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# Prospective Anti-Aging Benefits of Mackerel Scad Collagen Peptides Through Anti-Hyaluronidase Activity

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## Abstract
The increased activity of the hyaluronidase enzymes speeds up the degradation of hyaluronic acid in the skin, leading to reduced elasticity and the formation of fine wrinkles, eventually contributing to skin aging. Mackerel scad (*Decapterus macarellus*) is a promising candidate as a skin anti-aging substance due to its particular amino acid composition. Native collagen and collagen peptides from mackerel scad skin were extracted using pepsine soluble collagen and hydrolysis with collagenase II enzyme. The amino acid profile of collagen was determined using HPLC analysis. An anti-hyaluronidase activity test was done using the spectrophotometry assay to express the content of N-acetyl glucosaminoglycan, and $IC_{50}$ was calculated. Results showed that mackerel scad collagen contains 17 amino acids, with the highest content of glutamic acid, 8.20%; aspartic acid, 6.70%; glycine, 5.37%; arginine, 4.24%; and proline, 3.84%. The collagen from the extraction results had relatively low anti-hyaluronidase activity ($IC_{50}$ 326.05 ± 6.77 ppm). However, when it was broken down into smaller collagen peptides, the anti-hyaluronidase activity increased to $IC_{50}$ 100.78 ± 0.17 ppm. This indicated that the hydrolysis of collagen into collagen peptides with a smaller molecular weight increased its capacity to inhibit hyaluronidase. These findings suggest that *D. macarellus* collagen peptides have the potential to inhibit skin aging by inhibiting hyaluronidase enzyme activity.

**Keywords:** anti-hyaluronidase, collagen peptide, mackerel scad, skin aging.

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# Potensi Anti-Penuaan Peptida Kolagen dari Ikan Layang Biru Melalui Mekanisme Penghambatan Enzim Hyaluronidase

## Abstrak
Peningkatan aktivitas enzim hialuronidase mempercepat degradasi asam hialuronat di kulit, menyebabkan penurunan elastisitas dan pembentukan kerutan halus yang mengarah pada penuaan kulit. *Decapterus macarellus* merupakan kandidat agen anti penuaan kulit dengan komposisi asam amino tertentu. Kolagen dan peptida kolagen dari kulit ikan *D.macarellus* diekstraksi menggunakan metode kolagen larut pepsin dan hidrolisis dengan enzim kolagenase II. Kandungan asam amino pada kolagen diuji menggunakan HPLC. Aktivitas anti-hialuronidase diukur dengan metode spektrofotometri untuk mengekspresikan kandungan N-asetil glukosaminoglikan yang dinyatakan dengan $IC_{50}$. Kolagen *D. macarellus* mengandung 17 asam amino dengan kandungan tertinggi asam glutamat 8,20%; asam aspartat 6,70%; glisin 5,37%; arginin 4,24%; dan prolin 3,84%. Kolagen dari hasil ekstraksi memiliki aktivitas antihialuronidase relatif rendah ($IC_{50}$ 326.05 ± 6.77 ppm). Namun, ketika dihidrolisis menjadi peptida yang lebih kecil maka aktivitas antihialuronidase meningkat ($IC_{50}$ 100.78 ± 0.17 ppm). Hal ini menunjukkan bahwa hidrolisis kolagen menjadi peptida dengan berat molekul lebih kecil mampu meningkatkan kapasitasnya dalam menghambat hialuronidase. Temuan ini merepresentasikan bahwa peptida kolagen *D. macarellus* berpotensi memperlambat penuaan kulit dengan menekan aktivitas enzim hialuronidase.

**Kata Kunci:** anti-hialuronidase, *Decapterus macarellus*, penuaan kulit, peptida kolagen

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## 1. Introduction

Skin is the largest human organ, roughly one-sixth of the total body weight, and has an estimated area of 1.5-2 $m^2$.[1,2] Its main function is to serve as a protective shield that guards internal tissues against external factors. However, its structure and composition can change due to intrinsic (i.e., metabolic, hormonal, genetic) and extrinsic factors (i.e., UV exposure, pollution, poor nutrition, exposure to harmful chemicals, smoking), known as skin aging.[3,4] Skin aging is also associated with oxidation processes and enzyme activity abnormalities in the extracellular matrix (ECM), which increases reactive oxygen species (ROS). Overexpression of ROS will trigger the activation of mitogen-activated protein kinase (MAPK), NF-κB factor, and transcriptional activator (AP-1). Once activated, these receptors lead to increased matrix metalloproteinase (MMP) gene transcription, resulting in a deficiency of elastin fibers, collagen, and hyaluronic acid.[5]

Hyaluronic acid (HA) is a heteropolysaccharide that binds collagen and elastin fibers, facilitating the formation of water molecular bonds to increase viscosity and reduce extracellular fluid permeability. In normal skin, hyaluronic acid synthesis maintains the skin's moisture. However, the skin may lose the supporting fibers of water molecules caused by overproduction of the hyaluronidase enzyme that promotes ECM degradation. The enzyme hyaluronidase breaks down HA by hydrolyzing β-1,4-glycosidic bond connecting N-acetylglycosaminoglycan and glucuronic acid in the hyaluronic acid polymer.[6,7] This process decreases hyaluronic acid production in the skin, causing it to lose the water molecules' supporting fibers. As a result, skin tissue will weaken and lose integrity, with dry, wrinkled manifestations, and fine wrinkles will appear, so they cannot retain moisture.[8] Thus, inhibiting this enzymatic process by anti-hyaluronidase inhibitor compounds will be necessary to maintain the skin's hyaluronic acid balance.

Strategies used for skin anti-aging are complex and involve multiple treatments such as cosmetics, topical bioactive agents, preventive systemic agents, and lifestyle changes.[9] Exogenous nutrient intake is a promising treatment for cellular renewal and can remove aging effects. Collagen supplements are increasingly being used to fight the signs of skin aging. Collagen containing amino acids such as glycine, proline, and hydroxyproline can stimulate hyaluronic acid production in skin fibroblasts, promote fibroblast movement, and reinforce collagen fibers, leading to increased moisture retention in the stratum corneum.[10] Therefore, amino acids in the body can help maintain sufficient collagen in the skin. The nutraceutical industry has developed peptide-based supplement products as a promising nutraceutical agent that can increase collagen production by breaking down amino acids into small peptide molecules of 2-20 amino acids.[11] Indeed, collagen hydrolyzed into lower molecular weight peptides (3-11 kDa) has better bioactivity than the original collagen.12 The di- or tri-peptide form can increase the binding capacity of $Ca^+$ ions during the absorption process in the intestine, increasing its compatibility when circulated in the body.[12] Collagen peptides also provide several advantages, e.g., low viscosity, high emulsification and stabilization, low solubility, dispersibility, compressibility, and allergenicity.[13] Collagen from marine organisms is increasingly in demand because it has high bioactivity, abundant bioavailability, and a small risk of transmitting zoonotic diseases.[14] Fish, such as mackerel scad (*Decapterus macarellus*), is an excellent potential origin of collagen peptides.[15] Mackerel scad is a small pelagic fish abundant throughout the Indonesian Sea, namely the Java Sea, Makassar Strait, and the Eastern area. It has high economic value, with catches reaching 33,000 tons of small pelagic fish commodities.[16] On a global scale, average annual production reaches 1,199 thousand tons, with output rising to 1,336 thousand tons or 2% of total worldwide marine capture fisheries production in 2018.[17,18]

Production of mackerel scad in large quantities tends to produce waste such as skin, fins, heads, and viscera, accounting for more than 50% of the catch. Accumulating waste will eventually pollute the environment, so it is necessary to transform waste into useful products such as collagen source. For instance, mackerel scad skin with 10-25% protein concentration can be processed as an alternative collagen source.[19] Studies are needed to investigate the potential uses of mackerel scad skin, an alternative collagen source that can support the "Blue Economy" to encourage sustainable economic growth in the marine and maritime sectors that can reduce fisheries waste. A previous study[20] on the extraction and hydrolysis of mackerel scad skin has reported good in vitro antioxidant, antiglycation, and antityrosinase bioactivity as well as photoprotective properties[21], making it a perfect candidate for nutraceutical skin care products. This study aimed to determine the amino acid content and to test the collagen and peptide collagen from mackerel scad skin on inhibiting hyaluronidase enzyme.
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## 2. Materials and Methods

### 2.1. Materials

Mackerel scad fish was purchased from a local market

in Surakarta, Central Java, Indonesia. The isolation of collagen used pepsin (EC3.4.23.1; powder; 500 units/mg solids, Sigma-Aldrich, St. Louis, MO, USA), acetic acid, and membrane dialysis (Carolina Biological; 12 kDa, Burlington, NC, USA). The collagen hydrolyzation used powdered collagenase II 1% (w/w) (2-28-100 MG-PW; 125 units/mg solids, Sigma-Aldrich, St. Louis, MO, USA), ultrapure water. The amino acid analysis used o-phthaldialdehyde (OPA) and sodium acetate/triethylamine. The antioxidant activity test used DPPH (Sigma Aldrich, St. Louis, MO, USA). The anti-glycation test used HCL (Sigma-Aldrich, St. Louis, MO, USA), aminoguanidine, phosphate buffer saline, and TCA. The anti-tyrosinase activity test used tyrosinase enzyme (EC 1.14.18.1; lyophilized powder; ≥1000 units/mg solid, Sigma-Aldrich), L-DOPA used as a substrate, and kojic acid as the positive control. The anti-hyaluronidase activity test used lyophilized powder of Hyaluronidase (H3506-100 MG; 400-1000 units/mg solid; Sigma-Aldrich), hyaluronic acid, and p-dimethylaminobenzaldehyde (P-DMAB).

### 2.2. Instrument

Instruments used in this study were microcentrifuge Eppendorf 5810 (Eppendorf, Germany), Freeze dryer (Telstar® LyoQuest Plus Lyophilizers,Spain), UV-Vis Spectrophotometer (Hitachi, Japan).

### 2.3. Methods

#### 2.3.1. Collagen extraction and hydrolysis

Mackerel scad fish used in this study were medium-sized, with 15-30 cm body length and a weight of 70-200 grams. Fishes were washed in running water and skinned. Skin was cut into 1×1 $cm^2$ pieces, soaked in NaOH, and rinsed with running water until pH was neutral. Skin was then extracted using a mixture of acetic acid 0.5 M (0.1% w/w) and pepsin at a ratio of 1:8 (w/v), and the mixture was stirred for 48 hours at 4 °C. The filtrate was filtered and centrifuged for 60 minutes to separate the supernatant. The supernatant was precipitated and centrifuged for 20 minutes to collect pellets. The pellets were dissolved in 0.5 M (1:5) acetic acid (w/v) and subjected to gradual dialysis with a membrane. Phase I dialysis was performed using 0.2 M sodium phosphate buffer (pH 8) for 24 hours, and stage II dialysis was performed using Aquadest for 24 hours. The dialysate obtained was freeze-dried using a freeze-dryer.[20]

One gram of collagen obtained was dissolved into 200 ml of ultrapure water and immersed in a water bath at 37°C. The 1% collagenase II enzyme (w/w) solution was added and homogenized for 5 hours. The enzymatic reaction was ended by heating the mixture to 95 °C for 10 minutes. The mixture was cooled at room temperature and centrifuged at 3000 rpm for 30 minutes. The resulting supernatant (i.e., collagen peptide) was then freeze-dried for 90 hours and stored at 4 °C.[20]

#### 2.3.2. Amino Acid Content from Mackerel Scad Collagen

The amino acid analysis was done using the High Performance Liquid Chromatography (HPLC) method, according to AOAC (2005). Collagen was dissolved in 6 N HCl and incubated at 110 °C for 24 hours. The solution was centrifuged at 3500 rpm for 15 min, and the supernatant was neutralized using NaOH 1 N. The solution was diluted to a volume of 1:100 with milli-Q water before subjected to HPLC analysis with the Agilent 1100 assembly system after precolumn derivatization with OPA. The sample (1µl) was injected into the *Zorbax* 80 A C18 column at 40 °C at 338 and 262 nm wavelengths. Mobile phase A consisted of 7.35 mM/L sodium acetate/triethylamine (500:0.12, v/v) at pH 7.2, and mobile phase B consisted of 7.35 mM/L sodium acetate/methanol/acetonitrile (1:2:2, v/v/v) at pH 7.2. The amino acid composition of collagen was reported as a percetage of the total protein [22.23].

#### 2.3.3. Antioxidant Activity

A mixture of DPPH solution and samples (native collagen and collagen peptides) was prepared by combining 500 µL of DPPH with 500 µL sample. The mixture was incubated for 30 minutes in the dark at room temperature, after which the absorbance was measured at λ 517 nm using a UV-Vis spectrophotometer. All experiments were performed in triplicate, and the percetage of radical scavenging activity was calculated using the following formula: 100% × (As − Ax)/Ac, where As is the absorbance of the sample with DPPH, Ax is the absorbance of the sample with the DPPH solvent, and Ac is the absorbance of the DPPH solvent with DPPH[20].

#### 2.3.4. Antiglycation Activity

Antiglycation activity is measured by aminoguanidine HCl solution made in PBS (pH 7.4) and diluted to various concentrations. Samples (native collagen and collagen peptides) solutions were made on PBS (pH 7.4) with at concentration ranging from of 25–200 ppm. All test solutions were incubated at 60 °C for 72 h. Following incubation, solutions were added to 30 µL of TCA 100% to halt the reaction. The mixtures were incubated for 10 minutes at 4 °C. Solutions were centrifuged for 13 minutes at 4 °C to separate the pellets. Each pellet was dissolved in 1.2 mL of PBS until homogeneous. The entire solution was measured

for its absorbance at a wavelength of 350 nm[20].

2.3.5. Antityrosinase Activity

The inhibitory activity of the tyrosinase enzyme was evaluated using kojic acid solutions prepared in a phosphate buffer (pH 6.5) from various concentrations. Samples of native collagen and collagen peptides, initially at 1.000 ppm were diluted with phosphate buffer (pH 6.5) to obtain solutions with concentrations of 100, 150, 200, 250, 300, and 350 ppm. Then, 350 µL of each sample was added to 300 µL of tyrosinase solution. The mixture were incubated at room temperature for 5 minutes, followed by reincubation for 30 minutes. Absorbance was measured at 492 nm[20].

2.3.6. Hyaluronidase Inhibition Assay

The hyaluronidase enzyme was prepared by dissolving in acetate buffer pH 3.6, and 50 µl of it was added with 100 µl inhibitor compounds (collagen peptide/ DSCG) at different concentrations. The collagen concentration used was 100, 200, 300, 400, and 500 ppm. Meanwhile, the collagen peptide concentration used was 25, 50, 75, 100, 125, and 150 ppm). Chromoglutic acid served as a control and was used in the same concentration as collagen peptides. The solution was incubated at 37 °C for 20 minutes. Then, 20 µl of 12.5 mM $CaCl_2$ was added to the solution and reincubated at the same temperature and time. After incubation, 10 µl hyaluronic acid (1.2 mg/ml in acetic buffer) was added and incubated at 37 °C for 40 minutes. A positive enzymatic reaction was marked by color change by adding 2 µl NaOH 0.4 M and acetylacetone, then incubated in water at 100 °C for 3-5 minutes. A solution of p-dimethylaminobenzaldehyde was prepared by dissolving 4 g of DMAB in 35 ml of acetic acid and 5 ml of 10 N HCl was prepared. Finally, DMAB 600 µl was added to the mixture and incubated in two stages: 37 °C for 20 minutes and at room temperature for 15 minutes. Production of n-acetyl glucosamine from hyaluronic acid was quantified by measuring absorbance at 585 nm.[24.25] The following formula calculates the percentage of inhibition:

$$\% \text{ Anti-Hyaluronidase} = x\ 100\ \% \frac{(A-B)-C}{(A-B)}$$

Where A represents the absorbance of the negative blank solution containing enzyme, B is the absorbance of the negative blank without enzyme, and C denotes the absorbance of the sample solution with enzyme. The value of A-B is considered the production of n-acetylglucosamine without an inhibitor.

2.4. Data Analysis

The collected data in this study were tabulated, and the average and standard error for each test parameter (mean ± S.D.) were calculated. The concentration values for the anti-hyaluronidase activity parameter were converted into linear regression equation curves. The linear regression equation, represented as y = a + bx, was used to determine the $IC_{50}$ value and the percentage of inhibition.

## 3. Results

3.1. Bioactivity and Amino Acids Content of Mackerel Scad Collagen

The extraction of mackerel scad skin yielded 5.96 ± 0.41% (w/w) collagen with a pH of 7.55 ± 0.07. Tests were also carried out on parameters involved in anti-aging skin, as shown in Table 1. These enzymatic activities were based on spectrophotometric analysis applied to native collagen and its hydrolyzed form.[20] Based on this research, native collagen refers to the intact, full-length collagen protein extracted directly from mackerel scad skin, maintaining its natural triple-helix structure and large molecular size. Native collagen is extracted using the pepsin enzyme. In contrast, collagen peptides is the form of collagen that has undergone enzymatic hydrolysis (collagenase II enzyme), breaking it down into smaller peptides with lower molecular weight. In this research, $IC_{50}$ values were classified as follows: very strong, <50 ppm; strong, 50-100 ppm; moderate, 100-150 ppm; weak, 150-200 ppm; and very weak, 200 ppm. Notably, lower $IC_{50}$ values indicate better activity of anti-aging parameters.[26]

Peptides with lower molecular weight (as shown in Table 1) demonstrate greater bioactivity, although wide-ranging molecular weight distribution also gives marked differences in the properties of the anti-aging properties of the hydrolysates. [27] There were 17 types of amino acids identified from mackerel scad collagen in fairly high amounts. The highest content was shown by glutamate, alanine, glycine, and proline, which are common characteristics of collagen structure

Table 1. The anti-aging parameters of native and collagen peptide derived from mackerel scad skin.[20]

| Parameters | Native Collagen (ppm±SD) | Collagen Peptide (ppm ±SD) |
|---|---|---|
| Antioxidant | 148.55±3.14 | 34.96±0.51 |
| Antiglycation | 239.29±15.67 | 68.43±0.44 |
| Antityrosinase | 234.66±0.185 | 79.35±0.5 |

(Table 2). Other studies[28,29] reported collagen's amino acid content is generally dominated by proline, glycine, alanine, glutamic acid, and hydroxyproline. The abundant levels of these amino acids identified in this study confirm that mackerel scad skin has considerable potential as a source of collagen.

3.2. Inhibition of Hyaluronidase Enzyme Activity of Native Collagen and Collagen Peptides.

The hyaluronidase inhibition test measures the inhibitory capacity of collagen and collagen peptides against hyaluronidase based on the solution's turbidity. Dissolved hyaluronic acid increases turbidity, while its degradation reduces turbidity. The inhibitory activity of hyaluronidase is shown by a high N-acetylglucosamine concentration that remains after the reaction is stopped with DMAB. This enzymatic action is essential in delaying the aging process is caused by the degradation of extracellular matrix components.[32]

Results showed that collagen peptides have better inhibitory activity than collagen, which increase in a dose-dependent manner (Fig. 1). Collagen peptides have lower $IC_{50}$ values (100.78±0.17 ppm) than collagen (326.05±6.77 ppm). A study[33] mentioned that the $IC_{50}$ value for anti-hyaluronidase, ranging from 50-100 ppm, indicates a strong inhibitor. Therefore, collagen peptides extracted from mackerel scad in this study can be considered a potent hyaluronidase inhibitor.

## 4. Discussion

The native collagen obtained was a dry, odorless sponge with grayish-white color, meeting Indonesian national standard SNI 8076 2014 for quality (white, odorless, pH 6.5-8.25).[34] The extraction used acetic acid and pepsin; the acid cleaves peptides with intramolecular crosslinking, and pepsin preserves essential amino acids without damaging the non-helical peptide chain.[35] A previous study[20] identified mackerel scad collagen as type I collagen through FTIR and western blot analysis.

The amino acid content in collagen can be influenced by the extraction method and the amino acid composition in each species. Indeed, the amino acid composition of skin collagen obtained from mackerel scad differs from that of other fish, such as tilapia and yellowfin tuna (Table 2). The amount of glycine from tuna is greater, almost twice that of the amount found in mackerel scad and tilapia (Table 2). The collagen amino acid content of tilapia and yellowfin tuna skin is predominantly glycine. In contrast, the dominant amino acid in mackerel scad is glutamic. Extraction methods using different acid and acetic acid concentrations can also affect the amino acid composition. The acid solution in the yellowfin tuna skin extraction process was higher (0.75 M) than in mackerel scad and yellowfin tuna (0.5 M). Meanwhile, the amino acid

Table 2. Composition of amino acids from skin collagen obtained from mackerel scad (*D. macarellus*) compared to tilapia (*Oreochromis niloticus*)[30] and yellowfin tuna (*Thunnus albacares*)[31]

| Type of Amino Acids | Amino acids | *Decapterus macarellus* (%) | *Oreochromis niloticus* (%) | | *Thunnus albacares* (%) |
|---|---|---|---|---|---|
| | | 0.5 M* | 0.5 M* | 1.5 M* | 0.75 M* |
| Non-essential amino acids | Glutamic acid | 8.20 | 2.77 | 1.38 | 5.05 |
| | Aspartic acid | 6.70 | 1.55 | 0.77 | 2.73 |
| | Serine | 2.51 | 0.92 | 0.47 | 1.53 |
| | Glycine | 5.37 | 5.32 | 2.66 | 10.44 |
| | Arginine | 4.34 | 2.46 | 1.21 | 3.48 |
| | Alanine | 3.19 | 2.79 | 1.37 | 5.03 |
| | Proline | 3.84 | - | - | 5.10 |
| | Tyrosine | 1.57 | 0.18 | 0.08 | 0.21 |
| | Cysteine | 1.51 | - | - | - |
| Essential Amino Acids | Histidine | 1.18 | 0.24 | 0.12 | 0.31 |
| | Threonine | 1.89 | 0.65 | 0.32 | 1.48 |
| | Valine | 3.06 | 0.60 | 0.30 | 0.96 |
| | Methionine | 0.82 | 0.40 | 0.20 | - |
| | Isoleucine | 0.92 | 0.42 | 0.27 | 0.48 |
| | Leucine | 3.19 | 1.09 | 0.76 | 1.13 |
| | Phenilalanine | 1.13 | 0.51 | 0.27 | 0.95 |
| | Lycine | 2.90 | 0.98 | 0.87 | 2.24 |

*Collagen was extracted using various concentration of acetic acid

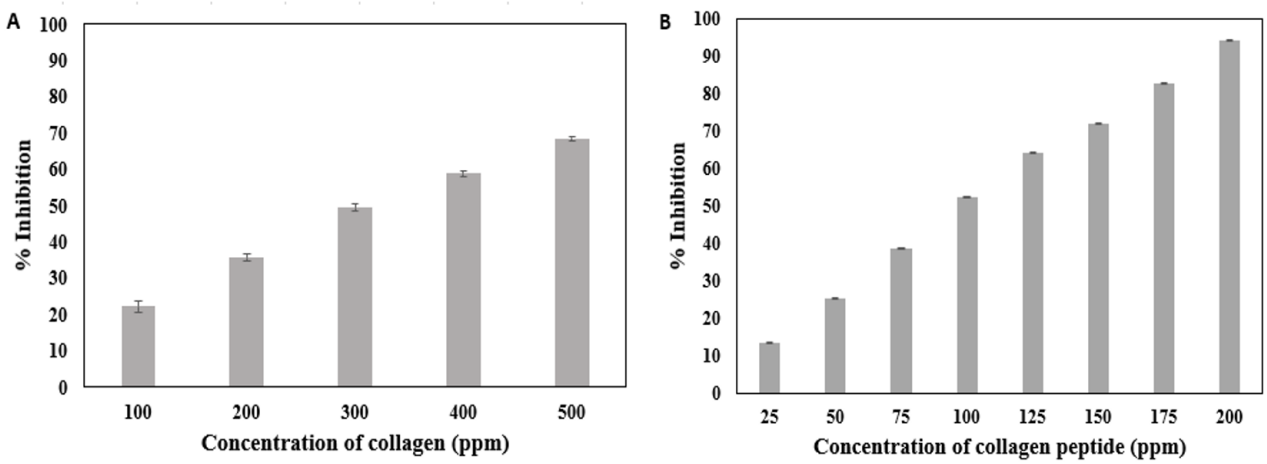


Figure 1. The hyaluronidase inhibition activity of collagen (A) and peptide (B) extracted from mackerel scad skin. Error bars represent SD; n=3.

content of collagen in tilapia with 1.5 M acid treatment has a lower composition than 0.5 M acid treatment. A high-concentration acetic acid solution can trigger the substitution of negative ions in salt with positive ions in acid faster to break the structure of proteins.[30]

Variations in amino acid composition, particularly hydroxyproline, generally cause differences in collagen characteristics, including stiffness, thermal stability, and denaturation temperature. The amount of proline and hydroxyproline in marine collagen is usually lower than in mammalian collagen. Nonetheless, it has a higher amount of serine and threonine residues, particularly in collagen sourced from cold-water fish species.[31] On the other hand, in warm-water fish, the amino acid content of proline and hydroxyproline is high, which can influence the thermal stability of collagen molecules.[36] However, the method employed in this study did not enable the detection of the amino acid hydroxyproline. The difference in amino acid content between marine fish and those in our study can be caused by differences in the fish's living environment.

Even though a strong hyaluronidase inhibition was shown by mackerel collagen peptide, it is still unclear whether it is a competitive or non-competitive inhibitor, as information on the inhibitory activity of hyaluronidase enzymes derived from marine products is still limited. Hyaluronidase inhibitors are derived from sulfated glycosaminoglycans and acids through non-competitive mechanisms.[25] Previous study on the hyaluronidase inhibition mechanism of collagen and collagen peptide extracted from blue swallowfish skin[37] showed that glycosaminoglycans did not bind to the enzyme's active site but interacted with amino groups on its surface, indicating that different types of amino acid compounds may suppress enzyme activity through different mechanisms. Nevertheless, differences in the applied test system may affect the reported data (e.g., incubation conditions, enzymes, and substrate concentrations).

Hyaluronidase inhibitors have been identified in various compounds such as proteins, polysaccharides, and plant-derived compounds, which can be synthesized and play a role in regulating the balance between the synthesis and degradation of hyaluronic fibers.[38] A reduction of HA levels leads to dry and wrinkled skin. Therefore, the inhibition of hyaluronidase is involved in improving skin morphology and delaying skin aging. There are limited studies on protein hydrolysates that specifically investigate hyaluronidase inhibitors. For instance, glycoproteins isolated from mammalian serum and medicinal plants have been shown to inhibit bovine hyaluronidase.[39] Other study[40] showed that collagen peptides extracted from fish scales can stimulate HA synthesis in HaCaT cells. The synthesis was induced through increased expression of the hyaluronate synthase 2 (HAS2) gene derived from the stimulation of CD44 and RHAMM receptors, resulting in a decrease in the hyaluronase 1 gene (HYAL1).

The bioactivity of collagen peptides is considered better than that of native collagen due to its lower molecular weight, which can facilitate the absorption process in the body.[41] Collagen peptides are derived from the denaturation process of the triple helix structure of collagen, resulting in peptides. Collagen peptides containing alanine, glycine, and proline binding to hydroxyproline can induce an increase of HA in the body. In addition, the arginine side chain plays an essential role in stabilizing the interaction of protein bonds with HA via the CD44 receptor-mediated transmembrane.[42,43] Cysteine can inhibit hyaluronidase activity, which is indicated by a decrease in the carbonyl compound synthesis that maintains the composition of thiol groups in the skin. The thiol group plays a role in chelating $Zn^{2+}$ at the active site of the hyaluronidase enzyme. Furthermore, the hydrophobic

bond on the active side of the hyaluronidase enzyme can bind strongly to the hydrophobic amino acid of collagen (i.e., leucine, isoleucine, proline, and phenylalanine).[44] The bond between the amino acid and the active side of the enzyme indicates that the amino acid of mackerel scad collagen can interfere with the enzyme's active site that binds to HA. The spontaneous inhibitory activity of hyaluronidase compounds by collagen and collagen peptides is possible through electrostatic forces, hydrophobic interactions, and hydrogen bonds. The presence of amino acids can alter the microenvironment and conformation of hyaluronidase. The interactions between amino acids affect the local environment of the hyaluronidase activity site, resulting in activity suppression.[45]

Besides the bioactivity, collagen's molecular weight also affects hyaluronidase inhibition. Collagen peptides with a lower molecular weight showed better inhibition than native collagen due to a higher absorption mechanism in the body. The absorption mechanism increases the production of HA and collagen in the body. The body absorbs peptides as free amino acids that provide scaffolding compounds that block collagen and elastin fibers. Collagen peptides, present as oligopeptides, function as ligands that attach to the surface of fibroblasts, then bind to receptors on the fibroblast membrane and promote the synthesis of new collagen, elastin, and HA.[46] Furthermore, the action of low molecular weight collagen peptides may decrease the expression of MMP genes. If gene expression decreases, then the breakdown of dermal collagen can be inhibited, decreasing skin wrinkles, improving hydration, and increasing skin elasticity.[46] Collagen and collagen peptides from mackerel scad containing amino acids can intervene with the hyaluronidase enzyme and maintain the structure of the skin's HA. The difference in molecular weight between the two forms of collagen also affects the inhibitory activity in preventing skin aging.

## 5. Conclusion

The extracted collagen from mackerel scad skin showed a high amino acid content of glutamic acid, aspartic acid, glycine, arginine, and proline. In vitro testing showed this collagen has moderate anti-hyaluronidase activity, with $IC_{50}$ values of 326.05±6.77 ppm. When collagen is hydrolyzed to collagen peptides, hyaluronidase inhibitory activity increases, showing an $IC_{50}$ of 100.78±0.17 ppm. These results indicate that collagen peptides from the skin of mackerel scad fish have the potential to be an inhibitory agent of hyaluronidase enzyme with higher effectiveness than native collagen.

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## Conflict of Interest

The authors declare no conflicts of interest.

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