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Isolation barracuda fish skin *Sphyraena jello* as a halal source of collagen

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ABSTRACT

Collagen is a large protein that makes up 30% of the total protein that makes up the human body. Fish skin is used mostly as a halal source of collagen, and various markets accept that. This study's main objective was to isolate collagen and see the amount of collagen obtained from barracuda fish's skin. The Barracuda's skin was extracted by immersion using a 1.5% acetic acid solution to obtain the filtrate, frozen in the freezer, and dried by freeze dryer until dry collagen is obtained. The results showed that the collagen could be isolated from Barracuda with a proportion value of 6.45 %%.

Key words : *Fish skin, Barracuda fish, Sphyraena jello, Collagen, FTIR*

Introduction

Collagen is one of the protein groups that make up 30% of the total protein that makes up the human body. Naturally, the collagen in the human body is reduced by 1% every year. Collagen decline begins to occur at the age of 25 years and over, and at the age of 30, people lose collagen around 15-20%, and aged 40 years, collagen loss can reach 35-40%. Collagens possess complex hierarchical structures and are present in various forms such as collagen fibrils (1.5–3.5 nm wide), collagen fibers (50–70 nm wide), and collagen bundles (150–250 nm wide), with distinct properties characteristic of each tissue providing elasticity to skin (Warsito and Kusumawati, 2019), softness of the cartilage (Sasamoto *et al.,* 2016), stiffness of the bone and tendon (Setiawati *et al.,* 2017), transparency of the cornea (Widiyanti and Prastyani, 2019), opaqueness of the sclera, etc. (Balasubramanian *et al.,* 2013).

Commercial collagen is extracted from cow skin and pork skin mostly. It is feared that the collagen made from pork skin contains swine flu and is generally not under certain religious beliefs. In contrast, the collagen made from cow skin is feared to be contaminated with Bovine Spongiform Encephalopathy (BSE) or mad cow disease (mad cow disease). For these various reasons, other alternatives are sought as halal collagen sources and can be accepted by various market consumers. One of the potential ones that can be developed is fish skin (Prahasanti *et al.,* 2018; Krimariono *et al.,* 2020).

The utilization of fish skin used as a collagen raw material is an alternative to overcome this problem. Fish skin and bones are a source of collagen that all consumers can accept in the market. Several studies have conducted collagen studies extracted from fish skin (Ahmad and Benjakul, 2010;

Kittiphattanabawon *et al.,* 2010; Singh *et al.,* 2011). One suitable type of fish is Barracuda,

Most of the use of Barracuda fish is only in the fish meat, while other uses such as skin, bones, head, and fins have not been used optimally. Based on the above background, it is necessary to manage fish skin waste to research collagen isolation from barracuda skin.

Materials and Methods

The research material is using fresh skin from Barracuda fish (*Sphyraena jello*) that obtained from Maccini Baji Fish Auction Place, Pundata Baji Village, Labakkang District, Pangkep Regency, Powder NaOH, acetic acid (CH_3COOH), butyl alcohol, Ethylene Diamine Tetra Acetic Acid (EDTA), distilled water, and other ingredients for analysis of collagen characteristics.

The equipment used for collagen extraction includes stirring hot plate with the Ika C-Mag brand, Hitachi U-2800 UV-VIS spectrophotometer, Haichuan LDGZ-15 freeze dryer, Heidolph WB2000 rotary evaporator, sartorius analytical balance, gauze, brand glassware. Iwaki Pyrex and thermometer. The tools used for the analysis of collagen characteristics include the Bruker Tensor 37 Fourier Transform Infrared Spectrophotometer.

Data collection and data analysis

This research was conducted in two stages, collagen preparation and extraction and physical characterization of collagen. The physical characteristics measured include the yield value and functional groups using FTIR (Fourier Transform Infrared).

Collagen Extraction and Isolation

The process of extraction and isolation of collagen from Barracuda fish skin samples using a modified method (Baehaki *et al.,* 2016; Nagai and Suzuki, 2000) isolation with acid, with the following steps:

1. Preparation of the primary raw material. The skin of the Barracuda is weeded clean and separated from any remaining flesh attached to the skin. The Barracuda fish skin sample was cut into 2 x 2 cm^2 sizes. Barracuda fish skin samples then weighed as much as 300 g.
2. The sample was then removed from the non-collagen protein and fat liberation (degreasing) by immersing the cut sample in 0.1 M NaOH solution. 1800 mL of NaOH solvent was used. This soaking is carried out for three days (the NaOH solvent is replaced every day with a new one. The fish skin is washed with distilled water until a neutral pH.
3. The sample was removed from minerals and fatty metal fish skin by immersing the sample in EDTA at 0.5 M pH 7.4 with a 1: 10 (w / v) ratio for 24 hours, then washed with cold distilled water pH was neutral or reached pH 7.
4. The sample was then removed fat by immersing the fish skin in 10% butyl alcohol with a 1: 10 (w / v) ratio for one day, then washed with cold distilled water to a neutral pH.
5. The collagen extraction process was carried out with a 1.5% CH3COOH solution at a concentration of 1.5% in a ratio of 1: 2 (w / v) for 24 hours. The solvent used is 1800 ml.
6. Fish skin is washed with cold distilled water to neutral pH before proceeding to this stage. The extraction with aquadest with the ratio between fish skin and distilled water is 2: 1 (w / v) for 3 hours at a temperature of 40-50oC to obtain liquid collagen.
7. The extraction results are in the form of liquid collagen, then lyophilized (spray drying) using a freeze dryer to obtain collagen in the form of cotton or powder.

Results

FTIR Test

The FTIR spectrum of Barracuda fish skin collagen shows absorption peaks in the amide absorption region, including amide A, amide B, Amide I, Amide II, and Amide III. The secondary structure of barracuda fish skin collagen in Amide A is found at wave number 3352.28 cm^{-1}, amide B at wave number 2929.87 cm^{-1}, Amide I at wave number 1660.71 cm^{-1}, Amide II at wave number 1546.91 cm^{-1}, and Amide III at wave number 1240.23 cm^{-1} (Table 1). Characteristics of Barracuda Fish Skin Collagen Functional According to FTIR Analysis Results can be seen in Figure 1.

Discussion

The peak of Amide A absorption at wave number 3352.28 cm^{-1} shows the NH stretching vibration (Muyonga *et al.,* 2004), the average wave number of Amide A is 3300 cm^{-1} - 3500 cm^{-1}. The FTIR spec-

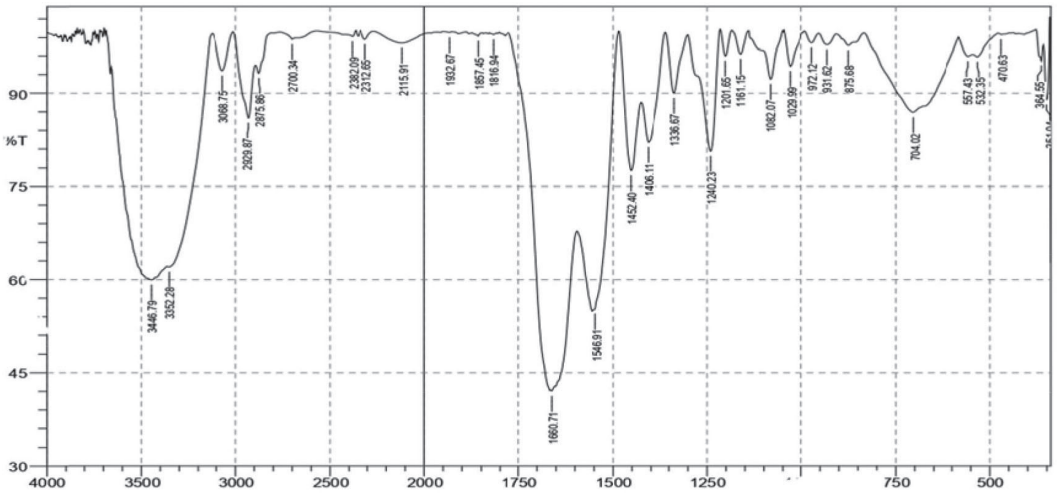

Fig. 1. Infrared Spectra from barracuda fish skin (*Sphyraena jello*)

Table 1. The FTIR spectrum of barracuda fish skin collagen shows absorption peaks in the amide absorption region

No.	Amida	Wavenumber (cm^{-1})	Absorband Area (cm^{-1})	Information	Reference
1	Amida A	3352.28	3300-3500	Vibrate *stretching* NH	(Muyonga *et al.*, 2004)
2	Amida B	2929.87	2915-2935	Asymmetrical *stretching* CH_2	(Coates, 2006)
3	Amida I	1660.71	1600-1690	Vibrate *stretching* C=O	(Kong and Yu, 2007)
4	Amida II	1546.91	1480-1575	CH *stretching*, NH bending	(Kong and Yu, 2007)
5	Amida III	1240.23	1229-1301	CH *stretching*, NH *bending*	(Kong and Yu, 2007)

trum of collagen in Wave number 2933.24 cm^{-1} which indicates a specific collagen group, namely Amide B. The amide group B with an absorption area wave number 2915 cm^{-1} - 2935 cm^{-1} (Coates, 2006). The wavenumber indicating amide B absorption is formed from asymmetrical stretching of CH_2 (Kong and Yu, 2007). The wave number indicating Amide A absorption is formed from amino acid bonds in collagen associated with the widening of vibrations in the NH group, which is in collagen (Nagai and Suzuki, 2000).

The Amide I wave number detected at 1660.71 cm-1 indicates a stretching vibration of the C = O group. Amide I is a distinctive functional group that makes up Himantura gerrardi collagen. Amide I was detected in the wavenumber range of 1600 cm-1 - 1690 bending cm^{-1}. Amide I is associated with the stretching vibration of the carbonyl group. Meanwhile, Amide II, a typical collagen functional group, was detected at wave number 1546.91 cm^{-1}. The amide II absorption region is related to CN stretching and NH bending groups in the range of 1480 cm-1 -1575 cm^{-1}. Amide III has an absorption area of 1229 cm^{-1} - 1301 cm^{-1} (Kong and Yu, 2007).

The collagen absorption region at wave number 1240.23 cm-1 indicates amide functional group III, which shows CH stretching and NH. Amide III's intensity is related to the triple helix structure (Muyonga *et al.*, 2004). It means that the collagen extraction of barracuda fish skin in the water at a temperature of 40 °C has not been degraded to form gelatin which is characterized by a triple helix structure and produces collagen. Collagen's denaturation due to the heating process causes the collagen triple helix chain to transform into a single á-helix (gelatin) chain completely (Gómez-Guillén *et al.*, 2011).

Conclusion

Based on the research on the isolation of Barracuda fish skin collagen (*Sphyraena jello*), it can be concluded that the Barracuda fish skin contains collagen. It's based on the results of FTIR analysis which shows the presence of amide A, amide B, Amide I, Amide II and Amide III groups, the triple

helical structure amide I and Amide III indicate that the resulting compound is collagen.

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