Scientific Journal for the Faculty of Science-Sirte University Vol. 6, No. 1 (2026) 16-23

Scientific Journal for the Faculty of Science-Sirte University

Journal home page: http://journal.su.edu.ly/index.php/JSFSU/index

DOI: 10.37375/issn.2789-858X

A Comparative Study of Sodium Sulfide and Sodium Hydroxide for Keratin Extraction from Waste Chicken Feathers Based on Response Surface Methodology (RSM)

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DOI: https://doi.org/10.37375/9t5jaj98

ARTICLE INFO:

Received: 22 February 2026

Accepted: 06 April 2026

Published: 17 April 2026

Keywords:

Separation Keratin, Chicken Feather waste, RSM, XRD.

A B S T R A C T

Keratin extraction from poultry feathers represents an environmentally sustainable approach for converting bio-waste into materials used in cosmetic and personal care applications, including shampoos, hair care formulations, skin creams, and related products. This study presents a comparative evaluation of sodium hydroxide (NaOH) and sodium sulfide (Na_2S) as extraction agents, with Response Surface Methodology (RSM) employed to optimize the operational parameters. The results demonstrated that NaOH was more effective in preserving peptide chain integrity and maintaining the structural stability of keratin, particularly under optimized conditions of 0.75 M concentration, 80 °C, and a 4 h, $R^2 = 0.9285$ extraction period. In contrast, the Na_2S achieved higher extraction yields and recovered mass; however, this advantage was accompanied by an increased tendency toward partial protein degradation. Structural characterization using FT-IR and SEM analyses confirmed that keratin extracted with NaOH retained its characteristic β-sheet fibrous morphology, supporting its suitability for cosmetic and personal care applications that require high structural integrity.

1. Introduction

The slow and unregulated decomposition of these biological wastes leads to the emission of ammonia and sulfur gases, posing severe risks of soil and groundwater contamination (Kidus Tekleab et al., 2020). However, given that keratin comprises approximately 91% of feather composition, this waste is increasingly viewed as a valuable resource for high-value bioproducts. These include medical substances, basic nutrients, and cosmetic ingredients for anti-aging creams, shampoos, and hair treatments (Gupta et al., 2012; Maurya & Singh, 2023).

The chemical and mechanical resilience of keratin is attributed to its high density of covalent disulfide (S-S) bonds, hydrogen bonding, and hydrophobic interactions, which confer a stable folded lamellar arrangement to beta-keratin (Parry & Fraser, 1985). From an inorganic chemistry standpoint, extracting keratin necessitates the cleavage of these disulfide bonds to transition the protein from a solid to a soluble state. Sodium sulfide (Na2S) is a highly effective reducing agent that generates hydrosulfide ions (HS-), acting as potent nucleophiles that selectively target (S-S) bonds while minimizing the degradation of long peptide chains (Schrooyen et al., 2001).

While various reducing agents, including (Na2S) and (Na2S2O5), have demonstrated extraction yields ranging from 76% to 84% (Marliyana et al., 2024; Gupta et al., 2012), alkaline hydrolysis using sodium hydroxide (NaOH) remains a cost-effective and widely applicable alternative. However, strong alkalis may cause excessive protein fragmentation into free amino acids and initiate lanthionation reactions, which can alter the chemical identity of sulfur-containing amino acids (Vasconcelos et al., 2008).

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Building on these comparative dynamics, the present study aims to optimize the extraction of keratin from waste feathers using Response Surface Methodology (RSM). This approach identifies the optimal operational parameters (concentration, temperature, and time) to maximize yield while preserving the protein's biological and structural integrity. The quality of the extracted keratin is rigorously verified through advanced analytical techniques, including Fourier Transform Infrared Spectroscopy (FT-IR) to monitor amide and carboxyl groups, X-Ray Diffraction (XRD) for crystallinity analysis, and Scanning Electron Microscopy (SEM) to evaluate the porous surface morphology (Amin et al., 2024; Dilnozakhon & Oytura, 2024). This study aims to investigate the relative effectiveness of sodium sulfide and sodium hydroxide in extracting keratin from chicken feathers, while analyzing the impact of each on the molecular weight and structural properties of the resulting protein. The present study aims to comparatively evaluate the efficiency of sodium hydroxide (NaOH) and sodium sulfide (Na_2S) in extracting keratin from waste chicken feathers, while optimizing the extraction conditions using Response Surface Methodology (RSM) to achieve maximum yield and preserve structural integrity.

2. Response Surface Methodology

Experimentation is integral to the fields of Science and Technology, involving the manipulation of experimental units and the subsequent determination of one or more responses. In every experiment, certain inputs (x) are transformed into outcomes that manifest as one or more observable response variables (y). Consequently, conclusions can be drawn from these results.

To ensure unbiased conclusions, researchers must meticulously plan and design the experiment and analyze the results obtained. When dealing with a continuous range of values, the precise relationship between y and x may not be known. In such instances, statistical analysis, such as Response Surface Methodology, becomes essential. This methodology combines statistical and mathematical techniques to design and analyze problems where a response of interest is influenced by several variables, with the objective of optimizing this response. It generally facilitates the examination of the empirical relationship between one or more measured responses and various independent variables through a polynomial equation. Mapping these responses within the experimental domain aids in developing an optimized method (A, 2023)

3. Materials and Methods

3.1. Preparation of feather powder

Initial treatment involved a thorough aqueous wash of the gathered feathers to eliminate residual lipids and contaminants. Following the cleaning phase, the material was subjected to solar drying and subsequently milled into a fine particulate form. For preservation, the resulting feather powder was kept in hermetically sealed polymer packaging.

3.2 Extraction of keratin by Sodium hydroxide

The extraction protocol utilized a 0.5 M to 1 M sodium hydroxide medium prepared within a 1L volumetric vessel. A 10 g aliquot of the prepared poultry feather powder was integrated into this alkaline environment. Thermal conditions were regulated between 30℃ and 80℃ , ensuring the alkalinity remained within a pH range of 10 to 13. This mixture was maintained under continuous mechanical agitation for a window of 2 to 6 hours. Post-reaction, the suspension was clarified via centrifugation 10,000rpm for 5 min and primary filtration, after which the keratin-rich supernatant was isolated for subsequent stages.

3.3. Precipitation of proteins

To induce protein fallout, the accumulated filtrate was treated with an ammonium sulfate reagent, introduced via a controlled dropwise technique in a 1:1 volumetric proportion. The resulting proteinaceous solids were harvested through high-speed centrifugation 20,000 rpm for a duration of 5 min., with the supernatant fraction being systematically reserved.

3.4. Protein Purification

The isolated solid fraction underwent a purification wash using 100ml of deionized water, followed by a 20,000rpm centrifugal cycle to recover the pellets. These purified solids were then re-solubilized in a more concentrated 2.0 M NaOH solution. A final centrifugal step at 20,000 rpm for 5 min. was employed to separate the clarified protein solution from any remaining insoluble debris. This entire purification cycle (precipitation, rinsing, and redissolving) was executed three times to maximize yield quality.

3.5. RSM Procedure Process

To model the extraction dynamics, Design-Expert software (version 7.0) was utilized to implement a Central Composite Design (CCD) framework. This statistical methodology was chosen to evaluate and contrast the efficacy of two distinct alkaline reagents on the resulting keratin protein yield. The experimental architecture was divided into two primary cohorts: the first employed Sodium Sulfide (Na_2S) as Design Parameter A, while the second utilized Sodium Hydroxide (NaOH). Complementing these, Temperature and Time were designated as Design Parameters B and C, respectively. To facilitate the optimization process, variable levels were codified as (-1), (0), and (+1),

representing the minimum, median, and maximum operational thresholds for each factor. The response variable in both studies was the quantity of keratin protein produced. Optimization was conducted using a desirability profile, with the objective of maximizing keratin protein yield while minimizing experimental time. The concentrations of NaOH and temperature were maintained within specified ranges. This comparative study aimed to determine the chemical agent that achieves the highest desirability for keratin production.

Table (1). The operational variables and their respective levels for the central composite design are summarized

Experimental	+1	0	-1
NaOH(M)	1	0.75	0.5
Temperature (°C)	80	55	30
Time (h)	6	4	2

To streamline the production of keratin protein, the study utilized the optimization capabilities of the Response Surface Methodology (RSM) profiler. The core strategy involved a careful selection of key experimental factors, prioritizing those with the highest impact on efficiency. Specifically, the research sought to strike a balance between maximizing the final protein yield and reducing the overall processing time. This was achieved by systematically adjusting thermal conditions and Na2S ، NaOH concentrations within predefined limits. The most 'desirable' configuration one that satisfied these competing requirements was identified as the optimal solution. Detailed constraints, including target objectives and operational boundaries for each factor, are tabulated in Table 2.

Table (2). Numerical limits and optimization criteria for keratin extraction factors

Operational Factor	Optimization Goal	Maximum Bound	Minimum Bound
NaOH (M)	Maintain within rang	1	0.5
Temperature (°C)	Maintain within rang	80	30
Time	Minimize	6	2

4. Results and Discussion

4.1. Observation

To verify the chemical composition of the extracted material, a Biuret test was conducted, yielding a positive result (Characteristic violet coloration), signaling the presence of peptide linkages in isolation protein. This qualitative confirmation aligns with the findings of Ibrahim et al. (2022), who utilized the Biuret method to quantitatively estimate keratin concentration while optimizing the extraction conditions. However, the primary methodological distinction between the two studies lies in the chemical agent employed for dissolution. Ibrahim et al. (2022) used sodium sulfide (Na_2S), a potent reducing agent that cleaves disulfide bonds to achieve maximum productivity. In contrast, the current research focuses on employing sodium hydroxide (NaOH) as a singular alkalizing agent. In addition to qualitative confirmation, UV-Vis analysis revealed a maximum absorption peak at 250 nm, indicating that NaOH treatment alone was sufficient to preserve the structural integrity of the aromatic amino acids.

This finding is corroborated by FT-IR analysis, which suggests that while Na_2S effectively enhances extraction yield by Ibrahim et al (2022), the use of NaOH prioritizes the structural integrity and chemical stability of the resulting protein.

4.1.1. UV-Vis Analysis of Extracted Keratin Protein

To evaluate the structural integrity and chromophoric composition of the isolated biopolymer, Ultraviolet-Visible (UV-Vis) spectroscopy was performed on a keratin solution standardized to 2.5 mg/mL in DMSO (Figure 1). The resulting spectral profile exhibited a well-defined absorption maximum (λ_{max}) at 250nm, with a recorded optical density of 1.2A.U. This prominent peak is fundamentally attributed to the $\pi \rightarrow \pi^*$ electronic transitions occurring within the aromatic side chains of amino acids, specifically Tyrosine and Tryptophan. The presence of this characteristic UV signature not only confirms the successful extraction of the protein but also underscores the preservation of its native chromophoric framework during the alkaline isolation process.

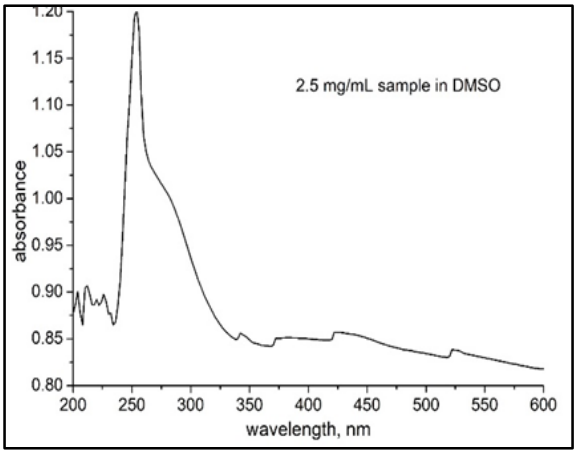

Figure (1) UV visible spectrum of extracted keratin

4.1.2. SEM Analysis of Keratin Powder

Advanced morphological characterization using Scanning Electron Microscopy (SEM) of the isolated keratin powder revealed a complex, highly agglomerated structure, where primary particles interconnect to form irregular geometric aggregates. Examination of the surface topography at high-resolution magnifications ranging from 10 to 25 kx highlighted prominent coarse and porous features, interspersed with fine foliated and laminar microstructures. These structural attributes are fundamentally attributed to the intrinsic fibrous architecture of keratin and its inherent propensity for molecular self-assembly. These characteristics are significantly influenced by the specific extraction protocols and dehydration conditions employed, ultimately resulting in an active surface area that governs the biopolymer's functional reactivity in targeted chemical and biomedical applications.

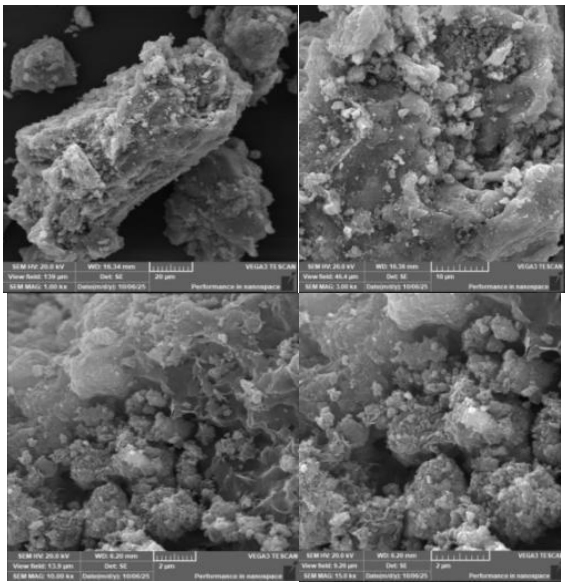

Figure (2) SEM Images of extracted keratin

4.1.3. FT-IR Analysis of Extracted Keratin Protein

Fourier-Transform Infrared (FT-IR) spectroscopy was implemented to elucidate the functional groups and secondary structural conformation of the isolated keratin (Figure 3). The resulting spectra exhibited a definitive proteinaceous profile, characterized by a broad absorption band at 3418 cm^{-1} corresponding to O–H and N–H stretching vibrations. Aliphatic C–H symmetric and asymmetric modes were identified within the 2920 - 2850 cm^{-1} range, signaling the presence of amino acid side chains. Crucially, the structural integrity was confirmed by the prominent Amide I peak at 1635.34 cm^{-1}, which specifically indicates a secondary structure dominated by β-sheet configurations. This was further substantiated by the Amide II band at 1540.85 cm^{-1} and the complex vibrational modes of the Amide III region (1350-1200 cm^{-1}), collectively validating the successful extraction of a structurally stable keratinous biopolymer.

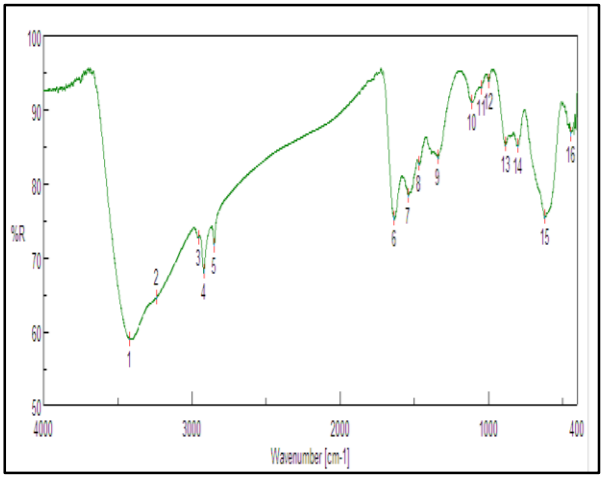

Figure (3) FT-IR spectra of keratin

4.1.4. XRD Pattern Features

The XRD pattern Figure 4 reveals sharp and well-defined diffraction peaks superimposed on a relatively broad background, which is characteristic of a semi-crystalline material. This indicates that the extracted keratin contains both highly ordered (crystalline) and less-ordered (amorphous) regions, typical of biopolymers like keratin.

Table (4). XRD diffraction peaks and structural parameters of extracted keratin

Pos. [°2θ]	[A°] *d*-spacing	Height [cps]	Rel. Int. [%]	[°2θ] FWHM
21.4303	4.14645	1.34	7.83	0.7085
35.8970	2.50173	17.09	100.00	0.4723
50.6160	1.80343	3.25	19.02	0.1771
57.4338	1.60451	2.42	14.18	0.9446

The XRD analysis reveals that the material derived from feathers exhibits a semi-crystalline structure, typical of β-Keratin. The prominent diffraction peak at 2θ = 35.8970° (d = 2.50173 Å) signifies the main crystallographic plane. Additionally, the narrow FWHM of 0.4723° for this peak indicates that the extracted keratin material has good crystalline quality.

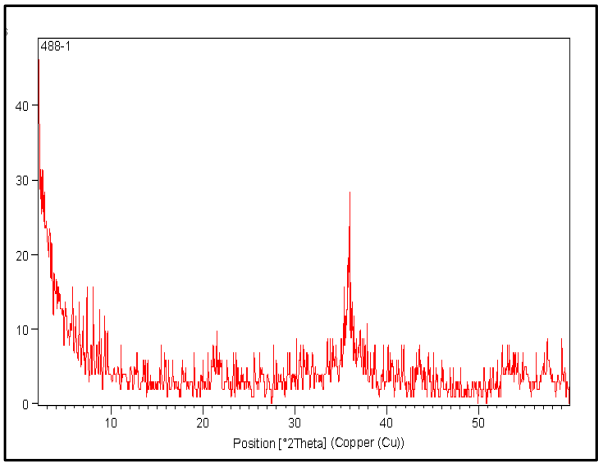

Figure (4) XRD of keratin

4.2. Optimization of Keratin Isolation via Response Surface Methodology (RSM)

To systematically evaluate the factors influencing keratin recovery, a statistical investigation was implemented using a Central Composite Design (CCD). Three critical process parameters alkali intensity (NaOH concentration), thermal conditions (temperature), and temporal duration (time) were strategically modulated to monitor their collective impact on protein yield. This experimental framework was specifically designed to facilitate the quantification and optimization of the isolated keratin. As summarized in Table (5), a total of eleven distinct experimental runs were executed. The recorded responses, derived from these varying parametric combinations, provide a comprehensive data set for modeling the extraction efficiency under the CCD configuration.

Table (5) The comprehensive CCD matrix, which integrate the strategic parametric adjustments with their respective experimental outcomes

Run	Independent factors			Response
	A:NaOH *(M)*	B:Temperature *(^0C)*	C: Time *(h)*	Amount of Keratin Protein
1	0.5	30	2	1.27
2	0.75	55	2	1.55
3	1	80	2	2.03
4	0.5	55	4	1.6
5	0.75	30	4	2
6	0.75	55	4	1.2
7	0.75	80	4	3.5
8	1	55	4	1.44
9	0.5	80	6	1.7
10	0.75	55	6	1.3
11	1	30	6	1.75

Table (5) The comprehensive CCD matrix, which integrate the strategic parametric adjustments with their respective experimental outcomes

Run	Independent factors			Response
	A:NaOH *(M)*	B:Temperature *(^0C)*	C: Time *(h)*	Amount of Keratin Protein
1	0.5	30	2	1.27
2	0.75	55	2	1.55
3	1	80	2	2.03
4	0.5	55	4	1.6
5	0.75	30	4	2
6	0.75	55	4	1.2
7	0.75	80	4	3.5
8	1	55	4	1.44
9	0.5	80	6	1.7
10	0.75	55	6	1.3
11	1	30	6	1.75

The experimental findings presented in Table 5 indicate that the keratin protein extraction ranged from 1.2 to 3.5g. Experiment No 7 achieved the highest yield of 3.5g at 80°C over 4h with a concentration of 0.75 M. Analysis of variance (ANOVA) demonstrated that the quadratic model was highly effective in prediction, as evidenced by an R2 value of 0.9285, indicating the model's accuracy in depicting the relationship between variables by over 92%. Statistical analysis revealed that temperature is the most influential factor in the process ($p = 0.0063$) high temperatures provide the catalytic energy needed to break the strong disulfide bonds in keratin, aiding its breakdown and transfer to the solvent. However, a significant quadratic effect of temperature ($B2 < 0.0001$) indicated a peak thermal efficiency, explaining the noticeable curvature in the 3D Surface Plots. This interpretation is evident when comparing Experiment No. 7 with Experiment No. 9. Although the temperature was constant at 80°C, extending the time from 4 to 6 h resulted in a significant drop in extracted weight to 1.7 g. This reduction is physically attributed to the reaction Temperature and time causing protein chain degradation rather than extraction, confirming that excessive operational factors surpass the optimal "balance point" and lead to negative outcomes. Optimization algorithms suggest that achieving the highest yield necessitates a precise balance between chemical concentration and physical conditions to ensure protein extraction while preserving its molecular structure.

This study is consistent with Research by Sienkiewicz et al. (2022) demonstrates that optimizing the NaOH concentration ideally around 1.0 M coupled with precise

thermomechanical control, significantly enhances the solubilization of keratinous biomass. Their findings highlight that the liquor-to-feather ratio serves as a critical determinant of extraction yield. Building on the functional utility of the extract, Maurya and Singh (2023) explored the structural integrity of the recovered protein, noting that alkali-extracted keratin maintains a high degree of crystallinity (approximately 83%). This structural stability facilitates its application in environmental remediation, specifically as a bio-sorbent for heavy metal sequestration from wastewater. Furthermore, recent advancements by Kamalam et al. (2023) validate that while alkaline treatment is vigorous, the resulting keratin biopolymers retain their essential peptide linkages, making them suitable candidates for fabricating biodegradable films and advanced biomaterials.

Comparing these to the results obtained with Ibrahim et al. (2022), we find the quantity of isolated keratin protein ranged from 2.4 to 4.9g. The analysis of variance (ANOVA) the sodium sulfide content (Na_2S) and the duration of exposure exerted a statistically significant effect ($p < 0.05$). Furthermore, the study identified a significant interaction between Na2S concentration and temperature, with a probability of < 0.0001. In a similar context, the statistical analysis revealed that temperature did not exhibit significance as an independent variable, nor was there a significant effect from the binary interactions between (concentration and time) and (temperature and time) were insignificant as the p-values were equal to 0.1499, 0.024 and 0.47, respectively.

Also when compared to previous studies (Maurya & Singh, 2023) We find recovery of keratinous protein from discarded broiler poultry feathers, addressing the environmental challenges associated with their disposal. Through a multi-stage chemical process involving alkaline hydrolysis with 0.5M sodium sulfide (Na_2S) and subsequent isoelectric precipitation via hydrochloric acid (HCl), a substantial protein recovery rate of 81.1% was achieved. Comprehensive analytical techniques, including FT-IR and XRD, verified the structural integrity and semi crystalline morphology of the isolated biopolymer. Furthermore, thermo gravimetric assessment TGA confirmed a robust thermal stability profile with a primary degradation threshold at 280°C, while SEM micro-analysis revealed a distinctively porous and non-homogeneous surface texture. These physicochemical attributes highlight the potential of feather-derived keratin as a sustainable biomaterial for high-value applications in tissue engineering and the cosmeceutical industry.

Table (6) Deconvolution of variance (ANOVA) for keratin yield via quadratic Response Modeling.

Variance Origin	Mean Square	F value	Significance level	Statistical Conclusion
Model	0.52	10.09	0.0030	Significant
A-NaOH	0.017	0.33	0.5840	
B-Temp.	0.77	14.80	0.0063	
C-Time	0.026	0.50	0.5012	
AB	0.99	19.04	0.0033	
AC	0.57	10.98	0.0129	
BC	0.88	16.99	0.0045	
A^2	0.12	2.25	0.1769	
B^2	3.43	66.31	<0.0001	
C^2	0.072	1.40	0.2758	

Revealed in, three-dimensional surface plots are used to visually represent the impact of the independent factors on the answers. One independent variable was kept constant while the other two variables affecting the answer were left unaltered in order to create these graphs. The amount of keratin protein in AB is displayed in Figure 5. According to the graph, a decrease in NaOH concentration has a negative effect on the reaction, whereas a rise in temperature has a beneficial effect. Nevertheless, the response is significantly impacted negatively when both parameters decline at the same time. This indicates that there is a significant connection between temperature and NaOH concentration.

As Shown in Figure (5) the 3-D surface plot further elucidates the interaction between NaOH concentration and temperature on keratin yield. The apex of the plot indicates that the maximum recovery, quantified at 3.5g, is achieved at 80° C and 0.75 M. This observation is consistent with the findings of Ibrahim et al. (2022), which underscore the critical role of temperature in expediting the dissolution process.

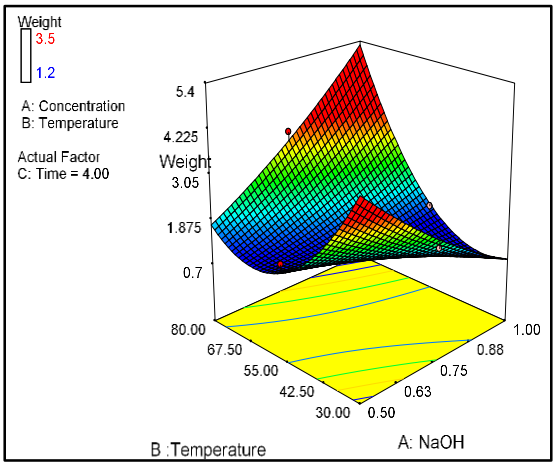

Figure (5) Three-dimensional (3D) response surface topography illustrating the synergistic interaction between alkali concentration (NaOH) and temperature on keratin yield

As seen Figure (6) the 3-D surface plot illustrating the influence of temperature and extraction time. The surface demonstrates an upward trend at elevated temperatures and moderate extraction durations 4 hours. This observation underscores the optimal duration necessary to maximize yield while mitigating the risk of protein degradation associated with extended heating.

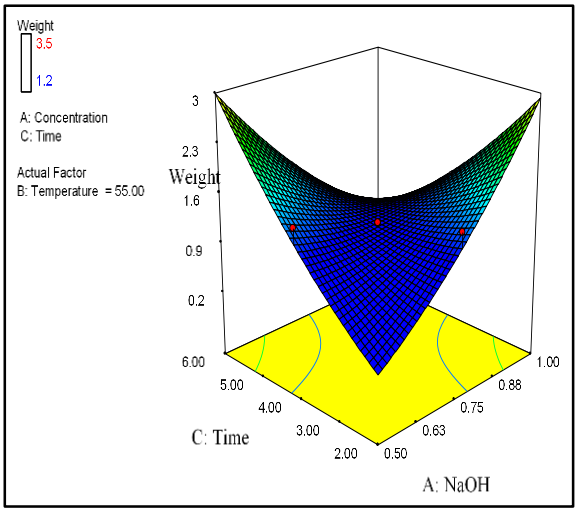

Figure (6) Three-dimensional (3D) response surface topography illustrating the synergistic interaction between alkali concentration (NaOH) and Time on keratin yield

Figure (7) Shows plot illustrating the interaction between NaOH concentration and extraction time. The observed curvature indicates that achieving an optimal balance between alkalinity and time is essential for maximizing yield, thereby demonstrating that NaOH can effectively serve as an alternative to sulphide.

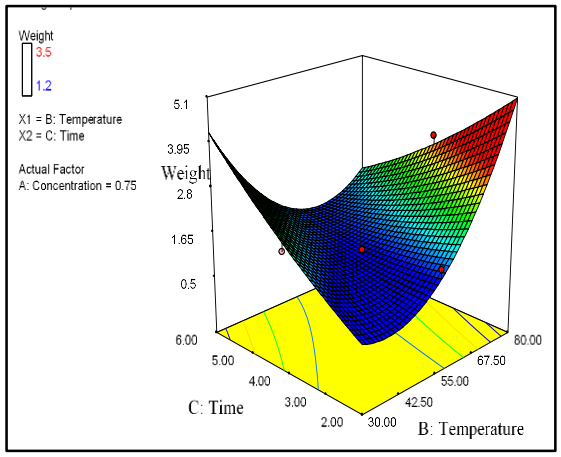

Figure (7) Three-dimensional (3D) response surface topography illustrating the synergistic interaction between Time and temperature on keratin yield

5. Conclusions

In conclusion, this study demonstrates that sodium hydroxide (NaOH) provides a more effective approach for preserving the structural integrity of keratin, while sodium sulfide (Na_2S) offers higher extraction yield. However, the balance between yield and structural stability favors NaOH as a more suitable and sustainable extraction agent. The application of Response Surface Methodology (RSM) successfully optimized the extraction conditions, confirming that temperature plays a critical role in keratin recovery. These findings support the potential of feather-derived keratin as a valuable biomaterial for industrial and biomedical applications.

Acknowledgements

Special thanks are extended to the research supervisor, Professor/Dr Dalal M. Ibrahim, for her invaluable guidance, expert supervision, and continuous support throughout this work. Deepest gratitude also goes to my father, Ahmed Bazeina, for his endless encouragement and unwavering support during my academic journey.

Conflict of interest: The authors declare that there are no conflicts of interest.

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