

Amino Acid and Proximate Analysis of Type-1 Collagen from Sea Cucumber and Tilapia-Skin and its Potential Application as Artificial Tendon

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ABSTRACT

Tendon injury is a medical indication that requires surgical therapy and prolonged recovery. Collagen plays a crucial role in the wound healing process. Collagen maintains homeostasis, interacts with platelets, and enhances cellular components. Most living organisms consist of collagen components as fibrillar proteins. In this study, the potential properties of type-1 collagen from sea cucumber and Tilapia-skin were evaluated depend on proximate analysis, amino acid composition, and moisture-absorption ability. The result showed that Tilapia-skin has a higher protein and fat composition compared to the sea cucumber. In contrast, moisture, ash, and yield composition showed higher for sea cucumber compared to Tilapia-skin. Meanwhile, based on amino acid analysis was revealed that proline, alanine, methionine, isoleucine, leucine, phenylalanine, and lysine were lower on sea cucumber compared to Tilapia-skin. In conclusion, both type-1 collagen material from sea cucumber and Tilapia-skin might be developed as a biomaterial for tendon injury. It is crucial that further studies be carried out to emphasize the potential of biodegradable and bio-replaceable materials using the *in vivo* model.

Key words: Artificial tendon, Marine biodiversity, Collagen, Sea cucumber, Tilapia-skin.

INTRODUCTION

Tendon rupture can occur acutely or chronically, either due to intrinsic or extrinsic factors. For trauma cases, extrinsic factors are more dominant. Trauma or injury can cause considerable morbidity for horses.¹ This case takes several months to recover to normal function. Tissue engineering has proven that scaffold-based cell construction can improve the tendon healing process.² Previous studies have shown better histological figures and biomechanical function in tendon defects filled with scaffold and powder coated mesenchymal stem cells taken from bone marrow compared to scaffolds without mesenchymal stem cells.³

Tendons have several variations and shapes and consist of both intracellular and extracellular matrix components. The tendon structure consists of collagen fibrils, collagen fibers, strands of tendon component fibers, tendon cells, and epitendon.⁴ Previous studies investigated tendon segments being replaced with tendon constructions that had undergone tissue engineering. In clinical applications, tendon ruptures or lacerations requiring primary repair are much more common than tendon injuries with defects requiring tendon graft.⁵

Collagen derived from organic material can be obtained from sea cucumber and Tilapia-skin. Sea cucumbers contain 70% collagen protein.⁶ Sea cucumbers are an important component in marine ecosystems scattered in the oceans throughout the world. The number of sea cucumber species currently available is about 125 species.⁷ Collagen

extraction methods are generally based on the solubility of collagen in neutral salt solutions, acids or with the addition of enzymes.⁸ Significant improvement in tendon healing has been achieved through advances in surgical technique and advances in rehabilitation, but there are still problems with rupture of the sutured tendons in addition to adhesion problems. This study aimed to determine the character of type-1 collagen in sea cucumber and Tilapia-skin based on proximate analysis, amino acid composition, and moisture-absorption ability.

MATERIALS AND METHODS

Ethical approval

Ethical approval was done by Animal Ethics and Use Committee Universitas Airlangga No 367/HRECC. FODM/VII/2021. However, this study did not involve animals extensively and required activities common to sea cucumbers and Tilapia during the study.

Extraction of sea cucumber and Tilapia-skin collagen

The dried sea cucumbers were washed with distilled water, dissected, and soaked in a solution of 0.5 N NaCl, 0.2 N -mercaptoethanol, 50 mM EDTA, and 0.1 N Tris-HCl. Next, the mixture was centrifuged at 8000 rpm and added with 0, 1 N NaOH. The sediment was centrifuged again and washed with distilled water. The supernatant obtained, added pepsin (1 g/100 g collagen) with 0.5 N acetic acid, centrifuged, and added NaCl to 0.6 M. By centrifugation, the sediment was redissolved in 0.5

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M acetic acid, dialyzed with 0.02 M Na₂HPO₄, and centrifuged to dissolve the precipitate in 0.5 M acetic acid solution. Finally, it was dialyzed with 0.1 M acetic acid solution, and freeze-dried to preserve collagen.

Interpretation

The collagen composition was then analyzed proximately using the AOAC method⁹ and the amino acid composition was analyzed using an amino acid autoanalyzer (L-8900, Hitachi).¹⁰ Meanwhile, the moisture-absorption ability was evaluated using the Huang method.¹¹

Statistical analysis

All data were expressed as mean ± standard deviation and analyzed using T-test independent sample ($p < 0.05$).

RESULTS AND DISCUSSION

Based on the proximate analysis, the results showed higher percentages of dry matter, ash, yield, and moisture in sea cucumbers compared to Tilapia-skin. In contrast, the percentage of protein and fat was higher in Tilapia-skin (Table 1). Meanwhile, evaluation of amino acid levels showed that Tilapia-skin collagen was higher than sea cucumbers for the following amino acids: alanine, proline, methionine, isoleucine, leucine, phenylalanine, and lysine (Table 2). This study revealed the potential of type-1 collagen obtained from sea cucumbers and Tilapia-skin.

Tilapia-skin has a fairly good nutritional content and is a high source of protein. However, from a nutritional point of view, sea cucumbers are considered a valuable food product, because they contain important nutrients. The organic and inorganic composition of fresh sea cucumbers varies depending on the species, season, habitat, and possibly on the stage of ontogenesis. The ontogenesis stage is the stage of organism development from the fertilized egg to its adult form.¹² Previous studies stated that the protein content in sea cucumbers can reach 40%,¹³ from several species of sea cucumbers analyzed the protein content is quite high, ranging from 31.11% to 42.32%.¹⁴ The high protein content confirms that sea cucumbers and Tilapia-skin have considerable potential as a source of collagen. Collagen is part of the protein that has many benefits. The amount of type-1 collagen content in sea cucumbers and Tilapia-skin can vary depending on the type.¹⁵

In contrast to previous studies, compared with other collagen sources such as catfish-skin, snakehead fish-skin, Tilapia-skin and from other mammalian skins, the percentage of collagen yield from sea cucumbers is slightly high.¹⁶ The source of collagen was found to be higher in some types of sea cucumbers. The collagen content in *H. cinerascens* was reported 72.2% compared to Tilapia-skin collagen of 67.33%. Optimal extraction has been shown to significantly affect the total yield of collagen produced.¹⁷

Table 1: Proximate analysis of sea cucumber and Tilapia-skin collagen.

Properties	Sea cucumber	Tilapia-skin	p-value
Dry matter (%)	95,1 ± 0,24	92,4 ± 0,06	0,000***
Ash (%)	0,9 ± 0,02	0,5 ± 0,03	0,000***
Protein (%)	10,3 ± 0,06	16,0 ± 0,26	0,000***
Fat (%)	0,3 ± 0,02	3,8 ± 0,10	0,000***
Calcium (%)	3,0 ± 0,12	2,9 ± 0,06	0,251
Yield (%)	72,3 ± 0,21	67,5 ± 0,26	0,000***
Moisture (%)	10,4 ± 0,42	8,9 ± 0,10	0,003**

Values are expressed in mean ± standard deviation. T independent test was carried out with a 95% significant threshold. Values are represented statistically when * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Table 2: Amino acid evaluation in sea cucumber and Tilapia-skin collagen.

Amino acid	Sea cucumber	Tilapia-skin	p-value
Aspartic	47,5 ± 0,06	30,0 ± 0,26	0,000***
Glutamic	85,4 ± 0,46	57,1 ± 0,25	0,000***
Serine	28,2 ± 0,60	28,9 ± 0,10	0,132
Threonine	31,4 ± 0,62	20,5 ± 0,82	0,000***
Arginine	49,3 ± 0,57	45,3 ± 0,55	0,001**
Glycine	31,3 ± 0,46	31,1 ± 0,20	0,527
Alanine	11,2 ± 0,10	11,5 ± 0,10	0,021*
Proline	10,6 ± 0,25	12,5 ± 0,40	0,003**
Tyrosine	5,1 ± 0,15	2,6 ± 0,26	0,000***
Valine	21,6 ± 0,57	17,3 ± 0,67	0,001**
Methionine	6,1 ± 0,25	9,7 ± 0,26	0,000***
Cysteine	0,6 ± 0,10	0,6 ± 0,06	0,643
Isoleucine	7,2 ± 0,25	8,6 ± 0,23	0,002**
Leucine	17,1 ± 0,30	19,1 ± 0,21	0,001**
Phenylalanine	10,4 ± 0,10	15,1 ± 0,44	0,000***
Lysine	8,3 ± 0,36	41,9 ± 0,35	0,000***

Values are expressed in mean ± standard deviation. T independent test was carried out with a 95% significant threshold. Values are represented statistically when * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Amino acid content varies depending on species, environment, and temperature.¹⁸ The most abundant amino acids in sea cucumbers and Tilapia-skin are serine and glycine. All types of collagens contain glycine as the main amino acid as well as other properties such as alanine, proline, methionine, isoleucine, leucine, phenylalanine, and lysine. The relatively high characteristic content of amino acids, such as glycine, hydroxyproline, and hydroxylysine, indicates the presence of collagen as the main protein component. Amino acids in sea cucumbers and Tilapia-skin which were shown to be similar were serine, glycine and cysteine. These results indicate that sea cucumber and Tilapia-skin collagen has high hydrophobic amino acids, so it can reduce the activity of the tyrosinase enzyme.¹⁹

CONCLUSIONS

In conclusion, sea cucumbers reported a higher percentage of dry matter, ash, yield, and water content compared to Tilapia-skin. In contrast, fish skin values showed higher protein and fat percentages. Based on the determination of amino acid levels, it was revealed that Tilapia-skin collagen was higher than sea cucumbers for the following amino acids: alanine, proline, methionine, isoleucine, leucine, phenylalanine, and lysine. Further studies can be carried out to synthesize type-1 collagen as a candidate for artificial tendons.

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DECLARATION OF INTEREST

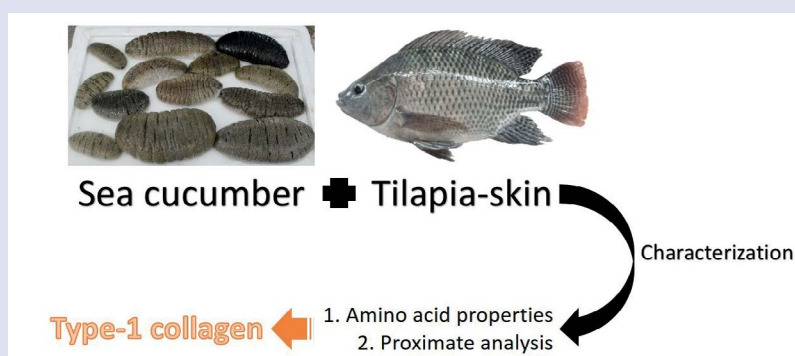
The authors declare no conflicts of interest.

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GRAPHICAL ABSTRACT



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