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Extraction and Characterization of Collagen from Payangka Fish Skin (*Ophieleotris aporos)*

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A B S T R A C T

Payangka fish (Ophieleotris aporos) is one of the most widely consumed fish by communities around Tondano, North Sulawesi, and can be used as a safe alternative source of collagen.
This study aims to extract and characterize collagen from payangka fish skin. The extraction was carried out in three stages, namely pretreatment with 0.05 M NaOH and 0.15 M acetic acid, and extraction with distilled water at 40 ^{0}C for 1, 2, 3, and 4 hours.
The highest yield was obtained at 4 hours of extraction, which was 0.0564%. FTIR analysis obtained amide A, amide B, amide I, amide II, and amide III functional groups, which are characteristic of collagen, and an unchanged triple helix structure, indicating type I collagen.

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Introduction

Payangka fish (Ophieleotris aporos) is one of the fish species found in Lake Tondanon, but it is only used as a daily food source. Collagen is the most abundant structural protein in animals, accounting for 30% of total protein. Traditionally, collagen can be obtained from mammalian products, but collagen sourced from mammals carries the risk of transmitting diseases and religious restrictions, so its use has decreased.

Currently, fish products are being developed as an alternative for collagen production because they are considered to pose no risk of disease transmission and have no religious restrictions. Fish skin has a high purity level (approximately 70%), and collagen from fish skin also demonstrates excellent water-holding capacity (approximately 6%)
and shows no potential for irritation, making it suitable for various applications[1].

Materials and Methods

The main material used in this study was payangka fish skin obtained from the Tondano market, South Tondano District, North Sulawesi. The materials used for the extraction process were NaOH p.a, CH_3COOH p.a KBr (Merck), biuret reagent, sulfuric acid (Merck), H_3BO_3 (Merck), HCl p.a, and distilled water. Collagen characterization was performed using Fourier Transform Infrared (FTIR) spectroscopy.

This study was conducted in four stages, namely sample preparation [2], preliminary analysis of raw materials [3], extraction [4] and characterization [5].

The payangka fish skin was first separated from the scales, meat, and bones, then cut into pieces measuring approximately 0.5 cm x 0.5 cm. It was then washed thoroughly in running water. After cleaning, the fish skin was stored in a freezer until it was ready to be used. The initial

analysis of raw materials included protein, moisture, and ash content analysis.

Before extraction, the samples were pretreated. The pretreatment stage was carried out using a 0.05 M NaOH solution for 2 hours with periodic stirring. The samples were washed with distilled water until they reached a neutral pH. The samples that had been pretreated were then treated with 0.15 M acetic acid for 3 hours, after which the fish skin was washed until it reached a neutral pH. In the next stage, the samples were extracted with a heating bath using distilled water at a temperature of 40^0C with immersion variations of 1, 2, 3, and 4 hours. The extraction results were water-soluble collagen, which was then oven dried at a temperature of 40^0C to obtain dry collagen. The hydroextraction process is carried out at a temperature of 40°C to prevent degradation into gelatin, as collagen converts to gelatin at temperatures above 45°C.

Collagen characterization was performed using FTIR instruments to observe the functional groups characteristic of collagen.

Results and Discussion

Tabel 1 Results of the analysis of the initial raw material of fish skin payangka

Analysis	**Value (%)[1] Result research**	**Value (%)[2] References**	**Value (%)[3] References**
Protein	25,44	30,29	18,96
Moisture	63,98	65,59	39,24
Ash	0,17	0,19	0,72

Note: Personal data (payangka fish skin)[1]; [6][2]; [8][3].

Protein content is an important parameter for determining the initial quality of raw materials. The presence of protein in payangka fish skin indicates better raw material quality and can be used as a source of collagen. The protein content obtained is slightly lower than that of patin fish skin [6], but higher than that of yellowfin tuna skin [7] and P. sutchi [8].

One important parameter in determining the quality of raw materials to be used is moisture content, which is related to the shelf life and freshness of the raw materials. High moisture content can affect metabolic activities such as enzyme activity, microbial activity, and chemical activity, which can affect nutrient conten [9]. The moisture content obtained from payangka fish skin was higher than that of P. sutchi fish, which was 39.24% [8] and yellowfin tuna skin, which was 57.42% [7], but this result was lower than the moisture content of catfish skin, which was 65.59% [6]. The moisture content obtained was still within the range of fish skin moisture content in general.

The ash content describes the cleanliness and purity of a material to be used [10]. The ash content of payangka fish skin obtained was 0.17%, which was almost the same as that of patin fish skin at 0.19% [6] and P. hypopohtalmus at 0.23%, but lower than that of Pangasius sutchi at 0.72% [8]. An ash content of less than 0.5% is considered a good quality raw material that can be used for collagen production.

Pretreatment of Payangka Fish Skin aims to remove impurities adhering to the fish skin. Soaking in a NaOH solution causes chemical changes, where glycine and NaOH react. The use of NaOH in the pretreatment process was chosen because it can cause swelling on skin payangka compared to other alkaline solution [10].

Pretreatment at 0,05 M is stated to be able to dissolve impurities removing collagen, which is in line with [11] which revealed that NaOH used at concentrations above 0.1 M can significantly dissolve collagen, while the use of NaOH at concentrations below 0.1 M can remove impurities without causing collagen loss in fish skin during the pretreatment process.

Payangka Fish Skin, Soaking using acid causes swelling of the fish skin, which is due to the entry of water into the collagen fibers. Water entering the collagen fibers is caused by electrostatic forces between the polar groups in the collagen fibers and H+ from the acid. Swelling of the fish skin is very important to facilitate extraction and increase collagen solubility.

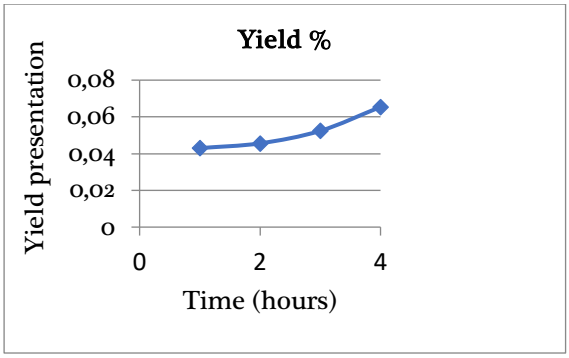


Figure 1 Collagen extraction results continue to increase as extraction time increases

Collagen extraction, hydroextraction during the isolation process utilize water a catalyst (heat transfer medium) and temperature as pressure (high temperature short time/HTST) so that it can dissolve collagen. Fish skin soaked in warm water causes damage to hydrogen and covalent bonds that initially occurred during the soaking process with acetic acid.

Based on the yield graph, collagen extraction results continue to increase as extraction time increases. Characterization of payangka fish skin collagen. Based on the FTIR spectrum, typical collagen absorption peaks are observed. The absorption peak of payangka collagen was detected at a wavelength of 3272 cm^{-1} which indicates NH stretching vibration. The absorption region for amide A is in the range of 3400-3440 cm-1 [12]. When the N-H group at peptide is affected by bonds Hydrogen will shift to a lower frequency [6], in studies [13], [14] comparing acid-soluble collagen and pepsin, the amide A absorption peaks were obtained at wavelengths of 3275 cm-1, 3286,67 cm-1, 3297,85 cm-1 respectively.

The FTIR spectrum of payangka fish skin collagen also shows an absorption peak at a wavelength of 2924 cm-1, indicating amide B absorption, where the absorption region is generally at a wavelength of 2924-2932 cm-1 indicating the presence of an asymmetric CH_2 stretching functional group [15]. 2924 cm^{-1} The characterization of collagen from Sandrinella longiceps obtained the amide B region at a wavelength of [16].

The amide I wave number detected in the collagen of payangka fish skin was 1634 cm-1, a result that was not significantly different from that obtained in study [12], which was1633,22 cm-1. The absorption region is generally located at 1600-1700 cm-1 [17]. Amide I indicates carbonyl group (C=O) stretching vibrations [18] which are characteristic of secondary structures in peptides. Amide I has four secondary protein structure components, namely α-helix, β-sheet, β-turn, and random coil, each with different absorption regions. The α-helix component is indicated in the absorption region of 1654-1658 cm-1, β-sheet in the range of 1624-1642 cm-1, β-turn in 1666-1672 cm-1, and random coil in 1648±2 [19].

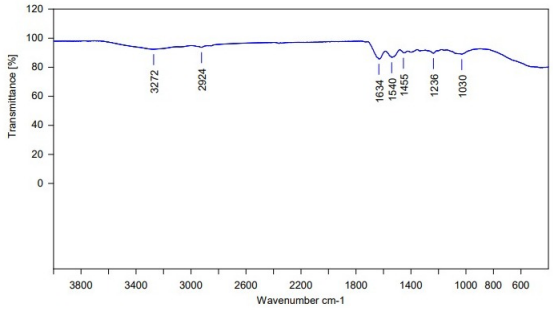


Figure 2. FTIR spectrum, typical collagen absorption

Tabel 2 Functional Group Characterization of Collagen

Amide	Wavenumber (cm^{-1})	Absorption Range (cm^{-1})	Assignment
Amide A	3272	3400-3440	*sreaching* NH
Amide B	2924	2915-1935	asimetri CH_2
Amide I	1634	1600-1658	*sreaching* C=O
Amide II	1540	1550-1600	*bending NH sreaching CN*
Amide III	1236	1229-1301	*bending NH sreaching* CH

Based on the results of the amide I wavenumber in fish skin collagen, it was found that the collagen produced has βsheet β-sheet structure, indicating that the compound

produced is at process extraction with distilled water at a temperature of 40^0C is collagen and has not degraded into gelatin.

The amide II band is generally observed in the range of 1550-1600 cm-1, indicating the presence of C-N stretching and N-H bending groups [18]. The FTIR spectrum of payangka fish skin collagen is 1540 cm-1. The shift of the amide II band to a lower frequency is caused by the large number of H bonds, which indicate collagen activity and a triple helix structure [20]. The results obtained are not much different from those for collagen from patin and parrotfish skin, which are 15536.66 cm^{-1} and 1541,29 cm^{-1} [13], [21]. Amide III from payangka fish skin is 1236 cm-1, indicating the presence of C-H stretching and N-H bending. The presence of amide III in payangka fish skin confirms the existence of a collagen triple helix structure [22].

The closer the intensity ratio between the amide III wave number and the wave number around 1451-1455 cm-1 to one, the more intact the triple helix structure of collagen obtained. In the FTIR analysis of collagen from payangka fish skin, the intensity ratio obtained was 1, which means that the triple helix structure of the collagen obtained did not change during the extraction process. The same was also obtained in the absorbance of yellow tuna, seer fish, and Asian sea bass, which were 0.98, 1.01, and 0.98, respectively. The unchanged triple helix structure determines the function of type I collagen [18].

Author Contribution

Veronika Salassa contributed to the conceptualization of the study, implementation of the study, data collection, data processing and curation, formal analysis, interpretation of the results, and preparation of the initial draft of the manuscript. Sofia Krisen contributed to the conceptualization and design of the research methodology, research supervision. Anderson Aloanis contributed to the supervision, evaluation of the research results, critical review, editing, and refinement of the substance of the manuscript before publication.

Conclusion

Based on the research results, it can be concluded that 1. The longer the extraction process, the higher the yield obtained. The yield of payangka fish skin produced at 4 hours was higher than that at 1.2 and 1 hour. 2. Characterization of payangka fish skin collagen using FTIR shows the II, presence of amide A, amide B, amide I, amide and amide III groups, which are characteristic of collagen. 3. The amide I, II, and III bands, which maintain the triple helix structure, indicate type I collagen.

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