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## Research Article

# Formulation Of an Epidermal Cream with Collagen Extracted from *Cynoglossus Semilaevis* Fish Skin

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### ABSTRACT

Collagen is the most abundant protein in the human body and also the key structural component of skin, bones, muscles, tendons, ligaments and connective tissue. It provides elasticity, strength and support to various body parts. There are various natural sources for collagen and for this project, marine collagen is used. Marine collagen has its unique properties and excellent invitro biodegradability and biocompatibility, making it ideal to repair tissue. This project develops an epidermal cream using collagen extracted from Tongue sole fish (*Cynoglossus semilaevis*), combined with beeswax and shea butter. Beeswax and shea butter has very good moisturizing effect, anti-inflammatory, act as a natural preservative and also biodegradable. The formulated cream was evaluated for physiochemical properties, showing a pH from 3.3 decreased to 3.0 on the 14th day and viscosities of 129.85 Pa.s (brown skin) and 206.04 Pa.s (white skin). Stability test confirmed no phase separation, with easy washability. The cream enhances skin hydration, improves skin tone and reduces wrinkles, making it a promising anti-aging formulation.


### INTRODUCTION

Collagen is widely used in regenerative medicine and tissue engineering and also has a positive impact in post-translational modification, which is necessary to maintain healthy skin structure (Yamada et al., 2013). Fish skin is a fine source of collagen and gelatin (Mala Nurilmala et al., 2022). The gelling ability of flat-fish species are more stable than those from cold-adapted fish.

(Gomez -Guillen et al., 2002). Marine collagen has very advanced application properties in cell regeneration and wound -healing properties (Lian Chen et al., 2022). Due to the decrease in the synthesis and changes in proteoglycans and collagen, integrity and health of these tissues also leads to a decline in skin elasticity, increased wrinkle formation, and other age-related skin concerns in old-age people. (Czajka et al., 2018).

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Investigating the viability of formulating a cosmeceutical emulsion incorporating piscine-derived collagen, with a particular emphasis on its therapeutic advantages and the intricate technical parameters essential for its successful development. (Yamada et al., 2013) (Salvatore et al., 2023) (Rahman et al., 2024).

## MATERIALS AND METHODS

Tongue sole fish is collected from Kelambakkam fish market. The other chemicals for the project is used Affyclone Laboratories. The tools used are glasswares, glass rod, pH meter, centrifuge. Weight balance, petri dish. Distilled water ,acidic pH buffer solution(4.0) and neutral pH buffer solution (7.0), 96% ethanol and the chemicals used are hydrogen peroxide, acetic acid, sodium chloride, sodium hydroxide .And other ingredients used are shea butter and beeswax .Next an evaluation of the cream prepared was carried out which includes: organoleptic test , pH test, spreadability test, viscosity test, emulsion test, sensitivity test, stability test. This research was carried out at Affyclone Laboratories in Chromepoet, Chennai.

### 1.Collagen Extraction

#### Preparation of collagen from fish skin

For the methodology of collagen extraction, referred to collagen extraction from tilapia fish (Romi Triadi et al ., 2022) isolation of collagen from Brama Australis(Sionkowska et al., 2015). In the beginning the skin of tongue sole fish was cleaned with distilled water and both white skin and brown skin are cut into pieces and kept overnight with NaoH. Next day adding acetic acid for stirring and grinding and kept aside for 10days.

After 10 days both skin types are centrifuged and preparing supernatant with certain amount of NaCl and after precipitation combining with pepsin to get collagen.

### 2. Cream Formulation

#### Basic Preparation Steps

For oil phase, melted shea butter, beeswax together in a double boiler. For water phase heated distilled water and glycerine in a separate container to a similar temperature as the oil phase. Slowly poured the water phase into the oil phase while continuously stirring until the mixture thickens for emulsification. And cooling phase , the mixture cools to about 40°C, add collagen, vitamin E . Finally, all these are mixed well and pour into clean, sterilized containers.

**Table 2.1 Ingredients for Cream Formulation**

	Ingredients	Function	Percentage (%)	Amount (40g)
1.	Shea Butter	Moisturizer, skin softener	20%	20g
2.	Beeswax	Thickener & Protective Barrier	7%	7g
3.	Vitamin E oil	Antioxidant & extends shelf life	1%	1G
4.	Hydrolysed collagen	Improves skin elasticity, hydration	10%	10 g



5.	Glycerine	Exfoliating	3%	3g
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## RESULTS AND DISCUSSIONS

### RESULT

#### 1. Organoleptic Test

The organoleptic properties like colour, odour, and phase separation were noted. Through basic observation, organoleptic tests for the formulated cream is analysed. Organoleptic tests includes mainly of colour , odour , texture. For both brown skin and white skin formulation, organoleptic test

**Table 1. Organoleptic Parameters**

Organoleptic Parameters		
7 <sup>th</sup> Day		
Appearance	Brown Skin	White skin
Colour	Sunset Yellow	Sunset Yellow
Odour	Pleasant	Pleasant
Texture	Smooth	Smooth
14 <sup>th</sup> Day		
Appearance	Brown Skin	White Skin
Colour	Sunset Yellow	Sunset Yellow
Odour	Pleasant	Pleasant
Texture	Smooth	Smooth

#### 2. pH Test

The results of analysing pH value of the prepared cream were obtained between 3.0 – 3.3. From the results of analyzing the pH value of the cream is 3.3 on 7th day of preparation and reduced to 3.0 on 14th day until cream prepared. If the pH is too alkaline it may cause dried skin and it is too acidic may cause irritation. Here based on the data above, it is found that the cream is nearly acidic and after some days the pH is decreased to acidic. The pH of the creams was determined using a pH meter (Hanna Instruments, India).

**Table 2.1 pH Of the Formulation**

pH		
Formulation	7 Day	14 Day
Brown Skin	3.3	3.0
White skin	3.3	3.0

#### 3. Spreadability

The Spreadability of the cream was evaluated by referring the methods of Kaushik et al., 2020 and Okafo et al., 2023.. In this analysis ,0.5g of cream was placed within 1cm diameter circle on a glass plate and another glass plate was positioned on top by applying a 300g weight for 5 minutes. The pressure from the weight caused the cream to spread which increase the diameter of the circle. The expansion was measured in centimetres and recorded as a approximate value for Spreadability

**Table 3.1 Spreadability of The Cream**

Spreadability				
7 <sup>th</sup> DAY				
	Wt. Tied to Upper Slide(M)	Length Of Glass Slide Moved(L)	Time Taken(T)	Average Spreadability, $S=(M \times L)/T$
Brown Skin	100 g	10.5 cm	1.85 sec	567.5675676
White skin	100 g	9.8 cm	1.92 sec	510.4166667



14 <sup>th</sup> DAY				
<b>Brown Skin</b>	100 g	8.8 cm	2.85 sec	308.7719
<b>White skin</b>	100 g	8.5 cm	2.62 sec	324.4275

#### 4. Ease of removal (Washability)

This was assessed by rinsing off the applied cream from a specific body area using running tap water. (Ashish et al., 2013). As the observation shows that the cream has easy wash off property which ensures user convenience.

**Table 4.1 Washability of The Cream**

Washability	
<b>Formulation</b>	
<b>Brown Skin</b>	Ease of removal
<b>White skin</b>	Ease of removal

#### 5. Viscosity

10ml of cream were taken and poured into the viscometer. The pressure was given to the opening valve and cream started to flow from initial to final point. Therefore, the time was noted with the help of a stopwatch.

To calculate  $\eta_2$  the viscosity of the sample, we can use the formula:

$$\eta_1 / \eta_2 = \rho_1 * t_1 / \rho_2 * t_2$$

Rearrange to solve for  $\eta_2$ :

$$\eta_2 = \eta_1 * (\rho_2 * t_2 / \rho_1 * t_1)$$

$$\eta_2 = 2.1 * (0.89 * 3960 / 1.5 * 3.8) = 129.85$$

The viscosity of the sample – Brown skin is approximately 129.85 ( Pa.s )

Sample – white skin

The viscosity of the sample – White skin is 206.04 ( Pa.s ).

#### 6. Emulsion test

When an emulsion is added to a dried filter paper soaked in cobalt chloride solution it turns from blue to pink indicating that the emulsion is O/W type. But here as per the observation the cream is W/O emulsion which shows this cream is moisture sealing and act as a protector to skin.

**Table 5.1 Emulsion of The Cream**

Emulsion Test		
Formulation	Observation	Type
Brown Skin	Brown Skin	Brown Skin
White Skin	Remains Same	W/O Emulsion
A W/O (Water-in-Oil) emulsion has water droplets dispersed within an oil phase, leading to a thicker, more occlusive (moisture-sealing) product that is commonly used in heavier creams and ointments		

#### 7. Stability Test

For the stability test of the cosmetic cream, samples were subjected to centrifugation at 3000 rpm for a duration of 30 minutes. The stability of the cream is observed as there is no phase separation on 7<sup>th</sup> day also phase separation is not found even after 14<sup>th</sup> day, which clear shows the cream has good shelf- life and the product maintains its effectiveness over time.

**Table 6.1 Stability of The Cream**

Stability Testing
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Formulation	7 <sup>th</sup> Day	14 <sup>th</sup> Day
Brown Skin	No Phase Separation	No Phase Separation
White Skin	No Phase Separation	No Phase Separation

## DISCUSSION

The present work is to prepare a cream incorporating collagen of the fish skin (*Cynoglossus Semilaevis*) which mainly focus on enhancing the skin tone, reducing wrinkles and improves hydration by retaining moisture. Cream analysis tests are done for this formulation, such as follows.

- The organoleptic tests are done for the cream formulation such as colour, odour and texture, As have done cream formulation for both brown and white skin Analysis have done on 7th day and also on the 14th day. The cream has sunset yellow colour with pleasant odour and smooth texture.

- pH testing is a crucial step in formulating and evaluating skincare creams, as it determines how the product interacts with the skin and its overall stability. The pH ranges from 0-14.

o Acidic: pH 0–6

o Neutral: pH 7

o Alkaline (Basic): pH 8–14

After the pH analysis of the cream, the results seems to be 3.3 which is slightly acidic and on 14th day analysis it again reduced to 3.0. Usually the skincare creams are slightly acidic for the skin.

- The spreadability refers to how easily and evenly the cream spreads over the skin. It plays an crucial role in determining the cream effectiveness .Here the cream is having good range of spreadability.

- Ease of removal for the cream is also in a good range. As well as viscosity.

- Stability is a key factor in determining the effectiveness and shelf-life of a cream. As per the results the formulated cream doesn't shows any phase separation on 7th day as well as 14th day, which concludes the cream have good shelf-life.

## CONCLUSION

In conclusion, Tongue sole fish collagen has been shown to be a valuable ingredient in the preparation of creams due to its ability to promote collagen synthesis and reduce the appearance of fine lines , enhances skin tone and increases hydration. Overall, the incorporation of Tongue sole fish collagen in cream has the potential to revolutionize the skincare industry. Its ability to promote collagen synthesis and reduce the signs of aging in a safe and natural way by enhancing skin tone makes it an exciting prospect for consumers looking for effective and sustainable skincare solutions. Thus it was found out that the cream preparation from the fish skin collagen which is isolated from tongue sole fish has simple methodology and is an inexpensive process. All of the done tests shows the effectiveness of the prepared cream formulation and a best remedy for hydration of skin and enhances skin tone.

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