Advanced Journal of Chemistry, Section A

Journal homepage: www.ajchem-a.com

Original Research Article

Development and Evaluation of Gelatin Nanoparticle Peel-Off Gel Mask from Milkfish (*Chanos chanos*) Bones: Formulation, Stability, Skin Safety, and Anti-Aging Activity

Rahmi Annisa, Jauharuddin Luthfi Al Jabbar, Wirda Anggraini*

Department of Pharmacy, Faculty of Medicine and Health Sciences, Maliki Islamic University, Malang, Indonesia

ARTICLEINFO

Article history

Submitted: 2025-12-22
Revised: 2026-01-10
Accepted: 2026-02-25
ID: AJCA-2512-2011
DOI: 10.48309/AJCA.2026.567198.2011

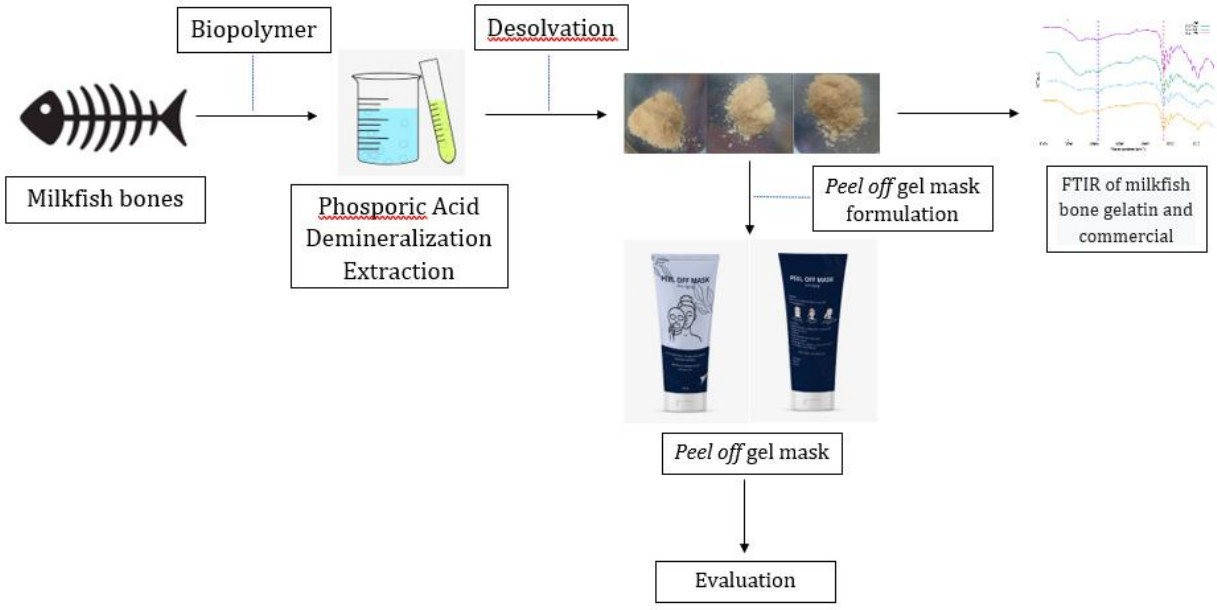
KEYWORDS

Gelatin nanoparticles
Peel-off gel mask
Milkfish bones
Preparation stability
Antiaging

ABSTRACT

This study aims to develop and evaluate a peel-off gel mask based on gelatin nanoparticles derived from milkfish (*Chanos chanos*) bones, a natural biopolymer with potential applications in the cosmetics industry. Gelatin was demineralized with phosphoric acid and converted into nanoparticles via desolvation. The gelatin nanoparticles were formulated into a peel-off gel mask preparation incorporating polyvinyl alcohol (PVA) and propylene glycol as film formers and humectants. Evaluation of the formulation included physicochemical characteristics (pH, viscosity, spreadability, adhesiveness, drying time, and organoleptic properties), particle size analysis, and stability testing using heating–cooling cycles, freeze–thaw cycles, and 90-day storage conditions. Skin safety was assessed using the Draize test, while anti-aging effectiveness was evaluated using tyrosinase enzyme inhibition. The results showed that the gelatin-based formulations produced particles in the nanometer range, with particle size varying with gelatin concentration, exhibited good physical stability, caused no skin irritation (PII ≤ 0.5), and demonstrated measurable tyrosinase inhibitory activity. These findings indicate that gelatin nanoparticles derived from milkfish bones have potential as safe, stable, and effective natural active ingredients for anti-aging peel-off gel mask formulations.

GRAPHICAL ABSTRACT



* Corresponding author: Anggraini, Wirda
✉ E-mail: wirdaanggraini@uin-malang.ac.id

Introduction

The cosmetics industry is experiencing rapid growth, driven by increasing public awareness of health and skin appearance. Products that maintain skin with functional benefits, such as anti-aging, whitening, and intensive hydration, have become a category with the highest demand in both the global and national cosmetics markets. One of the large stock forms in demand is the peel-off gel mask, known for its convenient application, ability to lift dead skin cells, and instant skin-tightening effects [1]. Although some large commercial peel-off gel masks still use synthetic polymers like polyvinyl alcohol and carbopol, they can potentially cause skin irritation and pose environmental sustainability issues. This condition requires the development of a safe, biodegradable, and environmentally friendly material base that adheres to halal principles [2]. Gelatin is a protein biopolymer derived from the hydrolysis of large amounts of collagen and is used in the food, pharmaceutical, and cosmetic industries. Due to its biocompatible nature, it can form films while providing elasticity and hydration to the skin [3,4]. Fish gelatin is a strategic option because it does not raise halal concerns, unlike mammalian gelatin. Indonesia has a potential source of abundant fish gelatin, one of which is derived from waste milkfish bones (*Chanos chanos*), which are known to have high collagen content and quality that are comparable to commercial gelatin in physicochemical properties [5]. Utilizing milkfish waste as a source of halal gelatin not only increases the market value of local materials, but also supports the development of sustainable cosmetic ingredients, which are a trend in the modern cosmetics industry. The development of technology in cosmetics and pharmaceuticals drives the implementation of nanotechnology to enhance the effectiveness of active materials in topical applications [6]. The use of nanoparticles in gelatin has been reported to enhance the stability of active materials, thereby expanding the particle surface area and facilitating penetration of the epidermis-dermis layer, resulting in more significant outcomes than conventional methods [7]. In the context of anti-aging, gelatin nanoparticles can deliver antioxidant compounds or peptide bioactives to enhance skin elasticity and inhibit pigment formation. Therefore, integrating nanoparticles into halal gelatin in stock peel-off gel masks can yield more innovative formulas that are stable and effective in delivering rejuvenation benefits to the skin. Although there is potential for large-scale studies on the scientific use of gelatin derived from milkfish bones in the formulation of peel-off gel masks, research in this area remains very limited. Research has previously focused on isolating or characterizing fish gelatin, essentially without optimizing the formulation using nanotechnology or comprehensively evaluating the stock's performance. Additionally, the stability of physicochemical preparations containing gelatin nanoparticles under various storage conditions, such as heating–cooling and freeze–thaw cycles, remains limited. Aspects of skin safety—which are very important for stock topical—have also not yet been thoroughly reviewed. However, material experience suggests that it can cause irritation reactions if not evaluated through standard tests, such as the Draize skin irritation test. Anti-aging activity based on a biological mechanism, such as the inhibition of tyrosinase, has not yet been reported in the area of systems made from fish gelatin nanoparticles, despite this mechanism being important for overcoming pigmentation and signs of aging. Recent studies have shown that the development of gelatin-based biomaterials using advanced technological approaches, such as electrospinning and nanoparticle reinforcement, has been widely applied in tissue engineering and wound healing. Co-electrospun scaffolds based on polycaprolactone (PCL), gelatin, and chitosan enriched with carbon nanotubes can improve

mechanical properties and biocompatibility, and support cartilage tissue regeneration *in vitro* [8]. These findings confirm that combining gelatin with nanomaterials not only enhances structural stability, but also optimizes biological activity. The development of gelatin scaffolds loaded with curcumin and TiO_2 demonstrated antibacterial activity and significant potential for skin tissue engineering applications [9]. This study demonstrated that gelatin serves as an effective matrix for carrying active compounds and nanoparticles, enhancing the material's bioactivity and functional performance. The study demonstrated that co-electrospun scaffolds based on gelatin:TiO_2/PCL: silk fibroin possess physicochemical, morphological, and biocompatibility properties that significantly support wound healing [10].

Although gelatin- and nanoparticle-based approaches have been widely applied in the biomedical field, particularly in wound healing and tissue engineering, their use in cosmetic formulations, especially as a base ingredient for halal peel-off gel masks, remains very limited. However, the same principles of increasing the stability, bioactivity, and effectiveness of active ingredient delivery through nanoparticle systems are highly relevant for functional cosmetic products such as anti-aging and skin care. Therefore, the development of gelatin nanoparticles from local halal sources, such as milkfish bones, for application in peel-off gel masks is an innovation with a strong scientific basis and high applicability potential. This approach is expected to leverage the success of the gelatin-nanoparticle biomaterial system in the biomedical field for the halal cosmetics industry, offering a safe, sustainable, and high-value solution. Although several studies have reported the extraction and basic characterization of fish gelatin for cosmetic applications, research on its development into peel-off gel mask formulations with comprehensive evaluation remains limited. Most previous studies have focused on raw material aspects or conventional gelatin-based formulations, without integrating nanotechnology-based approaches or systematically assessing formulation performance. The present study advances existing work by developing halal gelatin nanoparticles derived from milkfish bone waste and incorporating them into a peel-off gel mask formulation, which is evaluated in terms of its physicochemical characteristics, stability under stress conditions, *in vivo* skin safety, and anti-aging activity through tyrosinase inhibition. Additionally, a direct comparison with commercial gelatin was conducted to assess functional equivalence and potential performance enhancements. Through this integrated approach, although the novelty is incremental, the study provides a relevant and timely contribution to the development of safe, stable, and application-oriented halal cosmetic products based on nanotechnology. The literature highlights the need for research to bridge the gap by developing a peel-off gel mask based on milkfish-derived gelatin nanoparticles. This encompasses formulation and characterization, physicochemical stability assessment under various conditions, *in vivo* skin safety assessment, and anti-aging activity through the inhibition of the enzyme tyrosinase. Research is a high-urgency project because the potential for locally producing halal-based cosmetic biopolymers is safe, stable, and effective, while contributing to the scientific utilization of natural materials in Indonesia's development of nanotechnology-based cosmetics. Findings from the studyare expected to not only address the existing gap in scientific knowledge, but also support strengthening the cosmetics industry's competitiveness at both national and global levels.

Experimental

Research materials

Ingredients used in the formulation, unless otherwise stated, have pharmaceutical-grade purity. The main ingredients used in the study were halal gelatin nanoparticles obtained from the synthesis of gelatin from milkfish bones (*Chanos chanos*). gelatin, polyvinyl alcohol (PVA), propylene glycol, sodium hydroxide (NaOH), and potassium dihydrogen phosphate (KH_2PO_4) (Merck, Germany).

Instrument study

Instruments used in the research included a set of equipment, a glass laboratory, Ultra Turrax (IKA T25), particle size analyzer (PSA), Nanotrac Wave II (Microtrac, USA), digital pH meter (pH-700), viscosimeter (Brookfield Cone and Plate), UV spectrophotometer (Shimadzu UV-1800), hot plate stirrer (Heidolph), centrifuge (Hettich Rotofix 32), and sonicator (Sonica).

Synthesis of gelatin from milkfish bones

The process of making gelatin involved several stages: (1) Degreasing – Fish bones are cleaned from the remaining meat and fat using warm water (60–70 °C), and then dried. (2) Demineralization –Fish bones were soaked in a solution of sodium phosphate with concentrations of 3, 5, and 7% for 48 h at a ratio of 1:8 (w/v). (3) Extraction – The demineralization bones were extracted using distilled water at 70 °C for 6 h (ratio 1:3 w/v). (4) Drying – The filtrate from the extraction dried at 60 °C for 48 h until the gelatin powder was dry. The resulting gelatin was then tested for its physicochemical characteristics, including pH, water content, ash content, viscosity, and functional group analysis by Fourier transform infrared spectroscopy (FTIR).

Preparation of gelatin nanoparticles

Nanoparticle gelatin was made using desolvation method . The gelatin solution (1% w/v) was slowly added to 95% ethanol while stirring until sediment nanoparticles were formed. The suspension was then centrifuged and dried in a freeze dryer. Particle size, index of polydispersity, and zeta potential were analyzed using PSA and Zeta Analyzer.

Peel off gel mask formulation peel off gel mask

Formulation development in this study used a controlled trial-and-error approach rather than the design of experiments (DOE) method, as the study focused on evaluating the formulation's feasibility and initial performance.

Peel-off gel mask nanoparticles halal gelatin from milkfish bones (*Chanos chanos*) formulated in six different formulas (Table 1), consisting of three formulas using halal nanoparticle gelatin (F1, F2, and F3) and three formulas using gelatin synthesis (F4, F5, and F6). Manufacturing stock was prepared by dissolving PVA at a concentration of up to 10% of the formula weight in distilled water at a high temperature (±90 °C)

Table 1. Peel off gel mask formula

Material		Concentration % (w/w)					
		F1	F2	F3	F4	F5	F6
Nanoparticle halal gelatin	Anti-aging gelling agent	0.5	1	1.5	-	-	-
Synthetic gelatin	Anti-aging gelling agent	-	-	-	0.5	1	1.5
PVA	Polymer	10	10	10	10	10	10
Propylene glycol	Humectant	5	5	5	5	5	5
Aquadest	Solvent	ad 100					

while stirring with a magnetic stirrer until a clear and homogeneous solution was formed. The PVA solution was then cooled to 40–45 °C. Next, halal nanoparticle gelatin or gelatin synthesis was weighed according to the formula, specifically at concentrations of 0.5, 1, and 1.5%. Then, it was dissolved in distilled water at a high temperature (±60 °C) while stirring until completely dissolved. The gelatin solution was then cooled to 40–45 °C to maintain nanoparticle stability. After that, propylene glycol, up to 5%, was added to the gelatin solution while stirring slowly. This mixture was then gradually combined with the prepared PVA solution while stirring with a speed stirrer at low speed until a homogeneous gel was formed. After all materials were mixed, the preparation volume was adjusted by adding distilled water until it reached 100% of the total formula weight. All mixtures were stirred slowly to ensure homogeneity and prevent bubble formation. Homogeneity of the stock was checked visually with a smear of several small preparations on a glass object, then observed for the existence of particles, roughness, differences in color, or clumping. The gel mask was prepared homogeneously, and then stored in a closed receptacle at room temperature (±25 °C) for the physicochemical characterization test.

Peel Off Gel Mask

Organoleptic test

This test was carried out by observing the smell, texture, and color of the halal gelatin nanoparticle peel-off gel mask.

pH test

The pH test was performed using a previously calibrated pH meter. 1 g of the sample was taken and dissolved in 10 mL of distilled water. The electrode was then placed in the sample, and the reading on the pH meter screen was recorded.

Drying time test

A 1 g sample was applied to the back of the hand, measuring 5 cm in length and 5 cm in width. The time it took for the gel mask to dry and form a film layer was then measured using a stopwatch.

Spread power test

0.5 g of the sample was placed on a round glass with a diameter scale underneath, and then covered with another glass that had been weighed and left for 1 min. After this, the distribution diameter was measured.

Adhesion test

0.25 g of the sample is placed on two glass objects and then pressed with a load of 1 kg for 5 min. After that, the glass object is mounted on the tool. Test, then add a load of 80 g to the tool test. Then note the release time from the glass object.

Viscosity test

1 g of the sample was placed in the sample cup, ensuring that there were no air bubbles and the sample was evenly distributed on the surface. The cup was then reinstalled on the viscometer. The device was then turned on and left to stabilize for a short period to ensure the viscosity reading was accurate.

Homogeneity test

0.1 g of the sample was smeared on transparent glass, and it was observed whether any parts were not mixed well.

Particle size test using PSA

1 g of the sample was dissolved in 10 mL of distilled water. The sample was placed in a cuvette and loaded into the instrument's sample holder. The instrument was then turned on, and the particle size menu was selected. Data obtained

included the average droplet diameter, polydispersity index (PDI), and zeta potential. Measurements were performed three times.

Stability test

Heating–cooling cycle

Storage was alternated between 4 and 45 °C for 48 h per cycle, with up to 3 cycles performed.

Freeze–thaw cycle

Storage was alternated between -20 and 25 °C for 48 h per cycle, with up to 3 cycles performed.

Stability test storage

Storage was performed for 90 days at room temperature, with observations made per 30 days on the parameters pH, viscosity, homogeneity, particle size, zeta potential, and PDI.

Skin safety test (irritation test)

A safety skin test was conducted using the Draize Skin Irritation Test on test animals (albino rabbits). A total of 0.5 g of the preparation was applied to the shaved skin area on the back and observed for 72 h. Reaction erythema and edema were assessed based on the score of the Primary Irritation Index (PII), with a category of "safe" if PII ≤ 0.5 (meaning not irritating).

Peel-off mask release and penetration study using Franz diffusion cell

The release medium used was fluid with a physiological pH of 7.4. The membrane used in the release test was cellophane. Membrane penetration used in penetration testing was the skin of a mouse, a white male (Rattus norvegicus). Research: This study has met the ethical standards in accordance with Regulation Number 108/40/EC/KEPK-FKIK/07/2025. Testing was done using the Franz cell diffusion with physiological fluid. 22 mL of pH 7.4 ± 0.05 was added to the compartment containing the fluid receptors. A peel-off gel mask containing halal nanoparticles, weighing 1.0 g, was placed on the surface membrane. Then, the water temperature in the vessel was set at 37 ± 0.5 °C. The magnetic stirrer was turned on and set at a speed of 100 rpm. The samples were taken at time intervals of 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, and 180 to 360 min with taking the receptor medium as much as 1 mL taken and replaced with 1 mL of receptor medium from the outside. The retrieved sample was equalized across all tests. Samples were observed using a UV spectrophotometer at the maximum wavelength of gelatin milkfish bones at 204 nm and commercial gelatin at 206 nm. The resulting absorbance was measured as the concentration of released gelatin, as determined by the standard error regression curve. Testing was done with the same procedure for the peel-off gel mask with commercial gelatin.

Anti-aging effectiveness test

Anti-aging effectiveness is determined through testing the activity inhibition of the enzyme tyrosinase in vitro**.** A total of 80 µL of phosphate buffer solution (pH 7.4), 40 µL of solution sample, 40 µL of enzyme tyrosinase, and 40 µL of L-DOPA substrate were added to a microplate. The mixture was incubated for 10 min at 37 °C; then the absorbance was measured with a microplate reader at 410 nm. The percentage inhibition of tyrosinase was calculated using the formula:

$$\text{Inhibition (\%)} = (B-S)\ B \times 100 \quad (1)$$

Where B is the absorbance of the blank, and S is the absorbance of the sample.

Data analysis

Physicochemical test results data were analyzed using ANOVA in a one-way direction to determine

the significant difference between formulas. Statistical analysis was performed using one-way ANOVA to evaluate differences between formulation groups, followed by Tukey's post hoc test when necessary. Results are reported as F values, degrees of freedom (df), and exact p-values. Differences were considered statistically significant at the 95% confidence level ($p < 0.05$). All data are presented as mean ± standard deviation (SD). Analysis was performed using the SPSS software version 26.0.

Results

The gelatin used in this study was obtained from the synthesis of milkfish bones using a 5% phosphoric acid (H_3PO_4) solution, which showed the most optimal physicochemical characteristics for use as a base ingredient in cosmetic preparations. The resulting gelatin is shown in Figure 1.

This concentration was able to remove major mineral components, such as calcium phosphate and carbonate, without causing excessive degradation of the collagen structure. The selection of a 5% phosphoric acid concentration as the demineralization condition was based on a balance between the effectiveness of mineral removal and the preservation of collagen structure as a precursor to gelatin. At lower acid concentrations, the demineralization process of fish bones is reported to be suboptimal, so that mineral residues can hinder the gelatin extraction process. Conversely, using acid at too high a concentration can lead to excessive degradation of the collagen polypeptide chain, negatively affecting the yield and quality of the resulting gelatin. A concentration of 5% phosphoric acid has been widely used in the literature as an effective moderate condition for demineralizing bone materials without causing significant damage to the organic matrix. Therefore, in this study, a concentration of 5% was chosen as a representative and safe condition for producing gelatin with characteristics suitable for cosmetic applications. However, quantitative comparative studies between acid concentrations were not conducted within the scope of this study.

This FTIR spectrum analysis not only validates the effectiveness of the synthesis, but also provides information on molecular interactions within the gelatin matrix, underscoring its potential functional performance in halal gelatin nanoparticle peel-off gel masks. Gelatin has a chemical structure similar to that of collagen, which is often characterized by triplet repeats (Gly-XY)n, where X and Y are typically proline and hydroxyproline, respectively. The basic structure

Figure 1. Milkfish bone gelatin: Gelatin obtained from the milkfish bone synthesis process using 5% phosphoric acid solution (H_3PO_4)

of gelatin fragments is a triplet repeat (Figure 2). Furthermore, approximately 13% of the gelatin polypeptide chain consists of positively charged amino acid residues (*i.e.,* mainly lysine and arginine residues), approximately 12% of negatively charged amino acid residues (*i.e.,* mainly glutamic and aspartic acids), and approximately 11% of hydrophobic residues (*i.e.,* leucine, isoleucine, methionine, and valine). The cleavage of the polypeptide chains and their natural structural organization resulting from hydrolysis means that gelatin cannot be considered a single chemical compound with a precise molecular weight, but rather consists of a mixture of polypeptide chains with different molecular weights that can fall within a certain range.

Figure 3 shows that the fundamental functional group of the gelatin fragments is the amide group. Therefore, the FTIR spectrum will include vibrations arising from this functional group.

Based on FTIR data, gelatin, a denatured form of collagen, exhibits characteristic spectral fingerprints that reflect its protein properties. Functional groups in the typical gelatin FTIR spectrum are associated with peptide bonds and their aliphatic chains. Five peaks are characteristic of gelatin, namely at wave numbers 3,200-3,400 cm^{-1} which indicate NH groups; wave

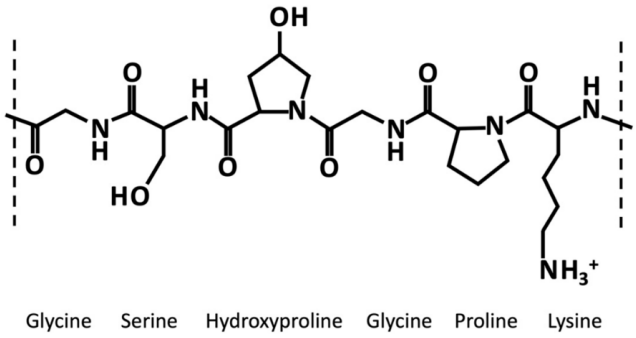


Figure 2. Basic structure of gelatin fragments

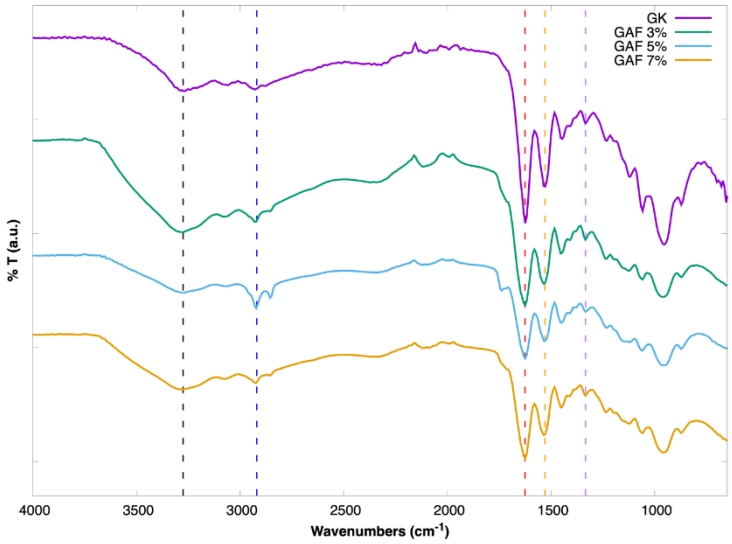


Figure 3. IR spectra of commercial gelatin (GK) and gelatin concentrations of 3, 5, and 7%

number 2,920 cm^{-1} which indicates CH_2 groups; wave numbers 1,620-1,650 cm^{-1} which indicate carbonyl groups (C=O); and fingerprint regions namely at wave numbers 1,500-1,550 and 1,220-1,300, which strengthen the indication of the presence of NH groups and CH groups.

Results of peel-off gel mask

Organoleptic results indicate that the halal gelatin nanoparticle peel-off gel mask made from milkfish bones (*Chanos chanos*) and the commercial gelatin peel-off gel mask exhibit similar characteristics, including a semisolid, colored preparation and a white, unscented appearance. The results of the peel-off gel mask are shown in Figures 4 and 5.

Peel-off gel mask preparation from milkfish bones (F1-F3) ranges between 204.00 and 478.00 nm, as shown in Figure 6, with averages F1: 241.67 ± 43.98, F2: 327.00 ± 50.31, and F3: 400.67 ± 126.23. All of these values are within the normal range of 50–500 nm, which is in accordance with the criteria for nanoparticles in topical preparations [11]. The higher the gelatin concentration, the greater the tendency to increase the particle size, as observed in F3 (1.5%), which has the largest average particle size. For comparison, the particle size of commercial gelatin-based peel-off gel masks (F4-F6) is relatively smaller, especially at a concentration of 0.5–1%, with an average particle size of F4: 121.03 ± 11.33, F5: 121.03 ± 17.38, and F6: 302.00 ± 178.26, as shown in Figure 7.

Figure 4. Halal gelatin nanoparticle peel off gel mask from milkfish bones (*Chanos chanos*) (A) F1-0.5%, (B) F2-1%, and (C) F3-1.5%

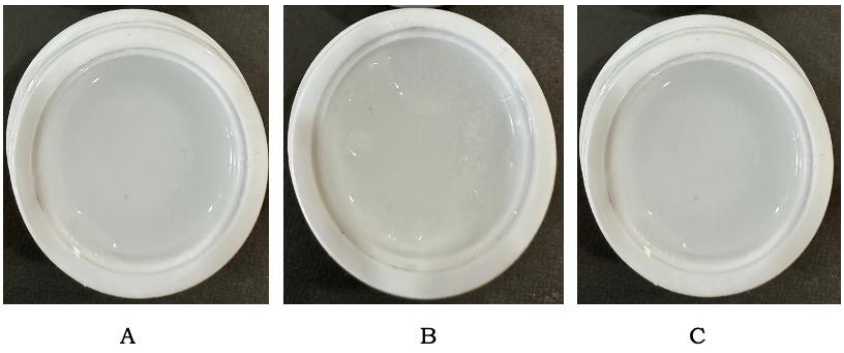


Figure 5. Commercial gelatin nanoparticle peel off gel mask (A) F4-0.5%, (B) F5- 1%, and (C) F6-1.5%

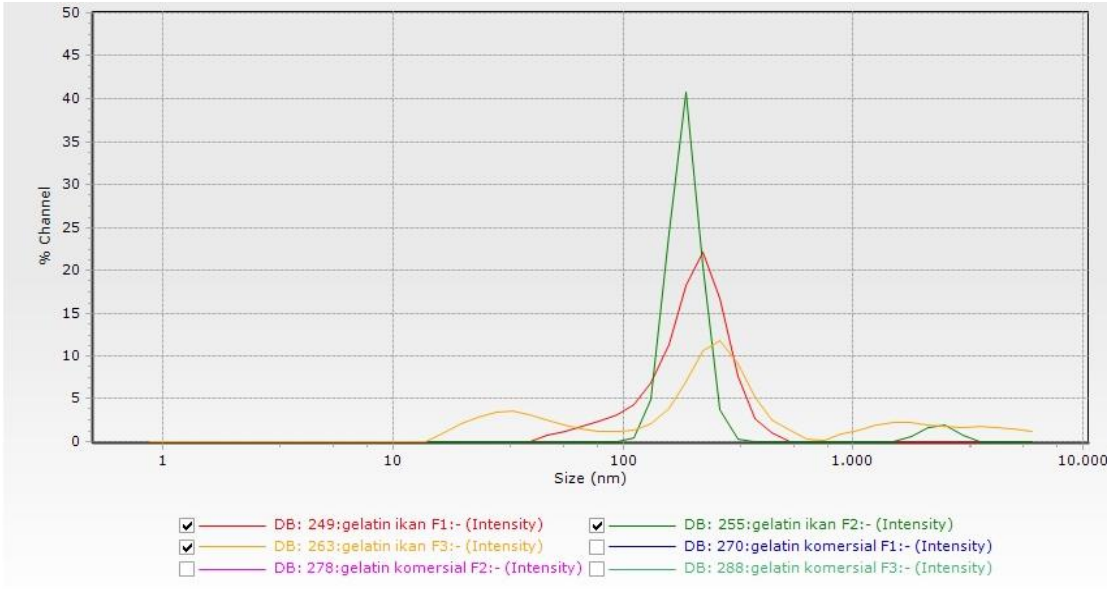


Figure 6. Size distribution of halal gelatin nanoparticle peel-off gel mask from milkfish bones (*Chanos chanos*)

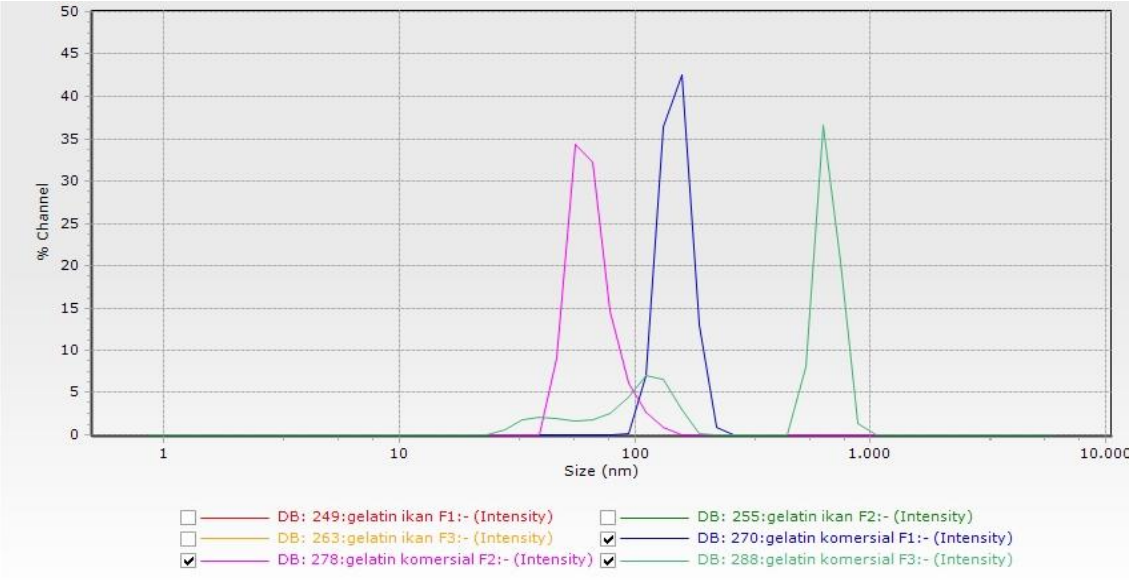


Figure 7. Particle size distribution of commercial gelatin peel-off gel mask

In general, an improvement in the concentration of gelatin tends to increase the size of particles in both types of gelatin. This results in a higher concentration of gelatin, which acts as a matrix that wraps nanoparticles, producing larger particles [12]. In halal gelatin, the particle size of milkfish bones increases significantly with rising concentration. Similar trends are observed in the commercial gelatin: although the values at 0.5% and 1% are relatively similar, they increase sharply at 1.5%. This is allegedly reflected in a viscosity difference between the solution and the gelatin, which affects particle agglomeration during sonication and encapsulation.

The potential value is an indicator of the stability of colloid nanoparticles. The ideal value for system nanoparticles is generally above ±30 mV to ensure the system's stability in an electrostatic

sense. However, in the study, this Formula F1–F3 has a zeta potential range 6.90-18.60 mV with F1 average: 7.47 ± 0.89 mV, F2: 13.83 ± 2.34 mV, F3: 15.70 ± 3.79 mV, temporary gelatin F4 commercial: 50.30 ± 15.04 mV, F5: 46.63 ± 13.53 mV, F6: 22.70 ± 4.99 mV. The zeta potential value shows a trend of increased halal gelatin along with an improvement in concentration. This indicates that a more concentrated gelatin solution can enhance the surface charge of nanoparticles, although it remains below the ideal value for electrostatic stability (>30 mV). On the other hand, in the commercial gelatin, the zeta potential precisely decreases as the concentration increases to 1.5%. This is possibly due to the cargo's shielding effect, which improves viscosity and reduces particle agglomeration, thereby decreasing the effective cargo on particle surfaces. The highest value was recorded at 0.5% concentration (50.30 mV), indicating excellent stability of the colloid. Although the measured zeta potential values of the gelatin nanoparticles were below ±30 mV, which is commonly associated with purely electrostatic stabilization, the observed stability of the system can be attributed to a combination of electrostatic and steric stabilization mechanisms. Gelatin is a macromolecular biopolymer that can provide steric hindrance through its polymeric chains, thereby reducing particle aggregation even at relatively low zeta potentials. Additionally, the presence of PVA in the formulation serves as a steric stabilizer, forming a hydrated polymer layer on the nanoparticle surface. This combined stabilization mechanism is supported by the absence of significant particle size growth, sedimentation, or phase separation during heating–cooling cycles, freeze–thaw cycles, and long-term storage, indicating satisfactory colloidal stability despite moderate zeta potential values.

The PDI value indicates the homogeneity of the particle size in preparation. A PDI value < 0.3 indicates a uniform particle distribution. The entire formula of halal gelatin milkfish bones still fulfills the condition (PDI < 1), with the most homogeneous values in F1 and F2. Formula F3 experienced an increase in PDI, along with improved concentration, but remains in the acceptable category for stock nanoparticles topical.

The PDI describes the degree of particle size distribution in a nanoparticle system; a value close to 0 indicates a narrow size distribution. In contrast, a value close to 1 reflects a broader distribution. In this study, the PDI value of the gelatin nanoparticles was in the mid-range, indicating a relatively heterogeneous size distribution, which is still acceptable for topical cosmetic applications. Since the nanoparticles were formulated in a polymer-based peel-off gel matrix, a completely monodisperse size distribution is not an absolute prerequisite. The consistency of physicochemical parameters primarily determines the preparation's stability and performance, as well as its absence of macroscopic aggregation during storage and under stress testing. Therefore, in the context of this study, the term "acceptable uniformity" refers to a stable and reproducible particle size distribution, not to an ideal monodisperse system.

Stability results

Physicochemical peel-off gel mask preparation

Stability evaluation of the peel-off gel mask preparation in this study focused on several physicochemical parameters, including pH, viscosity, homogeneity, particle size, and stability under different storage conditions and stress treatments. These parameters are important indicators to ensure that the formulation maintains its quality, effectiveness, and user acceptability during storage. Monitoring changes in these properties also helps to predict the shelf life and robustness of the formulation.

Table 2. Physicochemical results of halal gelatin nanoparticle peel off gel mask preparations from milkfish bones (*Chanos chanos*) and commercial gelatin peel off gel mask after storage at 25 °C for 90 days

Formula		Initial physicochemical characteristics					Final physicochemical characteristics				
		pH	Drying time test (min)	Spread power value (cm)	Adhesion value (sec)	Viscosity (cP)	pH	Drying time test (min)	Spread power value (cm)	Adhesion value (sec)	Viscosity (cP)
Halal gelatin nanoparticle peel-off gel mask made from milkfish bones (*Chanos chanos*)	F1	5.10 ±0.005	27.33 ±0.57	6.23 ±0.18	21.33 ±1.52	267.06 ±3.06	4.88 ± 0.04	26.90 ± 0.60	5.95 ± 0.09	20.80 ± 1.10	258.40 ± 3.80
	F2	4.74 ±0.05	22.33 ±1.52	5.73 ±0.14	34.67 ±2.51	469.33 ±4.06	4.65 ± 0.03	21.70 ± 0.50	5.60 ± 0.08	33.10 ± 1.25	458.50 ± 4.60
	F3	4.53 ±0.005	22.00 ±1.00	5.51 ±0.07	44.67 ±3.78	614.73 ±4.55	4.42 ± 0.04	21.10 ± 0.70	5.35 ± 0.10	43.20 ± 1.50	602.10 ± 4.80
	F4	6.12 ±0.02	30.00 ±0.00	6.05 ±0.05	28.67 ±4.04	345.96 ±9.02	5.98 ± 0.03	29.40 ± 0.45	5.90 ± 0.08	27.40 ± 1.20	336.30 ± 8.10
Commercial gelatin peel off gel mask	F5	5.61 ±0.01	29.60 ±1.52	5.41 ±0.20	53.33 ±3.51	680.76 ±15.55	5.48 ± 0.03	28.70 ± 0.55	5.30 ± 0.12	51.80 ± 1.60	662.50 ± 12.00
	F6	5.45 ±0.01	28.00 ±0.00	5.06 ±0.07	96.33 ±3.05	1070 ±24.26	5.30 ± 0.04	27.60 ± 0.60	4.95 ± 0.09	94.10 ± 2.10	1040 ± 18.50

All values are mean ± standard deviation (n=3).

The results of the stability test during storage at 25 °C for 90 days are summarized in Table 2. In addition, accelerated stability assessments were conducted using the heating–cooling cycle method and the freezing–thawing method to simulate extreme storage conditions. The results

of the heating–cooling cycle test are presented in Table 3, while the freezing–thawing stability results are shown in Table 4. These tests were performed to evaluate the ability of the peel-off gel mask preparation to maintain its physicochemical characteristics under temperature fluctuations and stress conditions.

Table 3. Results of physicochemical stability test of halal gelatin nanoparticle peel off gel mask preparations from milkfish bones (*Chanos chanos*) and commercial gelatin peel off gel mask method heating-cooling cycle

Formula		Initial physicochemical characteristics					Physicochemical characteristics after heating-cooling cycle stability				
		pH	Drying time test (min)	Spread power value (cm)	Adhesion value (sec)	Viscosity (cP)	pH	Drying time test (min)	Spread power value (cm)	Adhesion value (sec)	Viscosity (cP)
Peel-off gel mask made from milkfish bones (*Chanos chanos*)	F1	5.10 ±0.005	27.33 ±0.57	6.23 ±0.18	21.33 ±1.52	267.06 ±3.06	4.95 ± 0.03	26.80 ± 0.60	6.05 ± 0.15		261.80 ± 4.10
	F2	4.74 ±0.05	22.33 ±1.52	5.73 ±0.14	34.67 ±2.51	469.33 ±4.06	4.65 ± 0.04			33.10 ± 1.25	462.50 ± 5.20
	F3	4.53 ±0.005	22.00 ±1.00	5.51 ±0.07	44.67 ±3.78	614.73 ±4.55	4.45 ± 0.03	21.60 ± 1.10	5.55 ± 0.12	43.20 ± 1.50	608.10 ± 5.00
Commercial gelatin peel off gel mask	F4	6.12 ±0.02	30.00 ±0.00	6.05 ±0.05	28.67 ±4.04	345.96 ±9.02	6.00 ± 0.04	29.60 ± 0.20	5.90 ± 0.08	29.10 ± 3.90	340.20 ± 8.40
	F5	5.61 ±0.01	29.60 ±1.52	5.41 ±0.20	53.33 ±3.51	680.76 ±15.55	5.50 ± 0.03	28.90 ± 1.60	5.25 ± 0.18	54.20 ± 3.40	672.50 ± 14.80

	F6	5.45 ±0.01	28.00 ±0.00	5.06 ±0.07	96.33 ±3.05	1070 ±24.26	5.32 ± 0.03	27.50 ± 0.30	4.95 ± 0.09	97.10 ± 3.20	1060.00 ± 23.10

All values are mean ± standard deviation (n=3).

Table 4. Results of physicochemical stability test of halal gelatin nanoparticle peel off gel mask preparations from milkfish bones (*Chanos chanos*) and commercial gelatin peel off gel mask method freeze-thaw

Formula		Initial physicochemical characteristics					Physicochemical characteristics after freeze-thaw stability				
		pH	Drying time test (min)	Spread power value (cm)	Adhesion value (sec)	Viscosity (cP)	pH	Drying time test (min)	Spread power value (cm)	Adhesion value (sec)	Viscosity (cP)
Halal gelatin nanoparticle peel-off gel mask made from milkfish bones (*Chanos chanos*)	F1	5.10 ±0.005	27.33 ±0.57	6.23 ±0.18	21.33 ±1.52	267.06 ±3.06	4.90 ± 0.04	26.40 ± 0.55	5.95 ± 0.12	22.80 ± 1.30	255.60 ± 4.20
	F2	4.74 ±0.05	22.33 ±1.52	5.73 ±0.14	34.67 ±2.51	469.33 ±4.06	4.61 ± 0.03	21.50 ± 1.35	5.45 ± 0.10	35.70 ± 2.35	456.20 ± 5.10
	F3	4.53 ±0.005	22.00 ±1.00	5.51 ±0.07	44.67 ±3.78	614.73 ±4.55	4.40 ± 0.04	21.10 ± 1.00	5.30 ± 0.09	46.20 ± 3.55	600.50 ± 4.80
Commercial gelatin peel off gel mask	F4	6.12 ±0.02	30.00 ±0.00	6.05 ±0.05	28.67 ±4.04	345.96 ±9.02	5.95 ± 0.03	29.10 ± 0.25	5.85 ± 0.07	29.60 ± 3.80	334.40 ± 8.00
	F5	5.61 ±0.01	29.60 ±1.52	5.41 ±0.20	53.33 ±3.51	680.76 ±15.55	5.46 ± 0.03	28.50 ± 1.40	5.20 ± 0.16	54.80 ± 3.30	665.80 ± 13.70

Formula	Initial physicochemical characteristics					Physicochemical characteristics after freeze-thaw stability				
	pH	Drying time test (min)	Spread power value (cm)	Adhesion value (sec)	Viscosity (cP)	pH	Drying time test (min)	Spread power value (cm)	Adhesion value (sec)	Viscosity (cP)
F6	5.45 ±0.01	28.00 ±0.00	5.06 ±0.07	96.33 ±3.05	1070 ±24.26	5.28 ± 0.03	27.20 ± 0.25	4.90 ± 0.08	97.80 ± 3.10	1050.00 ± 22.90

All values are mean ± standard deviation (n=3).

Peel-off mask release and penetration test results: Gelatin in vitro using Franz diffusion cell

Release test results on formulas using gelatin from milkfish bones and commercial gelatin, as presented in Tables 5 and 6.

Table 5. Release test results: peel-off gel mask preparation

Time	Average amount cumulative halal gelatin from bones of milkfish (*Chanos chanos*) that are released (μg/cm²)± *SD*			Average amount cumulative gelatin commercial released (μg/cm² ± *SD*)		
	F1	F2	F3	F4	F5	F5
10	174.83± 0,01	209.37± 0,01	219.39± 0,01	163.6± 0,01	188.14± 0,01	198.18± 0,01
20	349.65± 0,01	384.19± 0,01	394.21	338.42± 0,01	362.96± 0,01	372.99± 0,01
30	524.48± 0,01	559.02± 0,01	569.04± 0,01	513.25± 0,01	537.79± 0,01	547.83± 0,01
40	699.30± 0,01	733.84± 0,01	743.86± 0,01	688.07± 0,01	712.61± 0,01	722.65± 0,01
50	874.13± 0,01	908.67± 0,01	918.69± 0,01	862.9± 0,01	887.44± 0,01	897.48± 0,01
60	1,048.95± 0,01	1,073.49± 0,01	1083.51± 0,01	1037.72± 0,01	1052.26± 0,01	1062.3± 0,01
70	1,223.78± 0,01	1,248.32± 0,01	1258.34± 0,01	1212.55± 0,01	1227.09± 0,01	1237.13± 0,01
80	1,398.60± 0,01	1,423.14± 0,01	1433.16	1314.55± 0,01	1401.91± 0,01	1411.95± 0,01
90	1,573.43± 0,01	1,597.97± 0,01	1607.99± 0,01	1387.37± 0,01	1576.74± 0,01	1586.78± 0,01
100	1,748.25± 0,01	1782.79± 0,01	1792.81± 0,01	1562.2± 0,01	1761.56± 0,01	1771.6± 0,01
110	1,923.08± 0,00	1,937.62± 0,01	1947.64± 0,01	1737.02± 0,01	1916.39± 0,01	1926.43± 0,01
120	2,097.90± 0,00	2,112.4± 0,014	2122.42± 0,01	1911.85± 0,01	2091.17± 0,01	2101.21± 0,01
130	2,272.73± 0,02	2,287.27± 0,01	2297.29± 0,01	2086.67± 0,01	2266.04± 0,01	2276.08± 0,01
140	2,447.55± 0,02	2,462.09± 0,01	2472.11± 0,01	2261.5± 0,01	22,98,56± 0,01	2450.9± 0,01
150	2,622.38± 0,01	2,636.92± 0,01	2646.94± 0,01	2436.32± 0,01	2440.86± 0,01	2625.73± 0,01
160	2,797.20± 0,02	2831.74± 0,01	2841.76± 0,01	2611.15± 0,01	2615.69± 0,01	2820.55± 0,01
170	2,972.03± 0,02	2,996.57± 0,01	3006.59± 0,01	2785.97± 0,01	2810.51± 0,01	2985.38± 0,01
180	3,146.85± 0,02	3181.39± 0,01	3191.41± 0,01	2960.8± 0,01	2975.34± 0,01	3170.20± 0,01
190	3,321.68± 0,00	3356.22± 0,01	3366.24± 0,01	3135.62± 0,01	3160.16± 0,01	3345.03± 0,01
200	3,496.50± 0,02	3531.04± 0,01	3541.06± 0,01	3310.45± 0,01	3334.99± 0,01	3519.85± 0,01
210	3,671.33± 0,01	3705.87± 0,01	3715.89± 0,01	3485.27± 0,01	3509.81± 0,01	3694.63± 0,01

Time	Average amount cumulative halal gelatin from bones of milkfish (*Chanos chanos*) that are released (μg/cm²)± *SD*			Average amount cumulative gelatin commercial released (μg/cm² ± *SD*)		
	F1	F2	F3	F4	F5	F5
220	3,846.15± 0,01	3880.69± 0,01	3890.71± 0,01	3660.1± 0,01	3684.64± 0,01	3869.5± 0,01
230	4,020.98±	4055.52± 0,01	4065.54± 0,01	3834.92± 0,01	3859.46± 0,01	4044.33± 0,01
240	4,195.80± 0,01	4230.34± 0,01	4240.36± 0,01	4009.75± 0,01	4034.29± 0,01	4219.15± 0,01
250	4,370.63± 0,01	4405.17± 0,01	4415.19± 0,01	4184.57± 0,01	4209.11± 0,01	4393.98± 0,01
260	4,545.45± 0,01	4579.99± 0,01	4590.01± 0,01	4359.40± 0,01	4383.94± 0,01	4568.8± 0,01
270	4,720.28± 0,01	4754.82± 0,01	4764.84± 0,01	4534.22± 0,01	4558.76± 0,01	4743.63± 0,01
280	4,895.10± 0,01	4929.64± 0,01	4939.66± 0,01	4709.05± 0,01	4733.59± 0,01	4918.45± 0,01
290	5,069.93± 0,01	5104.47± 0,01	5114.49± 0,01	4883.87± 0,01	4908.41± 0,01	5093.28± 0,01
300	5,244.76± 0,01	5,259.00± 0,01	5269.02± 0,01	5058.7± 0,01	5083.24± 0,01	5247.81± 0,01
310	5,419.58± 0,01	5,433.82± 0.01	5443.84± 0.01	5233.53± 0.01	5237.77± 0.01	5422.63± 0.01
320	5,594.41± 0.01	5,608.65± 0.01	5,618.67± 0.01	5408.35± 0.01	5,412.59± 0.01	5,597.46± 0.01
330	5,769.23± 0.01	5,783.47± 0.01	5,793.49± 0.01	5,583.18± 0.01	5,587.42± 0.01	5,772.28± 0.01
340	5,944.06± 0.02	5,958.30± 0.01	5,968.32± 0.01	5,758.01± 0.01	5,762.24± 0.01	5,947.11± 0.01
350	6,118.88± 0.02	6,133.12± 0.01	6,143.14± 0.01	5,932.83± 0.01	5,937.07± 0.01	6,121.93± 0.01
360	6,293.71± 0.02	6,333.12± 0.01	6,343.14± 0.01	6107.65± 0.01	6,111.89± 0.01	6,321.93± 0.01

All values are mean ± standard deviation (n=3).

Table 6. Penetration test results: Peel-off gel mask preparation

Time	Average amount cumulative halal gelatin from milkfish bones (*Chanos chanos*) with penetration (μg/cm²)± *SD*			Average amount cumulative gelatin commercial penetrated (μg/cm² ± *SD*)		
	F1	F2	F3	F4	F5	F5
10	2,914± 0.01	3.045 ± 0.545	4,242± 0.01	3,246± 0.01	2.136 ± 0.046	1,214± 0.01
20	3,246± 0.01	3.408 ± 0.569	4,408± 0.01	3,412± 0.01	2.254 ± 0.054	2,232± 0.01
30	3,412± 0.01	3.611 ± 0.733	4,741± 0.01	3,412± 0.01	2.374 ± 0.043	2,223± 0.01
40	3,578± 0.01	3.897 ± 0.423	4,907± 0.01	3,744± 0.01	2.495 ± 0.082	2,338± 0.01
50	3,744± 0.01	4,522 ± 1,262	5,239± 0.01	3,910± 0.01	2.642 ± 0.096	2,344± 0.01
60	4,242± 0.01	4,900 ± 1,170	5,405± 0.01	4,242± 0.01	2.795 ± 0.098	3,332± 0.01
70	4,907± 0.01	6.610 ± 0.195	5,737± 0.01	4,408± 0.01	3.967 ± 0.170	3,807± 0.01
80	5,405± 0.01	6.592 ± 0.121	5,903± 0.01	4,575± 0.01	2.167 ± 0.093	4,305± 0.01
90	6,401± 0.01	6.741 ± 0.737	6,235± 0.01	4,575± 0.01	2.365 ± 0.190	5,301± 0.01
100	6,733± 0.01	6,558 ± 2,443	6,567± 0.01	4,907± 0.01	2.599 ± 0.181	5,663± 0.01
110	7,563± 0.01	6.592 ± 0.121	6,899± 0.01	5,405± 0.01	2.821 ± 0.189	6,673± 0.01
120	7,729± 0.01	6,741 ± 0.01	7,231± 0.01	5,405± 0.01	3.053 ± 0.184	6,569± 0.01
130	7,895± 0.01	6,558 ± 0.01	7,563± 0.01	5,571± 0.01	3.303 ± 0.158	6,715± 0.01
140	8.061± 0.01	8.235 ± 0.912	7,895± 0.01	5,737± 0.01	3.408 ± 0.569	7,061± 0.01
150	8.061± 0.01	8.918 ± 0.792	8,393± 0.01	5,737± 0.01	3.611 ± 0.733	7,761± 0.01
160	8.393± 0.01	8.266 ± 0.768	9,057± 0.01	6,069± 0.01	3.897 ± 0.423	7,823± 0.01
170	8.725± 0.01	9,110 ± 1,887	9,389± 0.01	5,737± 0.01	4,522 ± 1,262	7,225± 0.01
180	8.891± 0.01	10,225± 0.01	10,385± 0.01	5,903± 0.01	4,900 ± 1,170	7,691± 0.01
190	9.223± 0.01	11,052± 0.01	11,050± 0.01	6,899± 0.01	5,018 ± 1,030	8,123± 0.01
200	9.887± 0.01	11,213± 0.01	11,216± 0.01	7,729± 0.01	5,339 ± 1,498	8,788± 0.01
210	10.053± 0.01	11,548± 0.01	11,548± 0.01	7,397± 0.01	5,557 ± 1,652	9,053± 0.01

Time	Average amount cumulative halal gelatin from milkfish bones (*Chanos chanos*) with penetration (μg/cm²)± *SD*			Average amount cumulative gelatin commercial penetrated (μg/cm² ± *SD*)		
	F1	F2	F3	F4	F5	F5
220	10.219± 0.01	12,312± 0.01	12,212± 0.01	8,061± 0.01	6.610 ± 0.195	9,219± 0.01
230	10.385± 0.01	13,342± 0.01	13,042± 0.01	8,725± 0.01	6.592 ± 0.121	9,385± 0.01
240	11,050± 0.01	13,340± 0.01	13,540± 0.01	9,389± 0.01	6.741 ± 0.737	9,050± 0.01
250	11.216± 0.01	14,370± 0.01	14,370± 0.01	9,555± 0.01	9,558 ± 2,443	10,010± 0.01
260	10.883± 0.01	14,336± 0.01	14,536± 0.01	9,721± 0.01	10.235 ± 0.912	10,883± 0.01
270	10,551± 0.01	14,336± 0.01	14,536± 0.01	9,887± 0.01	9.918 ± 0.792	11,548± 0.01
280	11,382± 0.01	14,602± 0.01	14,702± 0.01	10,717± 0.01	10.266 ± 0.768	11,714± 0.01
290	11,382± 0.01	14,368± 0.01	14,868± 0.01	11,548± 0.01	10,110 ± 1,887	12,046± 0.01
300	11,548± 0.01	15,334± 0.01	15,034± 0.01	11,714± 0.01	11.121 ± 0.092	12,212± 0.01
310	12,212± 0.01	15,300± 0.01	15,200± 0.01	12,046± 0.01	12,811 ± 4,084	12,212± 0.01
320	12,710± 0.01	15,398± 0.01	15,698± 0.01	12,212± 0.01	13.516 ± 0.629	13,540± 0.01
330	12,876± 0.01	16,330± 0.01	16,030± 0.01	12,710± 0.01	13,897 ± 1,715	14,370± 0.01
340	13,042± 0.01	16,328± 0.01	16,528± 0.01	13,042± 0.01	14,240 ± 0.663	14,536± 0.01
350	13,208± 0.01	16,394± 0.01	16,694± 0.01	14,204± 0.01	16.071 ± 0.352	14,536± 0.01
360	13.374± 0.01	17.326± 0.01	17,026± 0.01	14.536± 0.01	±16.901 0.385	14.702± 0.01

All values are mean ± standard deviation (n=3).

Table 7. Observation results of irritation tests on halal gelatin nanoparticle peel-off gel mask preparations from milkfish bones (*Chanos chanos*) and gelatin peel-off gel mask

Formula		Parameter	1 h	24 h	48 h	72 h	Mean ± SD	Category
Halal gelatin nanoparticle peel-off gel mask made from milkfish bones (*Chanos chanos*)	F1	Erythema	0.3	0.0	0.0	0.0	0.08 ± 0.15	Non-irritant
		Edema	0.0	0.0	0.0	0.0	0.00 ± 0.00	Non-irritant
	F2	Erythema	0.3	0.0	0.0	0.0	0.08 ± 0.15	Non-irritant
		Edema	0.0	0.0	0.0	0.0	0.00 ± 0.00	Non-irritant
	F3	Erythema	0.3	0.0	0.0	0.0	0.08 ± 0.15	Non-irritant
		Edema	0.0	0.0	0.0	0.0	0.00 ± 0.00	Non-irritant
Commercial gelatin peel off gel mask	F4	Erythema	0.3	0.0	0.0	0.0	0.08 ± 0.15	Non-irritant
		Edema	0.0	0.0	0.0	0.0	0.00 ± 0.00	Non-irritant
	F5	Erythema	0.3	0.0	0.0	0.0	0.08 ± 0.15	Non-irritant
		Edema	0.0	0.0	0.0	0.0	0.00 ± 0.00	Non-irritant
	F6	Erythema	0.3	0.0	0.0	0.0	0.08 ± 0.15	Non-irritant
		Edema	0.0	0.0	0.0	0.0	0.00 ± 0.00	Non-irritant

Note: Erythema and edema scores: 0 = no reaction, 1 = very mild, 2 = mild, 3 = moderate, and 4 = severe. No erythema or edema reaction was found after 24 h, so PII = 0 (non-irritant). The average value was calculated from three test animals per formula.

Observation results of the irritation test of the peel-off gel mask preparation

The irritation test was conducted to evaluate the safety of the peel-off gel mask formulation containing halal gelatin nanoparticles derived from milkfish bones (*Chanos chanos*) (Table 7). The assessment focused on two main parameters, namely erythema and edema, which were observed at 1, 24, 48, and 72 h after application.

These parameters are commonly used to determine potential skin irritation caused by topical preparations.

Irritation test results acute dermal on peel-off gel mask preparations based on nanoparticles halal gelatin from milkfish bones (F1–F3) and comparison formulas based on gelatin commercial (F4–F6) show that almost all observations of erythema and edema worth zero or only appear very mild erythema (score = 1) in the phase lost beginning within 24 h. Thus, the primary irritation index (PII) for each formula is at the value zero and is categorized as non-irritant. Findings indicate that the formulation causes an inflammatory reaction in rabbits under standard test conditions (0.5 g in an area of approximately 6 cm^2, with observations up to 72 h), and this effect is consistent throughout the CTh improvement. In practice, these data support the claim of safety for cosmetic products.

Results of the % inhibition effectiveness test anti-aging

Activity test results demonstrating anti-aging effects through the inhibition of the enzyme tyrosinase show that all formulations inhibit activity by 11%. Inhibition from milkfish bones (*Chanos chanos*) is slightly higher than commercial gelatin, with the F1 formula achieving the highest mark of 11.12%. In comparison, the F3 formula shows the lowest mark of 5.39%. Control positive using arbutin produces inhibition of tyrosinase by 25.64%, far more than all test formulas, whereas control negative shows no activity inhibition. The complete results of this test are presented in Table 8.

There is a difference in the groups (OVA). A significant difference was found between the groups. The t-test does not indicate significance in the formula-based p-value of 0.05 between the formulated group gelatin and the commercial gelatin. This suggests that the source gelatin has no significant impact on tyrosinase inhibition, despite the formula being based on halal gelatin with nanoparticles of a size that shows a trend of increased biological activity.

The IC_{50} value for tyrosinase inhibition was not calculated in this study because the test was conducted at a single concentration range tailored to the conditions of the peel-off gel mask formulation. Therefore, the results obtained are qualitative to semi-quantitative and are intended as a preliminary evaluation (screening) of the potential for tyrosinase inhibition. Although this limitation restricts direct comparison with other studies reporting IC_{50} values, this approach remains relevant for assessing the biological activity of cosmetic formulations in a practical context. Determining IC_{50} values through testing at various concentrations will be the focus of further research to strengthen the understanding of the mechanisms and biological effectiveness of the developed gelatin nanoparticle system.

Table 8. Results of the % inhibition effectiveness test anti-aging through inhibition of tyrosinase nanoparticle peel-off gel mask halal gelatin from milkfish bones (*Chanos chanos*)

Formula		Average absorbance	% Inhibition
Negative control		0.808± 0.06	-
Positive control		0.601± 0.09	25,639
Halal gelatin nanoparticle peel-off gel mask made from milkfish bones (*Chanos chanos*)	F1	0.719± 0.05	9,109
	F2	0.732± 0.06	5,606
	F3	0.765± 0.03	3,916
Commercial gelatin peel off gel mask	F4	0.735± 0.06	11,123
	F5	0.763 ±0.05	9,481
	F6	0.777 ± 0.03	5,399

All values are mean ± standard deviation (n=3).

Discussion

Characterization of the physicochemical properties of peel-off gel masks involves a series of tests and analyses conducted to evaluate the physical and chemical properties of the peel-off gel mask preparation, ensuring quality, stability, safety, effectiveness, and comfort when applied to the skin. Characterization involves testing physical parameters, such as viscosity, spreadability, power stickiness, drying time, homogeneity, and organoleptic properties, as well as chemical parameters, including pH. In the preparation of nanoparticles, characterization also includes analysis of particle size, zeta potential, and PDI to determine dispersion stability and the consistency of particle size in the supply. Through physicochemical characterization, it can be confirmed that the peel-off gel mask preparation has its own characteristics, meets the appropriate physical standards, and is safe for the skin, as indicated by the Indonesian Pharmacopoeia and a safe pH level.

Stability evaluation in this study focused on the physicochemical properties of the preparation, including pH, viscosity, homogeneity, particle size, and stability under various storage conditions and pressures. The results of the stability test after storage at 25 °C for 90 days are presented in Table 2. The stability results using the heating-cooling cycle method (Table 3) and freezing-thawing (Table 4). This approach aimed to assess the formulation consistency and stability of the gelatin nanoparticle system in the context of early-stage formulation development. Microbiological testing or preservative efficacy testing was not performed in this study, so product safety claims were limited to skin safety aspects evaluated through *in vivo* irritation testing. Evaluation of microbiological safety and preservative system efficacy is a crucial aspect of advanced cosmetic product development and will be the focus of further research prior to commercial application.

Organoleptic results of peel-off gel mask preparation after storage at 25 °C for 90 days showed that after storage for 90 days at 25 °C, the entire peel-off gel mask formula, both those that use halal gelatin nanoparticles from milkfish bones (*Chanos chanos*) and commercial gelatin, shows no existence changes in aroma, color, and shape compared to the initial. The preparation is still unscented, colored white, and shaped half-solid (semisolid). This result indicates that during storage, no chemical reactions, oxidation, or microbial contamination can cause a change in smell or color. Stability, shape, and color indicate that component gelling agents, such as gelatin and PVA, and other materials contribute to the overall sound and stable compatibility in temperature space. Thus, in a way, it can be concluded that all formulas are stable in an organoleptic sense after 90 days of storage. This also demonstrates that gelatin from milkfish bones has a stable physical equivalent to commercial gelatin, making it a viable alternative material in peel-off gel mask formulations.

Test results, including physicochemical properties such as pH, drying time, power spread, adhesive power, and viscosity, show a slight change after 90 days of storage. However, analysis of the results using a two-way ANOVA shows that the difference between the formulas is not statistically significant ($p > 0.05$). Generally, all parameters exhibit a decline in performance compared to their initial characteristics. The pH value has slightly decreased, indicating a reaction of hydrolysis in the gelatin component or the addition of material. However, the pH value remains within the range of 4.5–6.5, which is the physiological pH range of the skin, so the product is still safe to use. Drying time and power spread also show a slight decline, indicating a minor improvement in gel consistency due to the evaporation of free water during storage; however, this does not affect the product's ability

to spread and form a thin layer on the skin. On the other hand, the power sticky tends to exhibit slight improvement with minor consequences, strengthening the bond between the chain polymers (gelatin and PVA) that form during the storage process; however, this difference is also not statistically significant. Viscosity experience shows a slight decline that is still ongoing within reasonable limits, indicating a relaxation of the gel network structure, but with no loss of stability or film-forming ability in the mask. Overall, the changes are small across all parameters and still fall within the tolerance limits that can be accepted, not significantly influencing the quality of the product physical ($p > 0.05$). This indicates that the interaction between the material components remains stable, and 90 days of storage at 25 °C does not cause degradation in the gel system.

A test peel-off gel mask was prepared from halal gelatin nanoparticles derived from milkfish bones (*Chanos chanos*), and a commercial gelatin-based peel-off gel mask was used in a cell Franz diffusion experiment with pH 7.4 media for 360 min. The results showed that all formulas experienced improvements in the amount of cumulative released gelatin over time. Between the three halal gelatin nanoparticle formulas, F3 showed the highest cumulative release, namely 6,343.14 μg/cm^2 at the 360th min, followed by F2 (6,333.12 μg/cm^2) and F1 (6,293.71 μg/cm^2). In contrast, commercial gelatin exhibited a lower marked release, with F6 (6,321.93 μg/cm^2) as the highest formula, while F4 reached only 6,107.65 μg/cm^2.

Nanoparticles enhance the release of active substances by utilizing their small size and large surface area, thereby broadening the contact interface with the release medium and accelerating diffusion. Increasing the gelatin concentration in the stock further widens the concentration gradient between the gel matrix and the medium, thereby accelerating the release of active substances into the release medium [13].

Nanoparticle- or nanoliposome-based gel formulations significantly increase the release rate of bioactive substances because the particles penetrate the gel structure and facilitate efficient transfer of active compounds through membrane diffusion. In this study, the flux values confirm this pattern, with formulation F3 showing the highest flux (0.0414 ± 0.01 μg/cm^2.min^{-1}), followed by F2 (0.0378 ± 0.02 μg/cm^2.min^{-1}) and F1 (0.0355 ± 0.02 μg/cm^2.min^{-1}). Commercial gelatin shows lower overall flux, with F6 reaching only 0.0322 ± 0.05 μg/cm^2.min^{-1}. Research also shows that nanoparticle-based gels exhibit faster and more consistent release profiles than conventional gels, as their smaller particle size and more porous characteristics of the gel matrix enhance diffusion efficiency and reduce the barriers typically present in conventional systems [14,15].

Commercial gelatin was used in this study as a benchmark to provide context for the performance of milkfish bone gelatin-based formulations. The characterization of commercial gelatin was limited to parameters relevant to the application of peel-off gel mask formulations, specifically physicochemical behavior and stability, rather than comprehensive material characterization. Therefore, the comparisons made were functional and applicable, rather than comparisons of structurally or compositionally equivalent materials. This limitation has been taken into account in the interpretation of the results, and direct comparison claims focused on formulation performance, rather than equivalence to the basic properties of gelatin.

The overall results of this study strengthen the evidence that using halal gelatin nanoparticles derived from milkfish bones not only fulfills the requirements of a halal product in cosmetics, but also enhances the performance and release of active substances compared to commercial gelatin. Advantages. This is a significant advantage in developing safe, effective, and compliant halal cosmetics for modern Muslim consumers.

The emphasis on halal aspects in this study is based on the origin of the gelatin raw material, which is milkfish bones, which, in principle, do not raise halal issues, unlike gelatin sourced from certain land animals. However, it should be emphasized that this study does not include a formal halal certification process or specific analytical verification of the final product's halal status. Therefore, halal claims in the context of this study are limited to the source of the material and its conformity to conceptual halal principles. Official halal certification and other supporting testing are further stages beyond the scope of this study and are necessary before the product can be developed into a commercial product.

Franz diffusion testing reveals that halal gelatin nanoparticles derived from milkfish bones in the peel-off gel mask formulation exhibit higher cumulative release and greater flux than commercial gelatin-based gel masks. Formula F3 demonstrates the most optimal profile with a flux value of 0.0414 ± 0.01 μg/cm^2/min. Nanoparticles accelerate and enhance the release of active substances by increasing the contact area with the medium, while the optimal concentration gradient within the gel matrix further drives diffusion. These findings highlight the potential of this formulation as an innovative and superior halal cosmetic product in terms of effectiveness, delivery performance, and topical release characteristics [16].

The stability test of the peel-off gel mask using the heating–cooling cycle method shows that all formulations, both those containing halal gelatin nanoparticles from milkfish bones (*Chanos chanos*) and those prepared with commercial gelatin, maintain stable organoleptic characteristics after six cycles at 4 and 45 °C. All preparations remain white, non-aromatic, and semisolid. These findings indicate that the formulation components, including gelatin, PVA, glycerin, and other excipients, exhibit good thermal stability and preserve system homogeneity despite repeated temperature fluctuations. The absence of changes in color, odor, and consistency also demonstrates that no gelatin degradation, material oxidation, or microbial contamination occurs during testing, reflecting the strong hydrogen bonding within the gelatin–PVA matrix that supports structural stability at temperatures ≤ 50 °C [17].

Analysis of the physicochemical characteristics using two-way ANOVA reveals that although there is a slight decline in pH, drying time, spreadability, and viscosity, as well as an improvement in lightness on stickiness, there is no statistically significant difference ($p > 0.05$) between formulas and between testing time. The pH value tends to decrease slightly, allegedly due to the hydrolysis process occurring on the gelatin matrix during warming. Although this is the case, the final pH of the entire formula remains in the physiological pH range of 4.5–6.5, which is suitable for the skin, so the product is still safe to use. A decrease in spreadability and drying time occurs due to a reduction in free water during heating cycles. At the same time, an improvement in power stickiness indicates the existence of strengthening bonds between intermolecular gelatin and PVA. The viscosity value experiences a slight decline, consequence of polymer structure relaxation, but remains within the optimal range for peel-off gel masks (250–1,100 cP). Research indicates that gelatin-based gel preparations may undergo minor changes after the heating–cooling test; however, statistical analysis shows these changes are not significant. This study confirms that peel-off gel masks formulated with halal gelatin nanoparticles derived from milkfish bones maintain physicochemical stability equivalent to that of commercial gelatin, even under repeated temperature fluctuations [18,19].

Stability testing using the freeze–thaw method demonstrates that all formulas maintain good stability and organoleptic properties. The preparations do not undergo any changes in color, aroma, or consistency after three freezing–thawing cycles (−5 to 25 °C). Each formulation

remains homogeneous, white, non-fragrant, and semisolid. These findings demonstrate that the three-dimensional gelatin–PVA network remains stable under extreme conditions, including mechanical stress and temperature fluctuations. The matrix's integrity reflects the strength of hydrogen bonding and electrostatic interactions within the polymer chains, enabling the gel to withstand temperature variations without structural degradation [20].

Statistical analysis using two-way ANOVA reveals that the pH, drying time, spreadability, adhesiveness, and viscosity decrease slightly after treatment; however, these changes remain insignificant ($p > 0.05$). The slight reduction in pH likely results from the redistribution of H^+ ions during the freeze–thaw cycle; yet, the values remain within a safe range for the skin. Drying time and spreadability decrease as the gel matrix becomes slightly denser and water mobility is reduced. Conversely, adhesiveness increases as the polymer network strengthens during the reorientation process that occurs during thawing. Viscosity also decreases slightly, possibly due to partial molecular relaxation caused by repeated freezing, but the change remains statistically stable. These findings align with previous reports showing that peel-off gel preparations remain stable after freeze–thaw cycling, with changes in physical parameters of less than 5% and statistically insignificant differences [21].

When compared, the freeze–thaw method produces a slight decrease in physicochemical parameters, which is more significant than that of the heating–cooling method. This can be explained by the process of water crystallization at room temperature and at low temperatures during phase freezing, which can affect the deep-water distribution gel network and result in a slightly lower degree of homogeneity. However, changes to the 'no cause damage' system and the separation phase ensure that, in a way, the overall second method exhibits good stability.

The reduction in parameters across both stability methods remains within the acceptable tolerance range and shows no statistical significance ($p>0.05$). These findings demonstrate that the peel-off gel mask formulated with halal gelatin derived from milkfish bones maintains stability comparable to commercial gelatin. The reversibility of the physicochemical changes also confirms that temperature cycling does not compromise the overall quality of the preparation [22].

In a mechanistic sense, some formulation factors explain the low potential for irritation. First, the final pH levels in the neutral-to-acidic range for skin (around 4.5–6.5) reduce the risk of irritation from direct product acidity. Second, gelatin (especially after refinement and processing into nanoparticles) is generally considered biocompatible, although it may contain contaminants that can be irritating. The interaction of gelatin with polymer film formers (PVA), plasticizers, and humectants produces a soft film matrix, making contact with the epidermis more protective and less chemically aggressive. Third, the formulation is topical, with viscosity and power-appropriate distribution, which tends to reduce excessive penetration into the dermis layer, thereby reducing the opportunity for systemic inflammatory reactions. Additionally, no syneresis or separation phase was observed during stability testing, which supports the conclusion that no new substance (degradation) is released that could become a source of irritation.

The results of this study show that a body lotion formulated with areca nut skin extract achieved a maximum erythema/edema score of 1 and a PII value of 0, indicating it is safe and non-irritating (Table 7). Both studies employ BPOM RI/OECD 404–based protocols, which utilize identical scoring parameters (0–4 for erythema and edema, followed by PII calculations), allowing for a direct methodological comparison. The compatibility of pH and the use of non-irritant excipients further

enhance topical safety. Additional reports on gelatin-based topical preparations also show minimal erythema and edema after application, supporting the overall conclusion that purified fish gelatin does not exhibit inherent irritant properties [23].

Aging is a natural process that occurs. It is believed that one reason aging is a consequence of free-radical damage to cells. Hyperpigmentation can cause premature aging in the human skin and result from internal and external factors, such as exposure to UV rays, the consumption of certain medications, and the presence of certain chemical compounds in cosmetic products. Enzymes that play a crucial role in melanin synthesis include tyrosinase, which is involved in two key steps of melanin synthesis: the hydroxylation of L-tyrosine to L-DOPA (3,4-dihydroxyphenylalanine) and the oxidation of L-DOPA to dopaquinone. Then dopaquinone polymerizes to form dopachrome, which then polymerizes to form melanin. The mechanism of tyrosinase inhibition can cause skin bleaching by blocking melanin synthesis. In addition, inhibiting tyrosinase is crucial as a depigmentation agent in disorders of hyperpigmentation; inhibiting tyrosinase will hinder melanin formation or the browning reaction.

There is exposure to UV radiation, which leads to ROS accumulation. The ROS produced during the aging process activate mitogen-activated protein kinase (MAPKs) and induce the transcription of factors, including activator protein 1 (AP-1) and nuclear factor-kB (NF-κB). Activation increases MMP expression and inhibits the signal of transforming growth factor-β (TGF-β), leading to collagen fragmentation and a decline in collagen biosynthesis. This obstructs the mechanical interaction between fibroblasts and the extracellular matrix (ECM), resulting in reduced-sized dermal fibroblasts. Fibroblasts in aging skin produce a higher amount of ROS. The next step increases MMP expression and inhibits the TGF-β signal, creating a positive feedback loop that accelerates dermal aging. Apart from exposure to ultraviolet radiation, factors in other environments, such as smoke, influence skin changes and are closely related to wound healing, the development of carcinoma cells in squamous cancers of the mouth, the exacerbation of psoriasis, and early skin aging. Damage to the skin caused by tobacco is associated with oxidative stress, disorders of collagen biosynthesis, and MMP activation.

Milkfish bone gelatin inhibits tyrosinase activity through its collagen and peptide content. The bioactive peptides produced during gelatin hydrolysis contain amine, carboxyl, and imidazole groups that interact with Cu^{2+} ions at the tyrosinase active site, reducing enzyme activity and slowing the oxidation of tyrosine to dopaquinone. This inhibition suppresses melanogenesis, preventing excessive melanin formation in the skin. Additionally, the collagen in fish gelatin acts as an antioxidant, scavenging free radicals and inhibiting the generation of reactive oxygen species (ROS), which are significant contributors to skin ageing. Through antioxidant activity and metal-ion chelation, collagen supports anti-aging effects. Previous studies have demonstrated this mechanism, showing that fish collagen effectively scavenges hydroxyl and superoxide radicals, and that tilapia collagen peptides can reduce tyrosinase activity by up to 20% through copper-ion binding [24].

The anti-aging activity in this study was evaluated based on the formulation's ability to inhibit tyrosinase enzyme activity, which plays a crucial role in melanogenesis and the formation of hyperpigmentation, a sign of skin aging. It is essential to emphasize that the anti-aging mechanism is multifaceted and involves multiple biological pathways, including antioxidant activity, inhibition of collagen degradation, and protection against oxidative stress. Therefore, the anti-aging claims in this study are limited to the formulation's potential to support skin lightening and prevent hyperpigmentation by inhibiting

tyrosinase. Evaluation of other mechanisms, such as antioxidant activity or stimulation of collagen synthesis, is beyond the scope of this study and could be the focus of further studies.

Research on extract gel skin stem Taya (Nauclea subdita) suggests that inhibiting tyrosinase plays a role in enhancing skin elasticity and preventing early signs of aging. Research indicates that the IC_{50} value of 568.58 µg/mL for L-tyrosine substrate and 1,374.69 µg/mL for L-DOPA exhibits strong antioxidant activity (IC_{50} 48.78 µg/mL), as well as an effect on improving skin elasticity after four consecutive uses. The inhibition value in research, which ranges from 5 to 11%, indicates moderate category activity and suggests potential real-world applications in cosmetics and topical products. Compared with arbutin, which works as a competitive inhibitor potent on the enzyme tyrosinase through a mechanism of direct binding to the active site, fish gelatin works specifically through chelation of Cu^{2+} ions. It affects antioxidants, so that it powers the obstacle at a relatively lower level [25].

Even though the results show that halal gelatin nanoparticles from milkfish bones retain their own potential as a promising anti-aging agent, the structural peptide collagen in fish gelatin not only promotes melanin formation, but also stimulates the synthesis of new collagen in dermal tissue, increasing hydration and strengthening the ECM of the skin. Activity: This is closely related to the presence of high levels of the amino acids glycine, proline, and hydroxyproline, which play a crucial role in collagen triple helix formation in the skin. In addition, the nanoparticles in the halal gelatin formula are suspected to increase the surface area interaction between the peptide collagen and the enzyme tyrosinase, resulting in more pronounced inhibition than with commercial gelatin.

In a way, the study demonstrated tyrosinase inhibition. This is classified as moderate, but relevant in a physiological sense. In the support function, anti-aging occurs through mechanisms, namely the inhibition of the enzyme tyrosinase and protection against oxidative stress. The absence of a significant difference between the formulas indicates that both halal gelatin and commercial gelatin exhibit similar stability, activity, and biological equivalence, with a trend toward greater effectiveness in the fish gelatin nanoparticle formula. Thus, the halal gelatin-based peel-off gel mask preparation from milkfish bones can be categorized as having its own potential as an anti-aging natural, safe, and stable agent, even though its effectiveness still needs to be improved through optimization of the concentration of active material or combination with compound enlighteners, such as arbutin, vitamin C, or plant flavonoids.

The tyrosinase enzyme inhibition values obtained in this study were moderate; therefore, these findings should be viewed as preliminary. These results indicate that the gelatin nanoparticle formulation has the potential to support skin pigmentation control mechanisms, but cannot yet be categorized as a potent tyrosinase inhibitor. This is in line with gelatin's role as a natural biopolymer that primarily functions as a matrix or carrier system, rather than as a specific active compound. Therefore, the anti-aging activity reported in this study is limited to the initial potential of tyrosinase inhibition. Additional studies, including formulation optimization or testing of additional biological mechanisms, are needed to strengthen its effectiveness.

Conclusion

Gelatin nanoparticles derived from milkfish (*Chanos chanos*) bones were successfully formulated into a peel-off gel mask with acceptable particle size, good physicochemical properties, and stable performance under storage and stress conditions. All formulas showed a Primary Irritation Index of 0, indicating excellent dermal safety. The formulations also demonstrated moderate tyrosinase inhibition,

with F1 showing the highest activity. These findings confirm that milkfish-bone gelatin nanoparticles are safe, stable, and potentially effective as natural halal biopolymers for the development of anti-aging peel-off gel masks.

Acknowledgement

The authors would like to thank Bank Indonesia for full funding support through the 2025 Bank Indonesia Research Grant (RGBI). They would also like to thank the Pharmaceutical Research Laboratory at Maliki Islamic University for the facilities and technical support provided during the research process.

Disclosure Statement

No potential conflict of interest was reported by the authors in this work.

ORCID

Rahmi Annisa:
https://orcid.org/0000-0001-7536-5213
Jauharuddin Luthfi Al Jabbar:
https://orcid.org/0000-0001-5399-6751
Wirda Anggraini:
https://orcid.org/0000-0002-9837-155X

References

[1] Beringhs, A.O.R., Rosa, J.M., Stulzer, H.K., Budal, R.M., Sonaglio, D. Green clay and aloe vera peel-off facial masks: Response surface methodology applied to the formulation design. *AAPS Pharmscitech*, **2013**, 14(1), 445–455.

[2] Rawdkuen, S., Thitipramote, N., Benjakul, S. Preparation and functional characterisation of fish skin gelatin and comparison with commercial gelatin. *International Journal of Food Science and Technology*, **2013**, 48(5), 1093-1102.

[3] Karim, A.A., Bhat, R. Fish gelatin: Properties, challenges, and prospects as an alternative to mammalian gelatins. *Food Hydrocolloids*, **2009**, 23(3), 563–576.

[4] Roy, S., Siracusa. V. Multifunctional application of biopolymers and biomaterials, *International Journal of Molecular Sciences*, **2023**, 24, 10372.

[5] Choi, S.S., Regenstein, J.M. Physicochemical and sensory characteristics of fish gelatin, *Journal of Food Science*, **2000**, 65(2), 194–199.

[6] Silvipriya, K.S., Krishna Kumar, K., Bhat, A.R., Dinesh Kumar, B., John, A., Lakshmanan, P. Collagen: Animal sources and biomedical application, *Journal of Applied Pharmaceutical Science*, **2015**, 5(3), 123-127.

[7] Patra, J.K., Das, G., Fraceto, L.F., Campos, E.V.R., Rodriguez-Torres, M.d.P., Acosta-Torres, L.S., Diaz-Torres, L.A., Grillo, R., Swamy, M.K., Sharma, S. Nano based drug delivery systems: Recent developments and future prospects. *Journal of Nanobiotechnology*, **2018**, 16(1), 71.

[8] Irani, M., Nasirtabrizi, M.H., Ezzatzadeh, E., Sadeghianmaryan, A. In-vitro study of co-electrospun PCL/gelatin/chitosan scaffolds enhanced with carbon nanotube for cartilage regeneration. *Polymer Bulletin*, **2025**, 82(12), 6981–6999.

[9] Golipour, H., Ezzatzadeh, E., Sadeghianmaryan, A. The fabrication and characterization of a novel antibacterial curcumin and TiO_2 loaded gelatin/silk fibroin: Polycaprolactone scaffolds for skin tissue engineering. *International Journal of Polymeric Materials and Polymeric Biomaterials*, **2024**, 73(11), 974–986.

[10] Golipour, H., Ezzatzadeh, E., Sadeghianmaryan, A. Investigation of co-electrospun gelatin: TiO_2/polycaprolactone: Silk fibroin scaffolds for wound healing applications. *Journal of Applied Polymer Science*, **2022**, 139(27), e52505.

[11] Arunothayanun, P., Bernard, M.S., Craig, D.Q.M., Uchegbu, I.F., Florence, A.T. The effect of processing variables on the physical characteristics of non-ionic surfactant vesicles (niosomes) formed from a hexadecyl diglycerol ether, *International Journal of Pharmaceutics*, **2000**, 201, 7–14.

[12] Alipal, J., Pu'Ad, N.M., Lee, T.C., Nayan, N., Sahari, N., Basri, H., Idris, M., Abdullah, H. A review of gelatin: Properties, sources, process, applications, and commercialisation. *Materials Today: Proceedings*, **2021**, 42, 240–250.

[13] Prow, T.W., Grice, J.E., Lin, L.L., Faye, R., Butler, M., Becker, W., Wurm, E.M., Yoong, C., Robertson, T.A., Soyer, H.P. Nanoparticles and microparticles for skin drug delivery. *Advanced Drug Delivery Reviews*, **2011**, 63(6), 470–491.

[14] Mourtas, S., Fotopoulou, S., Duraj, S., Sfika, V., Tsakiroglou, C., Antimisiaris, S.G. Liposomal drugs dispersed in hydrogels: Effect of liposome, drug and gel properties on drug release kinetics. *Colloids and Surfaces B: Biointerfaces*, **2007**, 55(2), 212–221.

[15] Song, Y., Chen, W., Yin, Y., Li, J., Wang, M., Liu, Y., Ren, X. Advancements in the transdermal drug delivery systems utilizing microemulsion-based gels. *Current Pharmaceutical Design*, **2024**, 30(35), 2753–2764.
[16] Wang, Y., Tian, Y., Jia, L., Xu, S., Meng, X. Nano-mediated strategy: Recent advance in the application of drug delivery systems in melanoma treatment and diagnosis. *Biomedical Materials*, **2024**, 19(5), 052007.
[17] Sun, M., Wang, Y., Yao, L., Li, Y., Weng, Y., Qiu, D. Fabrication and characterization of gelatin/polyvinyl alcohol composite scaffold. *Polymers*, **2022**, 14(7), 1400.
[18] Ling, W.C. Thermal degradation of gelatin as applied to processing of gel mass. *Journal of Pharmaceutical Sciences*, **1978**, 67(2), 218–223.
[19] Dranca, I., Vyazovkin, S. Thermal stability of gelatin gels: Effect of preparation conditions on the activation energy barrier to melting. *Polymer*, **2009**, 50(20), 4859–4867.
[20] Michon, C., Cuvelier, G., Relkin, P., Launay, B. Influence of thermal history on the stability of gelatin gels. *International Journal of Biological Macromolecules*, **1997**, 20(4), 259–264.
[21] Waresindo, W.X., Luthfianti, H.R., Edikresnha, D., Suciati, T., Noor, F.A., Khairurrijal, K. A freeze–thaw PVA hydrogel loaded with guava leaf extract: Physical and antibacterial properties, *RSC Advances*, **2021**, 11, 30156–30171.
[22] Ahmed, E.M. Hydrogel: Preparation, characterization, and applications: A review. *Journal of Advanced Research*, **2015**, 6(2), 105–121.
[23] Li, J., Mooney, D.J. Designing hydrogels for controlled drug delivery. *Nature Reviews Materials*, **2016**, 1, 16071.
[24] Sae-Leaw, T., O'Callaghan, Y.C., Benjakul, S., O'Brien, N.M. Antioxidant activities and selected characteristics of gelatin hydrolysates from seabass (Lates calcarifer) skin as affected by production processes. *Journal of Food Science and Technology*, **2016**, 53(1), 197–208.
[25] Charissa, M., Djajadisastra, J., Elya, B. Uji aktivitas antioksidan dan penghambatan tirosinase serta uji manfaat gel ekstrak kulit batang taya (Nauclea subdita) terhadap kulit. *Jurnal Kefarmasian Indonesia*, **2016**, 6(2), 98–107.

HOW TO CITE THIS ARTICLE

R. Annisa, J.L. Al Jabbar, W. Anggraini. Development and Evaluation of Gelatin Nanoparticle Peel-Off Gel Mask from Milkfish (*Chanos chanos*) Bones: Formulation, Stability, Skin Safety, and Anti-Aging Activity. *Adv. J. Chem. A*, 2026, 9(10), 1816-1841.

DOI: 10.48309/AJCA.2026.567198.2011
URL: https://www.ajchem-a.com/article_242051.html