

## MOLECULAR STRUCTURE OF GELATIN EXTRACTED FROM PARROT (*Scarus sp*) FISH SCALES

Jeszy Novianti Andakke<sup>1</sup>, Hizkia H.Y. Nainggolan<sup>1</sup>, Lasma R.M.E. Parapat<sup>1</sup>, Inneke F.M.Rumengan<sup>1</sup>, Engel Pandey<sup>2</sup>, Pipih Suptijah<sup>3</sup>, Aldian H.Luntungan<sup>4</sup>

1 Marine Science Study Program, Faculty of Fisheries and Marine Science, Sam Ratulangi University Manado

2 Fish Processing Technology Study Program, Faculty of Fisheries and Marine Science, Faculty of Fisheries and Marine Science, Sam Ratulangi University Manado

3 Fish Processing Technology Study Program, Faculty of Fisheries and Marine Science, Bogor Agricultural University

4 Post Graduated of Sam Ratulangi University Manado

\*corresponding author: [innekerumengan@unsrat.ac.id](mailto:innekerumengan@unsrat.ac.id)

### Abstract

One of the protein molecules of fish scales is water soluble gelatin. Gelatin of fish scales could be best substitute of commercial available gelatin which derived from porcine and bovine. The purpose of this study was to determine the molecular structure of gelatin extracted from marine fish scale using Fourier transform infrared (FTIR) analysis, and to obtain the moisture content, pH and yield of gelatin. Samples were prepared from the wet and dried scales. As for the standard gelatin, the gelatin of the two samples are characterized with several types of amide groups. The two gelatin samples were slight different in absorption of wave length for amide A, B, I, II and III groups indicating the instability of the functional groups which may influence viscosity and gel strength. For the wet scales derived gelatin, the wave number absorption was found to be 3412  $\text{cm}^{-1}$  (amide A), 2421  $\text{cm}^{-1}$  (amide B), 1653  $\text{cm}^{-1}$  (amide I), 1400  $\text{cm}^{-1}$  (amide II), and 1001  $\text{cm}^{-1}$  (amide III), while for the dried scales derived gelatin was 3435  $\text{cm}^{-1}$  (amide A), 2920  $\text{cm}^{-1}$  (amide B), 1635  $\text{cm}^{-1}$  (amide I), 1404  $\text{cm}^{-1}$  (amide II), and 1036  $\text{cm}^{-1}$  (amide III). The wave number absorption of amide III of gelatin is smaller than the one of collagen, because gelatin is in form of single helix, not triple helix. The wet scales derived and dried scales derived gelatin show the moisture content of 15.0% and 13.7%, and yield of 2.33% and 2.43%, respectively. For both samples, the pH value was 7.

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*Key words : gelatin, fish scales, molecule structure, moisture, yield, pH*

### Abstrak

Salah satu dari molekul protein sisik ikan adalah gelatin larut air. Gelatin sisik ikan dapat menjadi pengganti terbaik dari gelatin komersial yang tersedia yang berasal dari babi dan sapi. Tujuan dari penelitian ini adalah untuk menentukan struktur molekul gelatin yang diekstrak dari sisik ikan laut menggunakan Analisis FTIR (Fourier Transform Infrared), dan untuk mendapatkan kadar air, pH dan rendemen gelatin. Sampel disiapkan dari sisik basah dan sisik kering. Adapun standar gelatin, gelatin dari kedua sampel ditandai dengan beberapa jenis gugus amida. Kedua sampel gelatin sedikit berbeda dalam penyerapan panjang gelombang untuk amida A, B, I, II dan III yang menunjukkan ketidakstabilan kelompok fungsional yang dapat mempengaruhi viskositas dan kekuatan gel. Untuk gelatin sisik basah, panjang gelombang serapan ditemukan pada 3412  $\text{cm}^{-1}$  (amida A), 2421  $\text{cm}^{-1}$  (amida B), 1653  $\text{cm}^{-1}$  (amida I), 1400  $\text{cm}^{-1}$  (amida II), and 1001  $\text{cm}^{-1}$  (amida III), sedangkan untuk gelatin sisik kering adalah 3435  $\text{cm}^{-1}$  (amida A), 2920  $\text{cm}^{-1}$  (amida B), 1635  $\text{cm}^{-1}$  (amida I), 1404  $\text{cm}^{-1}$  (amida II), and 1036  $\text{cm}^{-1}$  (amida III). Panjang gelombang serapan amida III pada gelatin lebih kecil dibanding kolagen, sehingga gelatin berbentuk single helix, bukan triple helix. Gelatin sisik basah dan sisik kering mengandung kadar air 15,0% dan 13,7%, rendemen 2,33% and 2,43%, secara berturut-turut. Untuk kedua sampel memiliki nilai pH 7.

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*Key words : gelatin, sisik ikan, struktur molekul, kadar air, rendemen, pH*

## Introduction

Gelatin is commercially used for food industry as a stabilizer, thickener, emulsifier, jelly formed, water binder, and settling down, while for pharmaceuticals industry it can be used as major material in capsule preparation (Damanik, 2005). In addition, gelatin could be prepared as a biodegradable matrix material in implantable delivery systems and microencapsulation of drugs (Rowe et al., 2009). Gelatin is chemically obtained by partial denaturation of the fibrous protein of collagen by heating in acid or alkaline pH (Haug and Draget, 2011; Lassoued et al, 2014).

Product of gelatin from pigskin is the highest (44%), followed by bovine hides (28%), bovine bones (27%) and other sources (1%) (Ahmad and Benjakul, 2011). However, the use of gelatin from pig and bovine sources have been problematic due to religious concerns (Nagarajan et al., 2012). A new source of gelatin can be obtained from marine fish scales. Fish scales are abundantly available as wastes of fish processing, especially in Manado, North Sulawesi which popularly known as a city surrounding with seafood restaurants. Parrot fish is one of the preferred marine fish species in various restaurants, and this fish was sold after removing the scales in local markets. Rumengan *et al.* (2017) have reported that parrot fish scale containing 32.30% of protein and it can be a potential sources of gelatin and can be accept by Islam, Judaism and Hinduism. The objectives of this study were to determine the molecular structure of gelatin from fish scale using FTIR analysis and detecting the functional group of gelatin by wave number absorption comparing to gelatin standard.

## Materials and Method

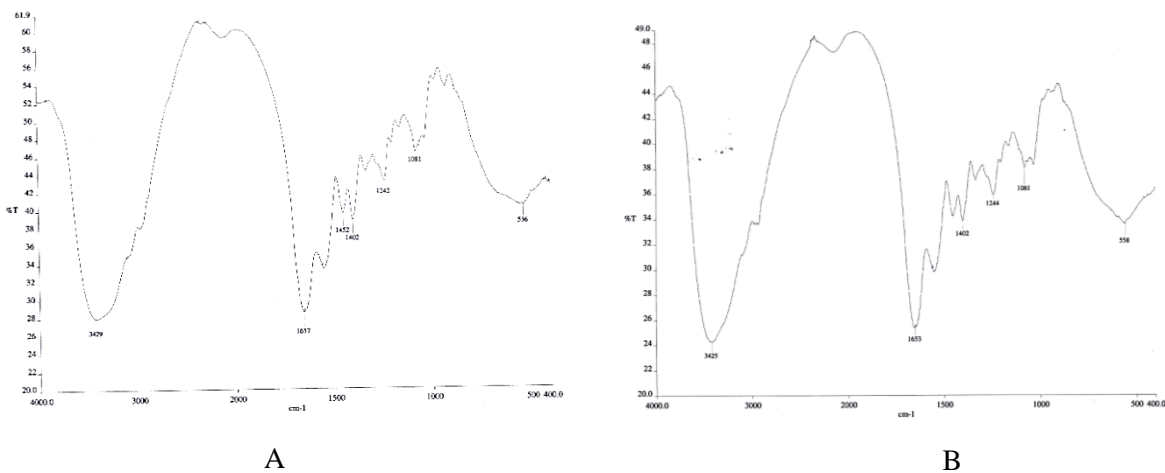
Fish scales were obtained from local markets, and samples were immediately washed with running tap water. The scales processed in Laboratory of Molecular

Biology and Marine Pharmaceutical, Faculty of Fisheries and Marine Science, Sam Ratulangi University since march 13, 2019. The scales were cut into pieces and soaked in NaOH 0,5N and distilled water for 48 hours to remove non-gelatinous protein. After soaking treatment, scales were washed with running water until pH of 7. The scales were soaked in HCl 0,75% and distilled water for 24 hours, and again neutralized with running water until pH of 7. Extraction procedure was conducted by heating the samples at 80°C for 3 hours, and then cooling at room temperature. Fourier transform infrared (FTIR) analysis was adopted from Khan et al. (2002) and Xu et al. (2005) method and moisture content from Gelatine Manufacture's Institute of America (GMIA) standard 2012. The yield of the dried scales derived gelatin was calculated using Equation 1.

$$\text{Yield (\%)} = \left( \frac{\text{weight of dried gelatin}}{\text{weight of dried fish scale}} \right) \times 100 \quad (1)$$

## Result and Discussions

Molecular structure of gelation is characterized by certain functional groups which could be detected by absorption in the wave number region. Figure 1 shows the wave number absorption of the two samples, wet and dried scales derived gelatin. The two gelatin samples were slight different in absorption of wave length for amide A, B, I, II and III groups indicating the instability of the functional groups which may influence viscosity and gel strength. For the wet scales derived gelatin, the wave number absorption was found to be 3412 cm<sup>-1</sup> (amide A), 2421 cm<sup>-1</sup> (amide B), 1653 cm<sup>-1</sup> (amide I), 1400 cm<sup>-1</sup> (amide II), and 1001 cm<sup>-1</sup> (amide III), while for the dried scales derived gelatin was 3435 cm<sup>-1</sup> (amide A), 2920 cm<sup>-1</sup> (amide B), 1635 cm<sup>-1</sup> (amide I), 1404 cm<sup>-1</sup> (amide II), and 1036 cm<sup>-1</sup> (amide III), where the standard of wave number absorption is presented in Table 1.



**Figure 1.** Wave number absorption of the wet scales derived gelatin (A) and the dried scales derived gelatin (B)

**Table 1.** Absorption region of gelatin derived from fish scales ( $\text{cm}^{-1}$ )

Type of Amida	Gs.wet	Gs.dry	Absorption region of Gelatin Standard	Note
Amide A	3412	3435	3478-3310	NH stretching
Amide B	2421	2920	2935-2915	Asymmetric $\text{CH}_2$
Amide I	1653	1635	1658-1653	C=O stretching
Amide II	1400	1404	1575-1480	$\text{NH}_2\text{CN}$ stretching, bending
Amide III	1001	1036	1240-1234	$\text{NH}_2$ bending CN stretching

Low peak of Amide III band was caused by the loss of triple helix while extraction process denaturation (Hashim et al., 2010). According to Muyonga et al (2004), the Amide III band intensity is responsible for the triple helix structure. The amide III absorption of gelatin is smaller than collagen amide III because the gelatin not producing triple helix but become a single helix. As explained by Kong and Yhun (2007) that the  $\alpha$  helix component was shown in the gelatin absorption region at wave number of  $1658\text{-}1653\text{ cm}^{-1}$ . Therefore, the gelatin of parrot fish scales shows a helix structure that corresponds to the  $\alpha$  helix. The amide II band functional group of gelatin parrot fish scales was found in gel soluble in wave absorption number of  $1400\text{-}1404\text{ cm}^{-1}$  are closely related in this case the triple helix structure. The range absorption number in the area of  $3478\text{-}3310\text{ cm}^{-1}$  indicates the

presence of NH Stretching bonds on hydrogen bonds of amide groups. Parallel polarized absorption with N-H bonds which are parallel to the  $\alpha$ -helix and perpendicular to the polypeptide chain on the b-sheet bond. The absorption at wave number of  $1658\text{-}1653\text{ cm}^{-1}$  shows the carbonyl bond C=O stretching with the contribution of NH and CN stretching bonds which are often referred as Amide I band. In the frequency range of  $1575\text{-}1480\text{ cm}^{-1}$  there was an absorbpt which shows the amide II group with  $\alpha$ -helical structure and b-sheet structure. Amide II band calibration is caused by deformation of the N-H bond. Absorption wave number of gelatin from parrot fish scale was suitable with the standard absorption number of gelatin where the amide I and II must be low intensity peaks and amide III peaks must be almost doesn't exist.

**Table 2.** The moisture content, pH and yield of the wet scales derived gelatin (Gs.wet) and the dried scales derived gelatin (Gs.dry) compared to the standard Gelatine Manufacture's Institute of America (GMIA)

Items	Gs.wet	Gs.dry	GMIA Standard 2012
Moisture content (%bb)	15.0%	13.7%	8-15%
pH	7	7	3.8-7.5
The yield (%bb)	2.33%	2.43%	-

As seen in Table 2, the moisture content of the wet scales derived gelatin was higher than the one of the dried scales derived gelatin and both of them are almost similar to the standard Gelatine Manufacture's Institute of America (GMIA). Low moisture determine the quality of gelatin. The lower the moisture content, the more concentrated gelatin produced. The pH value of the extracted gelatin from parrot fish's scales founds neutral pH (pH 7). The different of pH value is influenced by neutralization process. Zhou and Regenstein (2005) reported that mixed acidic and alkalic treatments in the extraction process resulted in neutral pH. The extraction yield of the dried scales derived gelatin was higher than the one of the wet scales derived gelatin.

### Conclusion

Absorption of wave number of gelatin from parrot fish scale was in range of the one of the standard gelatin, which was characterized by several types of amide group. There was only minor differences in the wave number absorption of the amide groups between the wet scales derived gelatin and the dried scales derived gelatin. The absorption wave number of amide III group for gelatin is smaller than the one of collagen indicating the molecular structure of gelatin is in single helix form, not triple helix.

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