116	Significant
Linear Mixture	0.0321	2	0.0161	9.00	0.0116	
Residual	0.0125	7	0.0018			
Lack of Fit	0.0099	3	0.0033	5.07	0.0753	not significant
Pure Error	0.0026	4	0.0007			
Cor Total	0.0446	9				

The ANOVA gave a Mixture Component coding as **L_Pseudo with a F-value** of 9.00 and **P-values** less than 0.0500 which indicates that the model terms are significant.

Table 7: Fit Statistics

Std. Dev.	0.0422	R²	0.7201
Mean	5.91	Adjusted R²	0.6401
C.V. %	0.7144	Predicted R²	0.4025
		Adeq Precision	7.4903

The **Lack of Fit F-value** is 5.07 which is significant, this is good because the model needs to fit. The **Adeq Precision** measures the signal to noise ratio of 7.490 which indicates an adequate signal. Thus, the model can be used to navigate the design space.

4.3 Viscosity analysis results

Table 8, 9, and 10 gives the model summary statistics, analysis of variance and the fit statistics for the viscosity response. Figure 4.3 and 4.4 gives a 3D graphical representation of the response and a graph relating the predicted and the actual value.

Table 8: Model Summary Statistics

Source	Std. Dev.	R ²	Adjusted R ²	Predicted R ²	PRESS	
Linear	4857.03	0.5855	0.4670	0.2731	2.896E+08	Suggested
Quadratic	5611.00	0.6839	0.2887			
Special Cubic						Aliased

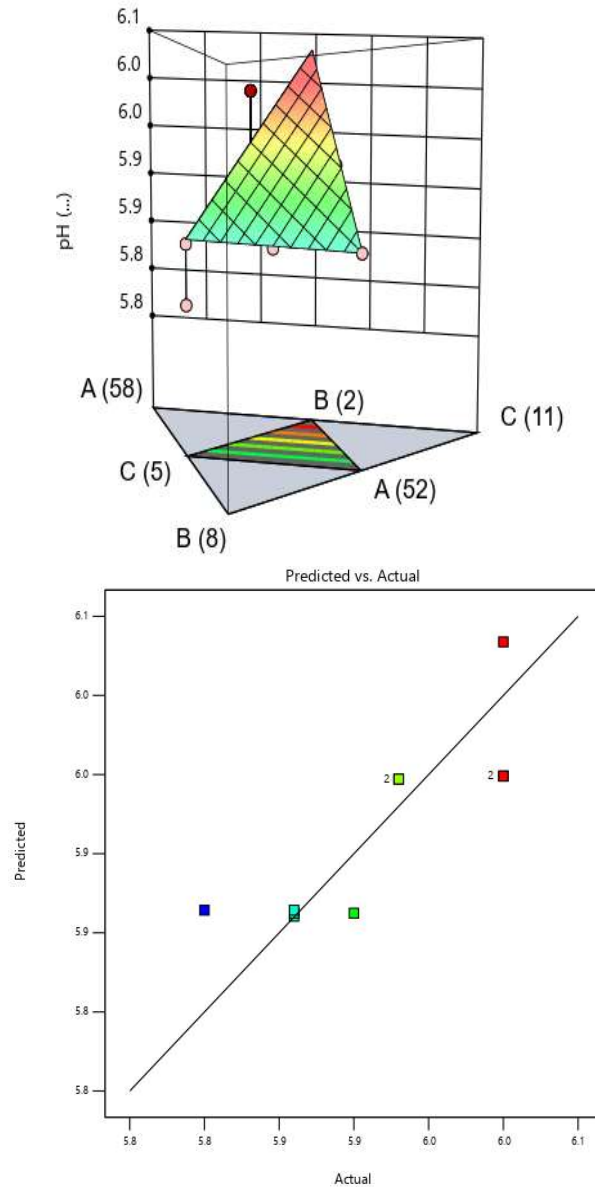


Fig 1: 3D Representation of pH Model Fig 2: Representation Predicted vs. Actual pH value

The suggested model for the viscosity is a linear model. The **Model F-value** is 4.94 and **P-values** is less than 0.0500 which indicates that the model terms are significant.

Table 9: ANOVA for Linear Model

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2.332E+08	2	1.166E+08	4.94	0.0459	Significant
Linear Mixture	2.332E+08	2	1.166E+08	4.94	0.0459	
Residual	1.651E+08	7	2.359E+07			
Lack of Fit	3.920E+07	3	1.307E+07	0.4151	0.7520	not significant
Pure Error	1.259E+08	4	3.148E+07			
Cor Total	3.984E+08	9				

The **Lack of Fit F-value** of 0.42 implies the Lack of Fit is not significant relative to the pure error which is good since the model needs to fit.

Table 10: Fit Statistics

Std. Dev.	4857.03	R²	0.5855
Mean	21222.00	Adjusted R²	0.4670
C.V. %	22.89	Predicted R²	0.2731
		Adeq Precision	5.8593

The **Predicted R²** of 0.2731 is in reasonable agreement with the **Adjusted R²** of 0.4670; i.e. the difference is less than 0.2. **Adeq Precision** measures the signal to noise ratio, a ratio of 5.859 indicates an adequate signal which is desirable. This model can be used to navigate the design space.

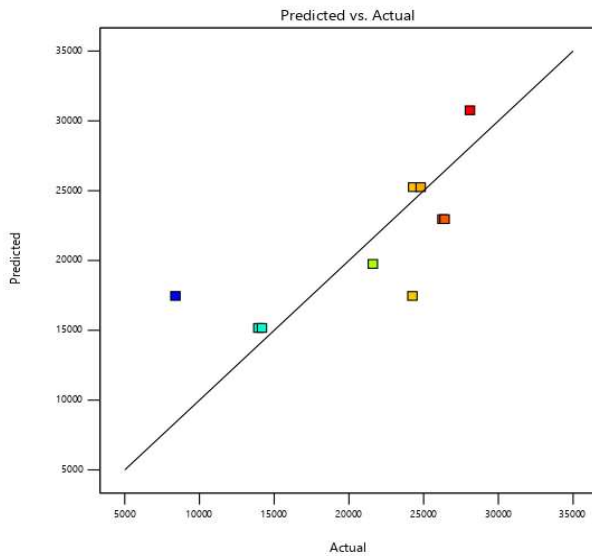
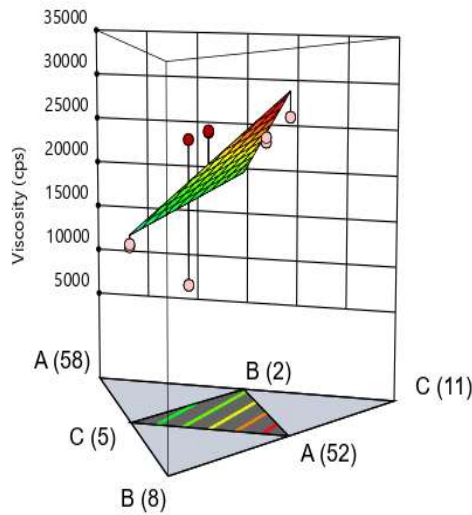


Fig 3: 3D Representation of Viscosity Model

Fig 4: Representation Predicted vs. Actual viscosity values

4.4 Numerical optimization solution

Equations 2 and 3 gives the final mathematical representations of the pH and Viscosity response model respectively in Terms of L_Pseudo Components.

$$pH = 6.04A + 5.69B + 6.03C \dots\dots\dots 2$$

$$Viscosity = 4164.76A + 26278.10B + 35340.00C \dots\dots\dots 3$$

Where;

A= Distilled water; B = Keratin Protein and C=Emulsifying wax

Table 4.9: Numerical Optimization Solutions

Distilled water	Keratin protein	Emulsifying wax	pH	Viscosity	Desirability	
54.544	2.588	7.868	6.0	21222.041	0.920	Selected

For the numerical optimization the amount of distilled water was kept in range, keratin protein was minimised and the emulsifying wax was maximised. These ranges give the highest desirability of 0.91% for the model as shown in Figure 5. Figure 6 shows the graphical optimization representation of the model.

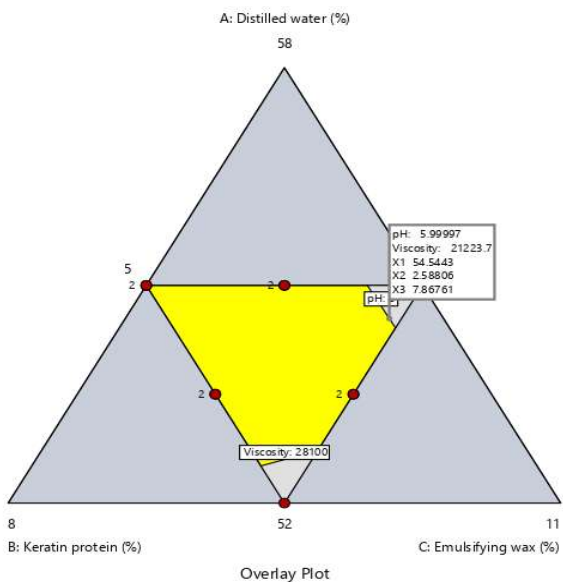
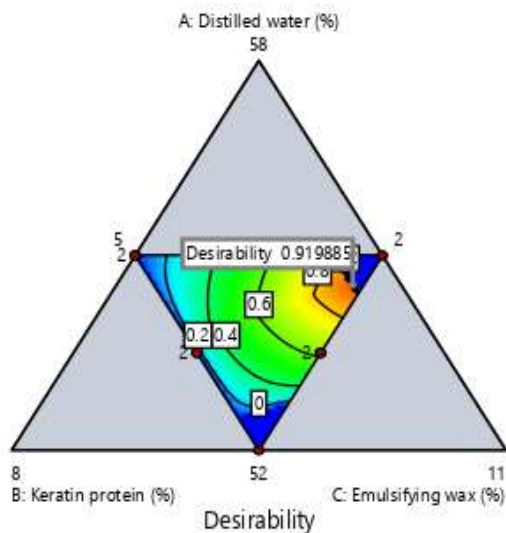


Fig 5: Contour Graph for Numerical Optimization Desirability

Fig 6: Graphical Optimization Representation

4.5 Stability Assay Results

The centrifugation test shows that each sample was stable enough to go through the preliminary stability analysis since no phase separation was observed after subjecting the formulations to a centrifugation cycle of 3000 rpm for 30 minutes, this is in accordance with ANVISA (2004) guidelines. The formulations were analyzed in the

laboratory for changes in organoleptic properties, phase separation, pH and spreadability during the stability assay. The physico-chemical properties of the formulations are stated in Table 11. Table 12 to 14 shows the results for the stability analysis of the formulations at day 5, 10 and 15 respectively. Table 15 shows the stability tests result at 50°C on day 7. There was no microbial growth observed throughout the duration of the stability test, which indicates that the preservative used was effective and stable.

Table 11: Stability Tests Result at Day 5

At 25°C	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Appearance	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Color	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Odor	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Phase separation	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Spreadability (cm)	25.52	24.63	27.34	28.28	25.52	20.43	20.43	21.23	27.34	28.28
pH	6.1	6.0	5.9	6.0	6.0	6.0	6.0	5.9	6.0	5.9
At -5°C										
Appearance	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Color	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Odor	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Phase separation	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Spreadability (cm)	27.34	23.76	25.52	24.63	25.57	21.23	21.23	23.76	25.52	28.28
pH	6.0	5.9	5.8	6.0	5.9	6.0	5.9	5.9	6.0	5.9

Where NC signifies no change and S1 to S10 stands for sample 1 to 10.

At 25°C, all 10 formulations showed no change in appearance, color and odor. There was also no phase separation. The change in pH and spreadability was also not significant. At -5°C there was also no change in color, odor, appearance and pH. The change in spreadability was also minute.

Table 12: Stability Tests Result at Day 10

At 25°C	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Appearance	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Color	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Odor	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Phase separation	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Spreadability (cm)	28.28	25.52	27.34	28.28	27.34	21.23	21.23	23.76	28.28	27.34
pH	6.1	6.0	6.1	5.9	5.9	6.1	6.1	6.0	5.9	6.0
At -5°C										
Appearance	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Color	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Odor	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Phase separation	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Spreadability (cm)	28.28	24.63	25.52	25.52	27.34	23.76	21.23	27.34	28.28	27.34
pH	6.0	5.9	5.8	6.0	5.9	6.0	5.9	5.9	6.0	5.9

Where NC signifies no change and S1 to S10 stands for sample 1 to 10.

At 25°C and -5°C respectively on day 10 of the stability analysis. At 25°C, all 10 formulations showed no change in appearance, color and odor. There was also no phase separation. The change in pH and spreadability was also not significant. At -5°C there was also no change in color, odor, appearance and pH. The change in spreadability was also minute.

Table 13: Stability Tests Result at Day 15

At 25°C	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Appearance	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Color	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Odor	NC	NC	NC	SC	SC	NC	NC	SC	NC	SC
Phase separation	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Spreadability (cm)	27.34	27.34	28.28	27.34	28.28	22.90	23.76	25.52	27.34	28.28

pH	6.0	6.1	5.9	5.9	6.0	6.1	6.1	5.9	6.0	6.1
At -5°C										
Appearance	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Color	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Odor	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Phase separation	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Spreadability (cm)	27.34	28.28	27.34	27.34	27.34	23.76	25.52	28.28	27.34	28.28
pH	6.0	5.9	5.8	6.0	5.9	6.0	5.9	5.9	6.0	5.9

Where NC is no change SC is slight change and S1 to S10 stands for sample 1 to 10

On day 15, at 25°C all 10 formulations shows no change in appearance, color, and phase separation. Sample 4,5,8 and 10 shows a slight change odor, this could be as a result of the fragrance wearing off. At -5°C there was also no change in color, odor, appearance and pH. The change in spreadability was also minute, this is shown in table.

Table 14: Stability Tests Result at 50°C on Day 7

Day 7	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Appearance	SVC	SVC	SVC	SVC	SVC	SVC	SVC	SVC	SVC	SVC
Color	SVC	SVC	SVC	SVC	SVC	SVC	SVC	SVC	SVC	SVC
Odor	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Phase separation	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Spreadability (cm)	9.62	9.34	8.55	9.46	9.62	8.92	9.72	8.55	9.34	9.62
pH	6.1	6.0	6.1	5.9	6.1	6.1	6.0	6.1	6.0	6.1

Where NC is no change, SVC is severe change and S1 to S10 stands for sample 1 to 10

All 10 formulations showed severe change in color, appearance and spreadability. There was no separation of the oil and water phases in the formulation and showed only slight change in the color and pH. This implies that the formulations are not stable and should not be stored at elevated temperature at high temperature.

4.6 Adjusting the pH of the formulations

As stated in literature, it is very rare to formulate cosmetics at the just the right pH, hence the need for the adjustment.

Table 15. pH Adjustment to Isoelectric pH of Hair

Samples	Quantity of Citric Acid Solution (ml)	Initial pH	Adjusted pH
1	0.5	6.0	3.9
2	0.5	6.1	4.1
3	0.5	5.9	4.3
4	0.5	5.9	4.0
5	0.5	6.0	4.2
6	0.5	6.1	4.3
7	0.5	6.1	4.5
8	0.5	5.9	4.2
9	0.5	6.0	4.0
10	0.5	6.1	4.1

The adjusted pH of the formulation using citric acid. The adjusted pH fell between the range of 3.9 to 4.5 which is in range as stated by Ursula *et al.*, 2002 and Michael & Marie, 2016. Which is acceptable and considered safe because it is between the isoelectric pH range of hair.

22. CONCLUSION

The results of the study demonstrate excellent compatibility among the ingredients in the hair cream formulations. The response surface models developed using Design Expert Version 13 were found to be linear and significant for both responses. Numerical optimization solutions revealed the optimal amounts of distilled water, keratin protein, and emulsifying wax to be 54.54%, 2.59%, and 7.87%, respectively, with a desirability score of 0.92. The formulations exhibited good stability at room temperature (25°C) and -5°C, but showed instability when exposed to high temperature (50°C), suggesting that storage above room temperature is not recommended. Throughout the stability analysis, there was no evidence of microbial growth, and the pH of the formulations fell within the isoelectric pH range of hair. Based on these results, the hair cream formulations can be considered both safe and effective for use.

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