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Original Article

Formulation of Dried Crude Papain in an Exfoliating Scrub Cream: Optimization Using Minitab 20 Response Optimizer and Evaluation of Proteolytic Activity via Release Test

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ABSTRACT

Papain is a proteolytic enzyme known for its exfoliating properties, capable of lysing dead skin cells on the skin's surface. This study aimed to formulate and optimize a scrub cream containing dried crude papain as an active exfoliant. A 2^2 factorial design was employed, with stearic acid (10%)–triethanolamine (3%–4%) and cetyl alcohol (2%–2.5%) as formulation variables. The scrub cream was evaluated for its physical and chemical characteristics, including viscosity, flowability, spreadability, cream type, pH, and proteolytic activity. Proteolytic release was assessed using a Franz diffusion cell. The resulting formulations exhibited viscosities ranging from 60,000 to 102,000 cP at 2 rpm, spreadability between 76.74 and 116.12 g•cm/sec, and pH values from 7.64 to 7.95. Proteolytic activity ranged from 1.02 to 4.24 TU/mg. Stearic acid–triethanolamine significantly influenced spreadability, proteolytic activity, and pH, while cetyl alcohol affected all measured parameters. The optimized formulation consisted of stearic acid–triethanolamine (10%; 4%) and cetyl alcohol (2%). The release mechanism of papain from the scrub cream was identified as a combination of Fickian diffusion and matrix relaxation. These findings support the potential of dried crude papain as a viable active ingredient in exfoliating scrub cream formulations.

Keywords*: Papain; scrub cream formulation; factorial design; proteolytic activity; Franz diffusion cell; exfoliating cosmeceutical.*

INTRODUCTION

The accumulation of dead skin cells on the skin's surface contributes to a dull complexion. To prevent this, exfoliators are commonly used. Papain, a proteolytic enzyme derived from the papaya plant (Carica papaya L.), is a natural exfoliant. It works by lysing dead skin cells adhered to the skin, resulting in a smoother and brighter appearance [1,2]. Kardono *et al.* reported that a 1% papain lotion can brighten the skin by inhibiting the tyrosinase enzyme [3], while other studies have shown that even 0.2% papain can enhance skin brightness through exfoliation [4].

Anggraini *et al.* formulated papaya gum into a water-in-oil (W/O) emulsion cream [5]. However, W/O creams are generally less comfortable for topical use due to their greasy texture and difficulty in removal [6]. To improve user comfort—ensuring ease of application, non-stickiness, a cooling sensation, non-comedogenicity, and ease of removal—papain coarse powder was formulated into an oil-in-water (O/W) cream base. This approach also enhances the stability and exfoliating efficacy of papain [7].

Exfoliating scrubs are designed to act through both enzymatic and mechanical mechanisms, with the addition of scrubbing agents to facilitate the removal of dead skin cells. To maximize enzymatic exfoliation, 10% crude papain

powder was incorporated into the formulation [6,8-10]. Triethanolamine-stearate was used as an *in situ* emulsifier to improve the stability of the O/W emulsion.

This study employed a 2^2 factorial design with two formulation factors: stearic acid–triethanolamine as the emulsifier and cetyl alcohol as the thickener.[11] The concentration ranges used were stearic acid–triethanolamine (10%; 3–4%) and cetyl alcohol (2–2.5%). The exfoliating scrub cream was evaluated for organoleptic properties, homogeneity, cream type, viscosity and flow behavior, spreadability, pH, and proteolytic activity. Proteolytic release was assessed using a Franz diffusion cell. To determine the effects of stearic acid–triethanolamine and cetyl alcohol on the formulation characteristics, factor effect and interaction analyses were conducted using Minitab 20 software.[12] The optimal formulation was identified using the Minitab 20 Response Optimizer [6,13]

MATERIALS AND METHODS

2.1. Materials

Crude papain was from Nanning Pangbo Biological Engineering Co., Ltd., China. Tyrosine and casein were purchased from Sigma-Aldrich, Germany, while cysteine HCl monohydrate and S-pack diffusion membranes were from Merck, Germany. The instruments used included a Brookfield viscometer (RV type), USA; a UV-Vis spectrophotometer (Shimadzu UV-1800), Japan; and a Franz diffusion cell (Logan Instruments), USA.

2.2. Methods

2.2.1. Preparation of Casein Substrate

One gram of casein was dispersed in 50 mL of 0.05 M sodium phosphate buffer and heated in a water bath at 40 °C for 30 min. After cooling to room temperature, the pH was adjusted to 6.0 ± 0.1 using 0.05 M citric acid. The casein solution was diluted with purified water to make a final volume of 100 mL (designated as Solution A). [14]

2.2.2. Preparation of Phosphate–Cysteine–Edetate Buffer

The preparation of phosphate–cysteine–edetate buffer involved dissolving 3.55 g of anhydrous sodium phosphate in 400 mL of purified water, adding 7 g of sodium edetate, and 3.05 g of cysteine HCl monohydrate. The buffer was mixed and the pH was adjusted to 6.0 ± 0.1 using 1 N HCl or 1 N NaOH. The final volume was 500 mL with purified water (designated as Solution B). [15]

2.2.3. Preparation of Crude Papain Solution

Exactly 100.00 mg of crude papain powder was weighed and dissolved in Solution B to a final volume of 100 mL. From this stock solution, 2 mL was pipetted into a 50 mL volumetric flask and diluted with Solution B to obtain 50 mL of working solution (designated as Solution C). [16][17][18]

2.2.4. Preparation of Tyrosine Standard Curve

Tyrosine (10.00 mg) was dissolved in purified water and diluted to a final volume of 100 mL, yielding Solution A. A series of working solutions, containing 25, 35, 45, 55, 65, 75, and 85 ppm tyrosine, was prepared from the stock solution. Absorbance was measured at the maximum wavelength using a UV-Vis spectrophotometer. A calibration curve was constructed by plotting absorbance against concentration, yielding the regression equation: [19][20][21]

$Y = -0.0059 + 0.0092X$, with a correlation coefficient $R^2 = 0.9985$.

2.2.5. Measurement of Proteolytic Activity (AOAC Method)

Proteolytic activity was measured using the AOAC method. One gram of casein was dispersed in 50 mL of 0.05 M sodium phosphate buffer and heated in a water bath at 40 °C for 30 min. After cooling, the pH was adjusted to 6.0 ± 0.1 using 0.05 M citric acid. The solution was then diluted with purified water to a final volume of 100 mL, designated as Solution A. Phosphate–cysteine–edetate buffer (Solution B) was prepared by dissolving 3.55 g of anhydrous sodium phosphate in 400 mL of purified water, followed by the addition of 7 g of sodium edetate and 3.05 g of cysteine HCl monohydrate. Solution B was mixed, and the pH was adjusted to 6.0 ± 0.1 using 1 N HCl or 1 N NaOH. The final volume was 500 mL, adjusted with purified water. Approximately 100 mg of dried crude papain was dissolved in 100 mL of Solution B. From this stock, 2 mL was pipetted into a 50 mL volumetric flask and diluted with Solution B to obtain 50 mL of working solution (designated as Solution C). Two test tubes were prepared and designated as the sample (S) and the blank (B). In the sample tube, 5 mL of Solution A was added and preincubated at 40 °C for 10 min. Then, 1 mL of Solution C and 1 mL of Solution B were mixed and incubated at 40 °C for an hour. Three mL of TCA solution was added, and the mixture was vortexed thoroughly. Both tubes were then incubated at 40 °C for an additional 30 min and filtered using Whatman No. 42 filter paper. The filtrate was collected, and absorbance was measured at the maximum wavelength using a UV-Vis spectrophotometer. [22]

Papain activity was determined using the tyrosine standard curve or its linear regression equation:
$Y = -0.0059 + 0.0092X$, $R^2 = 0.9985$

The proteolytic activity (A, in TU/mg) was determined using the following formula:

$$Cx\frac{100}{W}x\frac{50}{2}x\frac{10}{1}xA$$

Where: (C): concentration of tyrosine (mg/mL), (W): weight of dried crude papain (mg), and (A): proteolytic activity (TU/mg)

2.2.6. Formulation of Dry Crude Papain Scrub Cream

The oil and aqueous phases of the cream base were heated separately in a water bath at 70–75 °C. Methyl paraben and propyl paraben were added and mixed in propylene glycol. The oil phase was gradually added to the aqueous phase dropwise under continuous stirring. Homogenization was done at the optimal speed and duration until a stable cream base formed. [23][24]

2.2.7. Determination of Cream Type

A total of 0.1 g of scrub cream was dispersed and mixed in 10 mL of purified water. After thorough mixing, the sample was examined under a microscope. One drop of the resulting mixture was placed on a microscope slide, followed by the addition of methylene blue solution. The presence of a uniform blue coloration in the external phase indicated an oil-in-water (O/W) emulsion type. [24][25]

2.2.8. Viscosity and Flow Behavior

Viscosity was measured using a Brookfield viscometer (RVL type) equipped with an appropriate spindle. Measurements were conducted across a range of rotational speeds (rpm), progressing from the lowest to the highest setting. Each reading was recorded at 10-minute intervals, ensuring scale values exceeded 10 units for accuracy. Viscosity, expressed in centipoise (cP), was calculated using the following equation:

Viscosity = scale x multiplication factor (cP) (2)
Shear force (F) = scale x Kv (dyne/cm^2) (3)
Kvs = 7187.00 dyne/cm^2

The evaluation of flow behavior was conducted by constructing a rheogram, plotting shear force (F) on the x-axis against shear rate (rpm) on the y-axis using graph paper. [26][27][28]

2.2.9. Spreadability Test

Spreadability was evaluated by placing 0.5 g of scrub cream onto a watch glass or microscope slide positioned over graph paper. A petri dish was placed atop the sample, and weights of 50, 100, and 200 g were applied sequentially for 1 minute each. The diameters of the spread area were measured in multiple directions, and the mean value was recorded. [29]

2.2.10. pH Measurement

Before measurement, the pH electrode was calibrated using standard buffer solutions. A 10 g of scrub cream was dissolved in 100 mL of purified water, and the pH was determined using a calibrated pH meter. The acceptable pH range for the formulation was 3.5–8.0. [30]

2.2.11. Papain Release Test Using Franz Diffusion Cell

One gram of scrub cream was applied to an S-Pak membrane, which was then mounted in a Franz diffusion cell. The membrane was secured using a clamp ring to prevent air ingress. Phosphate buffer (5 mL, pH 7.4) was added to both the donor and receptor compartments. A magnetic stir bar was placed in the receptor chamber, and the system was sealed and maintained at 37 °C. After 30 minutes, a 5 mL aliquot was collected from the receptor compartment for papain release. [31][32]

2.2.12. Factorial Design Analysis

Experimental data, including viscosity, spreadability, pH, and proteolytic activity, were analyzed using Minitab 20 software to evaluate the effects of formulation factors and their interactions. (Table 1) The optimum formulation was determined using a response optimizer. The optimization criteria included minimizing viscosity, maximizing spreadability and papain activity, and maintaining the pH within the range of 3.5–8.0. [33][34]

Table 1. Formula of dried crude papain scrub cream with factorial design 22

Ingredient	Concentration (%)			
	F1	F2	F3	F4
Dried crude papain	10	10	10	10
Stearic acid	10	10	10	10
Triethanolamine	3	4	3	4
Setyl alcohol	2	2	2.5	2.5
Propylene glycol	10	10	10	10
Propyl paraben	0.05	0.05	0.05	0.05
Methyl paraben	0.15	0.15	0.15	0.15
Polyethylene	5	5	5	5
Aquadest ad	100	100	100	100

2.2.13. Optimum Scrub Cream Formulation

Based on the optimization results obtained using Minitab 20, the ideal formulation—comprising 10% stearic acid and 4% triethanolamine—corresponded to Formula 2. The optimized formulation was subsequently employed to evaluate the release profile of papain. [35]

2.2.14. Papain Release Analysis from Scrub Cream

Papain release from the optimized scrub cream (Formula 2) was evaluated using a Franz diffusion cell, a validated method for assessing enzyme diffusion from semi-solid matrices. [23][31] Samples were withdrawn from the receptor compartment at predetermined intervals (15–180 min), and papain activity was quantified via UV-Vis spectrophotometry, expressed in TU/mg. The cumulative release profile is presented in Table 2. [36]

RESULTS

Table 2. Cumulative papain release activity against variations in sampling time, using a Franz diffusion apparatus

Time (minutes)	15	30	45	60	90	120	150	180
Cumulative papain activity (Mt)	3.58	5.76	7.15	8.38	9.60	10.65	11.22	12.41

Table 3. Comprehensive measurement results for dried crude papain scrub cream

Formulation	Cream type	Flow properties	Viscosity (at rpm 2, cP)	Spreadability (g.cm/sec)	Ph	Proteolytic activity (TU/mg)
F1	O/W	Plastic	86,000±800	115.37±0.63	7.64±0.06	3.41±0.06
F2	O/W	Plastic	60,000±769	116.12±0.53	7.85±0.04	4.24±0.01
F3	O/W	Plastic	102,000±900	76.74±2.59	7.91±0.04	1.02±0.08
F4	O/W	Plastic	92,000±840	114.97±2.36	7.95±0.03	1.42±0.06

Table 4. Analysis results of factor effects and their interactions on viscosity response

Factor	**Effect**	**Significance (*p-value)***
TEA-stearic	-20.667	0.266
Cetyl alcohol	45.333	0.031
TEA-stearic*Cetyl alcohol	-6.667	0.710

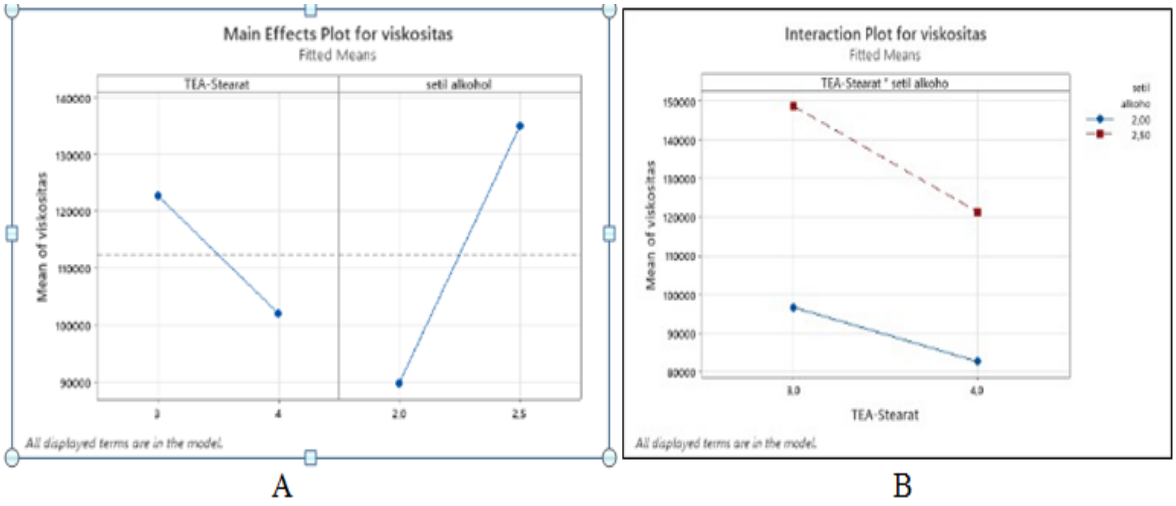


Figure 1. Plot showing main effect factors (A) and their interaction (B) on viscosity (viskositas) response

Table 5. Analysis results of factor effects and their interactions on the spreadability response.

Factor	Effect	Significance (*p-value)*
TEA-stearic	18.735	0,000
Cetyl alcohol	-19.492	0,000
TEA-stearic*Cetyl alcohol	19.888	0,000

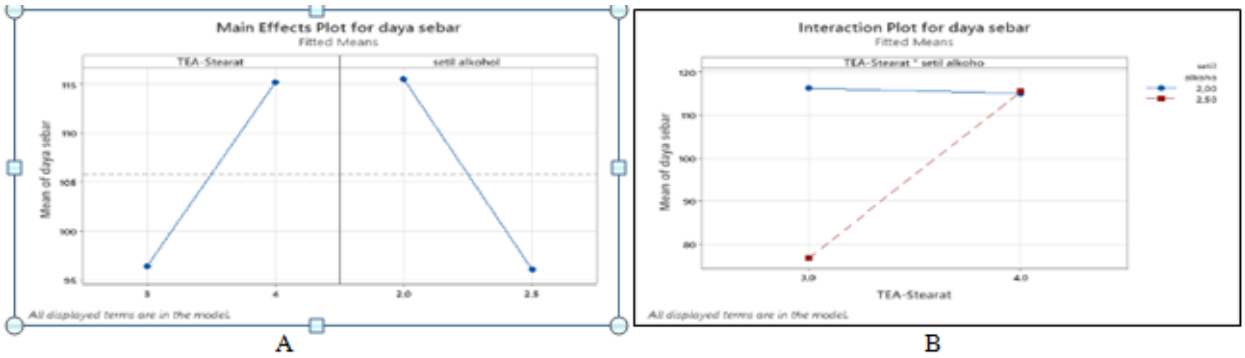
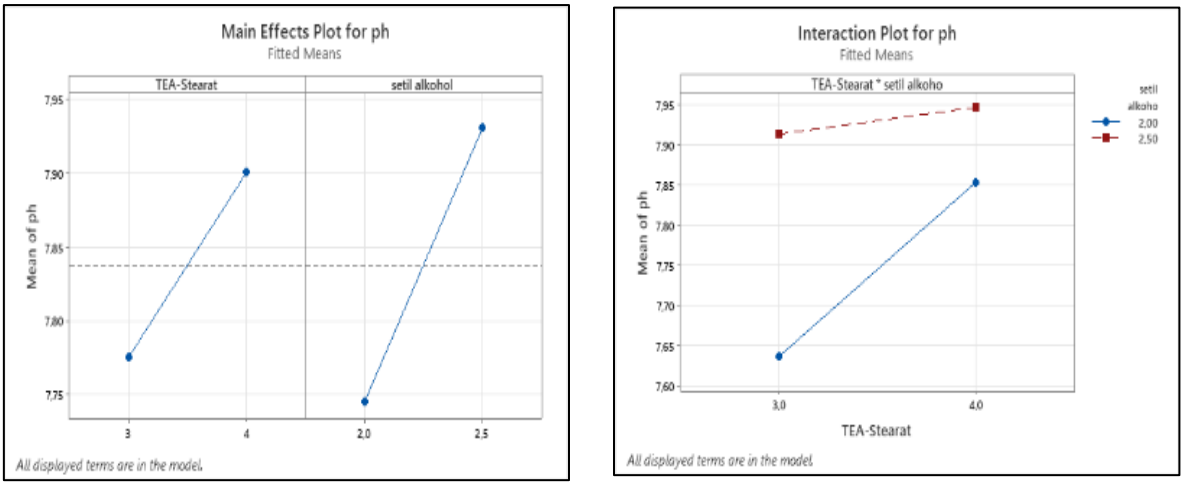


Figure 2. Plot showing main effect (A) factors and their interaction (B) on the spreadability (daya sebar) response.

Table 6. Analysis results of factor effects and their interactions on pH values

Factor	**Effect**	**Significance (*p-value)***
TEA-stearic	0.1250	0.001
Cetyl alcohol	0.1850	0.000
TEA-stearic*Cetyl alcohol	-0.0917	0.007

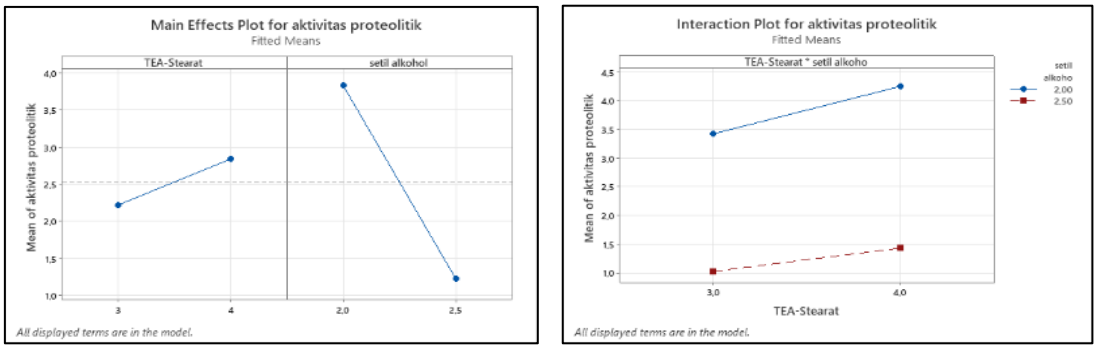


A B

Figure 3. Plot showing main effect factors (A, left)) and their interaction (B, right) on pH response.

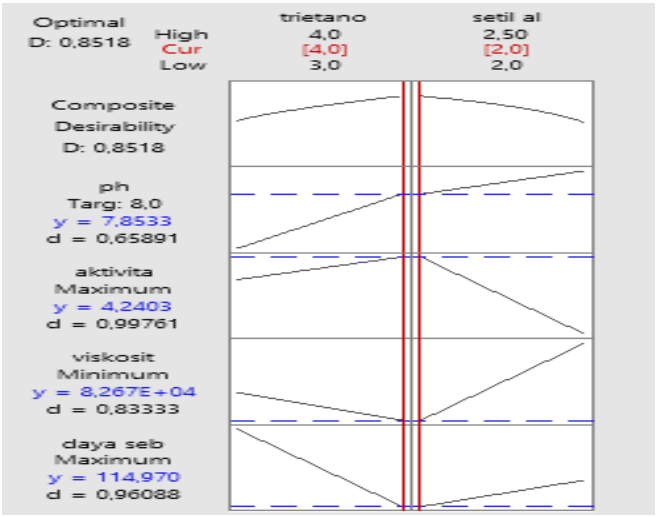
Table 7. Analysis results of factor effects and their interactions on Papain activity

Factor	Effect	Significance (*p-value)*
TEA-stearic	0.6140	0.000
Cetyl alcohol	-2.6037	0.000
TEA-stearic* Cetyl alcohol	-0.2114	0.000



A B

Figure 4. Plot showing main effect factors (A) and their interaction (B) on proteolytic activity (aktivitas proteolitik).



*) trietano (trietanolamine); setil al (cetyl alcohol); Targ (target); aktivita (activity); viskosit (viscosity); daya sebar (spraedability)

Figure 5. Analysis for determining the optimal formula using Minitab 20's response optimizer.

Tabel 8. Model, equation and constant values of papain release mechanism model

Model	Equation	R^2 & n
Zero-order	**M_t = 4.4623+0.0429t**	**0.9118**
First-order	**lnM_t = ln1.5528 + 0.0032t**	**0.7080**
Higuchi	**M_t = 0.9519 + 0.8745 √t**	**0.9754**
Korsmeyer-Peppas	**LnM_t/ M_∞ = Ln 0.0159+0.4768 Ln t**	**0.9745 & 0.4768**

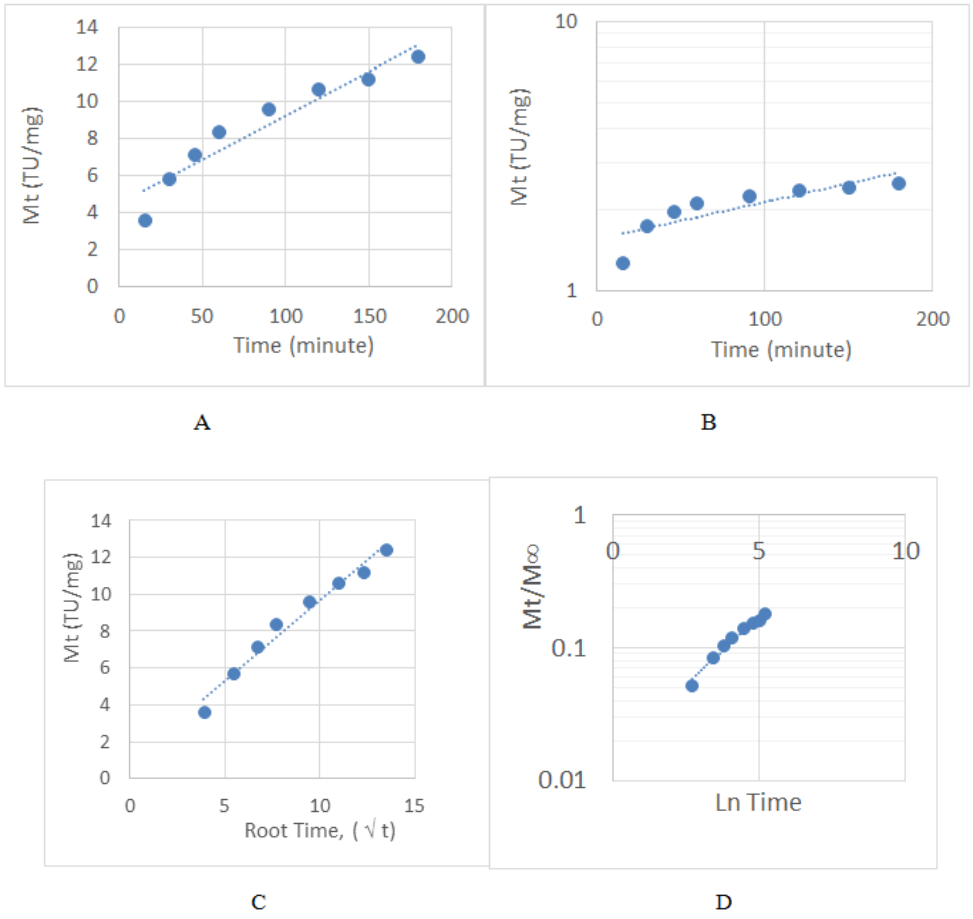


Figure 6. Mechanism of papain release from scrap cream: A, zero-order; B, first-order; C, Higuchi, and D, Korsmeyer-Peppas method.

DISCUSSION

Proteolytic activity was evaluated using Franz diffusion cells by measuring the enzymatic hydrolysis of casein substrates by papain, which releases tyrosine as a quantifiable product. The proteolytic activity values for formulations F1–F4 ranged from 1.02 to 4.24 TU/mg, indicating effective enzymatic function within the cream matrices (Table 2). [31]

Table 3 presents the comprehensive physicochemical characteristics of the dried crude papain scrub cream formulations. All formulations (F1–F4) exhibited a multiple emulsion type (M/A) and demonstrated plastic flow behavior. This rheological property is typical of suspension and emulsion systems and is attributed to flocculation. [22] Plastic flow is characterized by a yield value, below which the formulation behaves elastically. This allows the cream to remain adhered to the skin surface until it is physically removed, enhancing its topical retention. Viscosity measurements at 2 rpm revealed values of 86,000, 60,000, 102,000, and 92,000 cP for F1 through F4, respectively, confirming the semisolid consistency suitable for dermal application. [38] Spreadability, which reflects the ease of application [37] and uniform distribution on the skin, showed mean values of 115.37 ± 0.63, 116.12 ± 0.53, 76.74 ± 2.59, and 114.97 ± 2.36 mm for F1–F4, respectively. The pH values of the formulations ranged from 7.64 to 7.95, aligning with the optimal pH range for

papain activity (typically pH 6.0–8.0). [34] The slightly alkaline pH may also be attributed to the presence of triethanolamine, a commonly used emulsifier and pH adjuster with basic properties.

The Impact of Different Factors and Their Interactions on Viscosity

Table 4 and Figure 1 summarize the effects of individual formulation components and their interactions on the viscosity response of scrub cream formulations. Statistical analysis revealed that cetyl alcohol had a significant positive effect on viscosity (effect = 45.333; p = 0.031), while TEA-stearic acid showed a non-significant negative effect. The interaction between TEA-stearic acid and cetyl alcohol was also non-significant. Figure 1 illustrates the main effects (A) and interaction effects (B) of these factors on viscosity. The plot clearly shows that increasing the concentration of cetyl alcohol leads to a marked rise in viscosity. This increase is attributed to cetyl alcohol's role as a consistency enhancer. As a fatty alcohol, cetyl alcohol interacts with both the aqueous and oil phases, forming structured networks that trap water and increase the internal resistance of the emulsion. This behavior is particularly pronounced in oil-in-water (O/W) systems, where cetyl alcohol contributes to emulsion stability and thickening by reducing interfacial tension and promoting gel-like consistency. Studies have shown that cetyl alcohol enhances the viscosity and texture of cosmetic emulsions by forming lamellar structures and increasing water retention within the matrix. [27][39][40]

The Impact of Different Factors and Their Interactions on Spreadability

The spreadability of the scrub cream formulations was significantly influenced by both individual factors and their interaction, as shown in the factorial design analysis. The interaction plot between TEA-stearate and cetyl alcohol revealed that increasing the concentration of TEA-stearate emulsifier at 2.5% cetyl alcohol led to a marked improvement in spreadability (Table 5 and Figure 2). This enhancement is attributed to a corresponding decrease in viscosity, which facilitates easier application and distribution of the cream on the skin surface. In contrast, when cetyl alcohol concentration was reduced to 2.0%, the spreadability decreased. This behavior is consistent with the role of cetyl alcohol as a consistency enhancer; lower levels result in less structural integrity and reduced water-binding capacity, thereby increasing viscosity and limiting the cream's ability to spread uniformly. These findings align with previous studies demonstrating that emulsifier concentration and fatty alcohol content directly affect the rheological and sensory properties of oil-in-water (O/W) emulsions. TEA-stearate contributes to emulsification and stability, while cetyl alcohol enhances texture and modulates viscosity, both of which are critical for achieving optimal spreadability in topical formulations [27][34][41].

The Impact of Different Factors and Their Interactions on pH values.

Figure 3 illustrates the main effects (A) and interaction effects (B) of formulation variables on the pH response of the scrub cream. As shown in Table 6, both the individual factors—TEA-stearic and cetyl alcohol—and their interaction significantly influenced the pH of the formulations.

An increase in TEA-stearic concentration led to a corresponding rise in pH. This effect is attributed to the presence of triethanolamine (TEA), which functions as a neutralizing agent. TEA reacts with stearic acid to form TEA-stearate, a soap-based emulsifier that contributes to the alkalinity of the system. Consequently, higher concentrations of TEA result in elevated pH values in the final preparation. [27]

The interaction plot in Figure 3B further demonstrates that the pH increase was more pronounced at a cetyl alcohol concentration of 2.5% compared to 2.0%. This suggests that cetyl alcohol may modulate the buffering capacity or emulsification efficiency of the TEA-stearate system [39][41], thereby influencing the final pH. These findings are consistent with previous reports highlighting the role of TEA in pH modulation and the stabilizing effect of fatty alcohols in emulsion system.

The Impact of Different Factors and Their Interactions on Crude Papain Activity.

Table 7 presents the analysis of variance results, indicating that all three formulation factors significantly influenced the proteolytic activity of the crude papain scrub cream. An increase in cetyl alcohol concentration led to a reduction in proteolytic activity. This effect is attributed to the corresponding increase in viscosity, which impedes the diffusion of papain from the cream matrix. This relationship aligns with the Stokes–Einstein diffusion theory, which states that the diffusion coefficient (D) of a solute is inversely proportional to the viscosity (η) of the medium. As viscosity increases, molecular mobility decreases, resulting in a lower diffusion coefficient and, consequently, a slower release rate of the active compound. As illustrated in Figure 4A, increasing the concentration of the TEA-stearic emulsifier significantly enhanced the stability of the papain enzyme, leading to a notable increase in proteolytic activity. This may be due to improved emulsion stability and microenvironmental protection provided by the emulsifier, which helps preserve enzymatic functionality during formulation and application. [41][43][44]

Determining the Optimal Formula for Crude Papain Scrub Cream.

Optimization of the crude papain scrub cream formulation was performed using Minitab 20 response surface methodology. Four key parameters—viscosity, spreadability, pH, and proteolytic activity—were selected as critical quality attributes. Each response was assigned a target range to guide the optimization process: viscosity was minimized to enhance ease of application, spreadability was maximized to ensure uniform distribution, pH was constrained between 3.5 and 8.0 to maintain enzyme stability and skin compatibility, and proteolytic activity was maximized to preserve enzymatic efficacy. Following the establishment of these targets, the response optimizer tool in Minitab 20 was executed. The resulting optimal formulation consisted of 10% stearic acid, 4% triethanolamine (TEA), and 2% cetyl alcohol, yielding the following predicted responses: Viscosity of 82,666 cP; Spreadability of 114.97 g·cm/s; Proteolytic activity of 4.2403 TU/mg; pH of 7.8533, and Desirability index of 0.8518. [45]

These results indicate a well-balanced formulation with high desirability, reflecting the simultaneous achievement of multiple performance criteria. The selected concentrations of stearic acid and TEA contribute to emulsion stability and appropriate alkalinity, while cetyl alcohol enhances consistency without excessively increasing viscosity. The pH value falls within the optimal range for papain activity, which typically lies between pH 6.0 and 8.0, ensuring enzymatic function is retained. [46]

The desirability function approach used here is widely recognized for multi-response optimization in cosmetic and pharmaceutical formulations, allowing for the integration of diverse performance metrics into a single predictive model. This method supports rational formulation design by balancing trade-offs between competing attributes such as texture, bioactivity, and skin feel. [47]

Papain Release Kinetic Analysis

The release kinetics of papain from the scrub cream formulation were evaluated by analyzing the cumulative percentage of papain released (Mt) over time. Four kinetic models were applied to determine the release mechanism: zero-order, first-order, Higuchi, and Korsmeyer–Peppas. The corresponding equations, regression coefficients (R^2), and release exponent (n) values are presented in Table 8.

Based on the regression analysis, the Higuchi and Korsmeyer–Peppas models provided the best fit to the experimental data, with R^2 values of 0.9754 and 0.9745, respectively. The release exponent (n = 0.4768) from the Korsmeyer–Peppas model indicates a Fickian diffusion mechanism, suggesting that papain release is primarily governed by diffusion through the cream matrix [48].

The Higuchi model further supports this conclusion, as it describes drug release from a homogenous matrix system where the rate is proportional to the square root of time. This behavior is consistent with the physicochemical properties of the scrub cream, where viscosity and matrix structure influence the diffusion of papain [49].

These findings are critical for understanding the release dynamics of enzymatic actives in semisolid formulations and optimizing delivery systems for topical applications.

CONCLUSION

The study successfully formulated and optimized a crude papain scrub cream using response surface methodology and multi-response desirability analysis. Among the tested formulations, Formula 2, composed of 10% stearic acid, 4% triethanolamine (TEA), and 2% cetyl alcohol, demonstrated the most favorable balance of physicochemical and functional properties:

- Viscosity: 82,666 cP — suitable for dermal application without compromising spreadability
- Spreadability: 114.97 mm — ensuring ease of application and uniform skin coverage
- pH: 7.85 — within the optimal range for papain activity and skin compatibility
- Proteolytic activity: 4.24 TU/mg — indicating effective enzymatic function
- Desirability index: 0.8518 — reflecting strong multi-parameter optimization

The findings confirm that cetyl alcohol concentration inversely affects proteolytic activity due to increased viscosity, which limits enzyme diffusion, consistent with the Stokes–Einstein diffusion theory. Conversely, TEA-stearic emulsifier enhances papain stability, likely by improving emulsion integrity and providing a protective microenvironment. This optimized formulation offers a promising base for cosmeceutical applications, particularly in exfoliating and enzymatically active skincare products. The integration of statistical modeling with experimental validation supports rational design and scalability of enzyme-based topical systems. The most relevant mechanism for the release of papain from scrap cream preparations is a combination of Fickian diffusion and relaxation of the cream matrix.

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Competing interests
The authors have no conflicts of interest to declare**.**

Authors' contributions
Author JIF designed the study, wrote the protocol, performed the statistical analysis, and wrote the first draft of the manuscript. Author K.K. and Author MFA managed the analyses of the study. Author MFA, JIF, and K.K. managed the literature searches. All authors read and approved the final manuscript.

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