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Characterization of Collagen Hydrolysate Gel from Mackerel Scad Skin (*Decapterus macarellus*)

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Abstract: The skin of mackerel scad (*Decapterus macarellus*) is a novel source of marine bioactive collagen hydrolysate that has the potential to improve skin health. Collagen hydrolysate represent the major constituent in cosmetic industry because of its excellent moisturizing properties and multiple health benefits. The purpose of this study is to investigate the application of mackerel collagen hydrolysate (mackerel CH) as a component of cosmetic gel preparations by examining the gel's physical and chemical properties which include viscosity, spreadability, adhesiveness, gel strength, gel texture, pH, and organoleptic. Evaluation of physicochemical properties is necessary to ensure that the quality and safety of the gel preparation meets the intended specifications. The formulation of the mackerel CH resulted in a gel with higher viscosity and spreadability compared to the standard national guideline (SNI). The gel exhibited favorable adhesiveness and pH levels, indicating its potential to efficiently deliver active substances to the skin without causing any irritation. The results indicated that the gel had a soft consistency in terms of gel strength and texture. Organoleptic assessment described the gel as having a clear, yellowish appearance with a subtle fishy aroma. Future studies should focus on further optimizing the formulation. Additionally, it is recommended to assess the efficacy of this gel in addressing medication-related issues using an animal model.

Keywords: Collagen Hydrolysate; Gel; Mackerel Scad; Physicochemical Characteristics

Introduction

People's desire for nature-based skin and health care is growing. This can be seen in the number of products made from natural ingredients. Several marine biotas have been identified as safe sources of bioactive ingredients, one of which is collagen. Collagen is a major connective tissue of animal proteins and is widely used in the cosmetic and biomedical industries. Marine collagen has recently been reported as a source of new biomaterials for cell and tissue culture and is considered an alternative to common mammalian collagen, such as bovine, chicken, and porcine collagen (Jafari et al., 2020; Kulkarni & Maniyar, 2020). Marine collagen derived from scales, skin and bones has excellent bioactive properties such as biocompatibility (Lim et al., 2019), low antigenicity (Brunt & Burgess, 2018), high biodegradability (Yamamoto et al., 2014), and good cell growth potential (Zhou et al., 2013).

Mackerel (*Decapterus macarellus*) is an understudied source of marine collagen. It is pelagic fish species which has economic value, wide distribution and abundance (Silooy et al., 2020). This species is usually found in tropical and subtropical waters, especially in eastern Indonesia. The peak production of this fish occurs twice a year, in January-March and July-September (Kusumanigrum et al., 2021).

In general, the use of mackerel is still limited for consumption purposes. However, based on the research of recent years, it is known that mackerel scad-derived collagen and collagen hydrolysate have the potential to be used as a good antiaging bioactive agent (Herawati et al., 2022). They showed antioxidant, antityrosinase, and antiglycation activities which can help protect the body from free radical attacks that cause premature aging,

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collagen damage, and skin cancer (León-López et al., 2019). Furthermore, mackerel collagen hydrolysate (mackerel CH) can provide a protective effect against UVB radiation on mouse embryonic fibroblast cell cultures in vitro (Pratiwi, 2022). Thus, the discovery of collagen bioactivity obtained from mackerel scad fish brings merits such as biomedical applications, development of functional food and nutraceuticals, as well as advancements in the cosmetic and skincare industry.

Gel preparations are among the most commonly used medication in the cosmetic industry. It has several advantages over other topical preparations such as cream, ointment, and lotion. The gel has high viscosity and adhesion, so it does not flow easily on the skin, good spreadability on the skin, does not affect the physiological function of the skin, and is thixotropic, so it is easy to spread when applied and does not peel off scars, easily washes off with water and feels cool after use (Aryantini et al., 2020). The properties of the gel should be suitable for the intended use so that the gel can maintain a good solid form during storage and is not easily damaged when applying force by shaking in a jar or when applying topically (Sidiq et al., 2018).

Despite the increasing commercial importance of marine collagen in cosmetics, no scientific studies have been published on the gel preparation from mackerel CH. This study aimed to investigate several physicochemical characteristics of mackerel CH gel, including viscosity, spreadability, adhesiveness, pH, gel strength, gel texture, and organoleptic test. It allows for a comprehensive understanding of its structure, mechanical properties, stability, interactions, and performance. This knowledge facilitates the optimization of gel formulation, processing techniques, and storage conditions to achieve desired functional outcomes and enhance the efficacy and safety of collagen-based biomaterials.

Method

Pretreatment

Mackerel scad (*Decapterus macarellus*) was purchased locally from Surakarta, Indonesia. Fish scales were cleaned, washed under running water, and skinned manually using a knife. The skin of the fish was cut into small cubes measuring and immersed in 0.1 M NaOH solution with a ratio of 1:10 (w/v) for 6 hours at 4 0C. The NaOH solution is changed every two hours (Herawati et al., 2022).

Pepsin-Soluble Collagen (PSC) Extraction

After soaking in NaOH, the skin was neutralized with running water for 15 minutes or until neutral pH. The skin is then soaked in pepsin (EC 3.4.23.1; powdered; 500 units/mg solid, Sigma-Aldrich, St. Louis, MO, USA) containing 0.5 M acetic acid at a concentration of up to 0.1% (w/w) 1:8 (w/v) and stirred at 4°C for 48 hours. The filtrate was then filtered and centrifuged (XXX) for 60 minutes using Eppendorf 5810R (Eppendorf, Germany) to separate the supernatant. The precipitated supernatant was then centrifuged again for 20 min to obtain a pellet. The pellet was dissolved in 0.5 M acetic acid (1:5 w/v) and loaded onto a dialysis membrane (Carolina Biological; 12 kDa, cut off, Burlington, NC, USA) for stepwise dialysis. Phase I dialysis was performed for 24 h against 0.2 M sodium phosphate buffer (pH 8). Phase II dialysis was performed for 24 h using distilled water. The resulting dialysate was then dried for 90 hours using a freeze dryer (Telstar® LyoQuest Plus Lyophilizers, Barcelona, Spain (Herawati et al., 2022).

Collagen Hydrolysis

The collagen (1 g) was soaked in 200 ml of ultrapure water and immerse in a 37 °C water bath. Collagenase II (2-28-100 MG-PW; powdered; 125 units/mg solid, Sigma-Aldrich, St. Louis, MO, USA) (1% w/w) was then added and homogenized for 5 h. The enzymatic reaction was terminated by heating the mixture to 95 °C for 10 min. The resulting mixture was then cooled to room temperature and centrifuged at 3000 rpm for another 30 minutes. The resulting supernatant was then lyophilized (called mackerel collagen hydrolysate) for 90 hours and stored at 4°C (Herawati et al., 2022).

Formulation of Collagen Hydrolysate Gel

The composition of mackerel CH gel was modified from the study (Safaruddin et al., 2019) and shown in Table 1. Hydroxypropyl methylcellulose (HPMC) was stirred in warm distilled water until it swelled and became homogeneous. A mixture of glycerin solution, methylparaben, citrus fruit oil and mackerel CH was added to the resulting gel matrix, then mixed until a homogeneous gel mass is formed.

Table 1. Mackerel CH Gel Formulation

Material	Function	Amount (%)
Mackerel CH	Active ingredients	5
HPMC	Gelling agent	2
Glycerin	Humectants	10
Methyl Paraben	Preservative	0.2
Citrus Oleum	Fragrance	0.5
Aquades	Solvent	Added up to 100%

Evaluation of the Physical Properties of Collagen Hydrolysate Gel

1. *Viscosity Test*

Viscosity measurements was determined using

Rheosys Merlin VR II with cone and plate system. A total of 1 gram of sample was placed on a plate, then a spindle was installed on the viscometer and lowered to a distance of 1 mm from the gel. The rotor ran at 2.0 rpm for 60 seconds. Viscosity test was carried out on freeze thaw cycle (Wibowo, 2017).

2. *Spreadability* Test

A sample of 500 mg was placed on a round glass with another glass placed on top of it. An extra 125 grams of load was added afterward before it was allowed to stand for 1 minute and measured for its constant diameter. Spreadability test was carried out on freeze thaw cycle (Wibowo, 2017).

3. *Adhesiveness Test*

Gel weighing 500 mg was placed between 2 object glasses then pressed by a weight of 500 gram for 5 minutes. After that, the load was lofted and another load of 80 gram was given to the adhesiveness test tool. The time required for the 2 object glasses to separate was recorded (Wibowo, 2017).

4. *pH*

A sample of 1 gram was diluted in 10 ml aquadest. Universal pH sticks were dipped into diluted samples of the gel. Once they were completely immersed, the change in the color of the universal pH was observed and matched with universal pH standard.

5. *Gel Strength*

Gel strength was analyzes using Lloyd texture analyzer set with 4.5 gram load cell, cross-head speed 0.5 mm/s and 4 mm in diameter. The bottle of bloom containing the sample was placed in the middle and then the penetration of the probe until its submerged in the gel. The value that appears on the monitor is the gel strength in grams (Mufida et al., 2020).

6. *Gel Texture*

Gel texture was analysed with the Lloyd Texture Analyzer. The samples were previously cooled in the refrigerator, then the sample was incubated for 30 minutes at refrigerated temperature (15°C). The sample was pressed with a 12.7 mm probe until a deformation of up to 10 mm occurs at a speed of 1 mm/s. Gel texture parameters include hardness and elasticity (Atma et al., 2018).

7. *Organoleptic*

Organoleptic parameters include aroma, texture and colour of the gel, based on the organoleptic test specified according to SNI 8076:2014. Tests carried out by involving 25 to 30 respondents.

Result and Discussion

Viscosity

A viscosity test is used to determine the thickness of a gel formulation. In this study, the resulting viscosity of mackerel CH gel ranged from 35,970 to 930,090 cps (Table 2). According to the Indonesian National Standards (SNI-16-4399-1996), the viscosity of a good sunscreen gel is 2,000 to 50,000 cps, thus, the viscosity of mackerel CH gel was classified as high. This may be due to the amount of gelling agent (HPMC) used in the gel formulation. The advantages of HPMC as a gelling agent is that it can form transparent gels, easily soluble in water, and has good stability even when exposed to heat and humid conditions, so that it does not undergo significant changes in homogeneity, pH, consistency and rheological properties of the gel. However, since the resulting viscosity is higher than SNI standard, the amount of HPMC in the mackerel CH gel formulation needs to be reduced. Nevertheless, mackerel CH gel exhibits stable physical properties as indicated by a decrease in viscosity value accompanied by an increase in shear stress (Table 2).

HPMC is a polymer derived from cellulose. In the event of dispersion, the polymer molecules will enter the cavity formed by the water molecules and form hydrogen bonds between the hydroxyl groups of the polymers with the water molecules. Hydrogen bonding plays a role in hydration in the swelling process. The higher concentration of HPMC used allows more hydroxyl groups to bind resulting in a high viscosity value (Noval et al., 2020).

Spreadability

Spreadability test determine the ability of the mackerel CH gel to spread when applied to the skin. The result showed that the gel has a spreading area of 11.730 ± 0.450 cm g/s, which exceeds the SNI-16-4399-1996 value of a sunscreen gel (5-7 cm g/s). On the other hand, if a gel has high gel spreadability, it means that it can spread smoothly and evenly over the surface with minimal effort. High gel spreadability is a desirable characteristic for many cosmetic and personal care products. It offers several advantages: ease of application, uniform coverage, enhanced absorption, and reduced product wastage. The spreadability of a gel can be influenced by various factors, including its viscosity, consistency, formulation ingredients, and the presence of suitable spreading agents or surfactants (Hayat et al., 2023; Khan et al., 2022). Balancing spreadability with other desired product attributes is essential in formulating effective and user-friendly cosmetic gels. Therefore, skincare product in the form of a gel with good spreadability is an important

characteristic to obtain the ideal properties in topical application (Atma et al., 2018).

Adhesiveness

Adhesiveness refers to the adhesion of a gel on the skin. The longer adhesion time indicates that the gel preparation adheres well to the skin and allows more active substances to diffuse into the skin (Sani et al., 2021). The adhesion test results of mackerel CH gel were 1,561 ± 195 s. These results showed that the mackerel CH gel formulation met the requirements for semi-solid adhesion, which was longer than 10 seconds (Voigt, 1994). The adhesiveness of the gel preparation is attributed to the composition of the gelling agent. The higher concentration of HPMC leads to a greater adhesion capacity. This is because HPMC has strong binding ability and hence can stick for a long time (Putri & Anindhita, 2022). There are no special requirements for the optimal value of the adhesion test. The greater the adhesiveness of a gel formulation, the more optimal the drug delivery capability achieved.

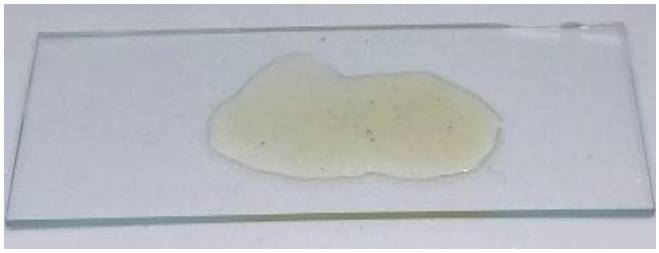

Figure 1. Mackerel collagen hydrolysate gel

Table 2. Viscosity of Mackerel CH Gel

Shear Stress (Pa)	RPM	Time (s)	Viscosity (cps)	SD
279.03	0.1	20.1	930,090	88.3
1062.1	2.6	40.2	136,170	0.99
1160.06	5.1	60.3	75,820	1.97
1153.13	7.5	80.4	51,250	1.81
1079.07	10	100.5	35,970	2.3

pH

According to SNI-16-4399-1996, a good gel preparation has a pH that corresponds to the physiological pH of the skin from 4.5 to 7. A pH lower than 4.5 can cause skin irritation, a pH higher than 4.5 can cause skin irritation 7 may impair the barrier function of the skin (Kuo et al., 2020). The pH of Mackerel CH gel was 6-7, which was within the range of the physiological pH of the skin and meets the national standards of Indonesia (SNI). According to SNI-16-4399-1996, a good pH for a cosmetic product is 6.5-8. This pH value shows that the mackerel CH gel is unlikely to cause skin irritation.

Gel Strength

Gel strength refers to the ability of a gel to maintain its shape and structure under applied forces. In this study, the gel strength of mackerel CH is 5.5 g.bloom and classified as low Bloom. Several factors such as the nature of the species, the extraction method and the composition of the collagen hydrolysate can be associated with the strength value of the gel. It is important to note that the appropriate gel strength or bloom value of gel depends on the specific application. Gel product for face and body, often aim for a softer and more lightweight texture. These gels may have lower gel strengths to provide a smooth, easy-to-spread consistency and enhance the application experience.

Gel Texture

The rheological properties studied included the hardness and springiness of the mackerel CH gel. The hardness value is the amount of force resistance of a non-deforming material to break apart due to an applied compressive force (Atma et al., 2018). Mackerel CH gel's hardness ranging from 10.5 g/cm^2 to 11 g/cm^2 which indicates a soft gel texture. Collagen hydrolysate has a high-water absorption capacity and thus becomes a good ingredient to give shape and strength to the gel (Nining, 2020). This ability of collagen hydrolysate increases the amount of free water in the gel, thus softening its texture.

Springiness is the value at which a material will resist breaking due to tensile strength (Atma et al., 2018). The result showed that mackerel CH gel has springiness with a value of 11 mm. This result is quite high compared to studies of gel preparations of other species, namely *Catla catla* (0.925 mm), *C. mrigala* (0.903 mm), and *L. rohita* (0.846) (Kumar et al., 2017). The springiness of a gel is directly proportional to the viscosity. The higher elasticity value of mackerel CH gel might be attributed to the high viscosity of the gel.

Organoleptic

The organoleptic test revealed that the mackerel collagen CH exhibited a light-yellow hue, emitted a subtle fishy aroma, and possessed a consistently dense and gel-like texture. Overall, the respondents expressed relatively positive acceptance towards the texture and color of the gel. However, there was a consensus among the participants that the aroma could be further enhanced to improve its sensory appeal.

Conclusion

This study characterized the physicochemical properties of a gel incorporating mackerel collagen hydrolysate (CH), a marine-based biomaterial known for its potential benefits in cosmetic applications. The formulation of the mackerel CH gel resulted in a high viscosity and spreadability compared to the standard national guideline (SNI). The gel exhibited good adhesiveness, suggesting its ability to effectively deliver active substances into the skin. Regarding gel strength and texture, the results indicated that the gel had a soft consistency. In terms of organoleptic assessment, the gel was described as having a clear, yellowish appearance with a subtle fishy aroma. Further optimization of the formulation is recommended for future studies. Additionally, assessing the efficacy of this gel in addressing medication-related issues using an animal model is warranted.

Author Contributions

The authors of this article consist of three people. Completion of this article is done together at each stage.

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Conflicts of Interest

The authors declare no conflict of interest.

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