Journal of Aquaculture and Fish Health Vol. 12(2) - June 2023
P-ISSN: 2301-7309 / E-ISSN: 2528-0864
doi : 10.20473/jafh.v12i2.35022

The Characterization of Collagen Isolated from Red Snapper Fish Skin (*Lutjanus* sp.) by Hydroextraction Method with Different Concentration of Acid Solution

R. Rahardyan Prasetyo[1], Ahmad Shofy Mubarak[2*], Eka Saputra[2] and Juni Triastuti[2]

[1]Study Program of Fisheries Product Technology, Faculty of Fisheries and Marine, Universitas Airlangga, Jl. Mulyorejo, Mulyorejo, Surabaya, East Java 60115, Indonesia
[2]Department of Marine, Faculty of Fisheries and Marine, Universitas Airlangga, Jl. Mulyorejo, Mulyorejo, Surabaya, East Java 60115, Indonesia

*Correspondence :
mubarak.as@fpk.unair.ac.id

Received : 2022-04-14
Accepted : 2022-11-15

Keywords :
Acetic acid, Collagen, Hydroextraction, Isolation

Abstract

Collagen is a connective tissue protein that is mostly produced from cattle and pigs. The use of cows and pigs in the manufacture of collagen causes a disease case, among others are bovine spongiform encephalopathy and transmissible spongiform disease. One of the alternative materials in the isolation of collagen is the use of fish skin. The purpose of this study was to determine the effect of the concentration of the acetic acid solution on the characteristics of red snapper collagen produced by the hydro extraction method and to determine the optimum concentration of the acetic acid solution in the red snapper skin collagen isolation process using the hydro extraction method. This study was experimental with a Completely Randomized Design (CRD) consisting of 3 acetic acid treatments: (P1) acetic acid with concentration 0,1 M; (P2) 0,15 M and (P3) 0,2 M. This study showed that the use of different acetic acid had a significant effect ($p<0.05$) on the yield parameters that is 1.72 – 2.46%, water content 11.12 – 12.8%, and protein content 83.66 – 84.81% and had no significant effect on the pH. The best treatment was P3 which uses a higher concentration of acetic acid that produced a higher yield of 2.46% and protein content of 84.81%. P3 with a concentration of acetic acid 0,2 M can be used for found the best result for the characterization of collagen.

INTRODUCTION

Collagen is a connective tissue protein. The basic molecule that forms collagen is three polypeptide chain units that are twisted together to form a triple helical structure which is better known as tropocollagen (Gelse *et al*., 2002). The uses of collagen include wound healing and body tissue repair, as a food softener and to reduce wrinkles on the face or it can be injected into the skin to replace damaged skin tissue (Gómez-Guillén *et al*., 2011). The current problem of collagen isolation is the low yield. Therefore, an innovation in collagen isolation is needed, namely the use of different acid concentrations to produce a high yield of collagen (Woo *et al*., 2008).

Cite this document as Prasetyo, R.R., Mubarak, A.S., Saputra, E. and Triastuti, J., 2023. The Characterization of Collagen Isolated from Red Snapper Fish Skin (*Lutjanus* sp.) by Hydroextraction Method with Different Concentration of Acid Solution. *Journal of Aquaculture and Fish Health*, *12*(2), pp.226-232.

The most common sources of collagen used today come from cows and pigs. The use of cows and pigs as raw materials for the manufacture of collagen causes a problem, namely the emergence of cases of bovine spongiform encephalopathy, transmissible spongiform disease and oral and nail diseases in cattle. Another problem also arises, collagen from cows and pigs is prohibited for certain circles (Gómez-Guillén *et al*., 2002). Therefore, alternative materials are needed in the manufacture of collagen, one of which is the use of fish.

Gómez-Guillén *et al*. (2002) stated that collagen can be extracted by chemical or enzymatic methods. The acid that is often used in collagen extraction is acetic acid and the collagen product extracted with acid is called Acid Soluble Collagen (ASC). The enzyme that is often used in collagen extraction is pepsin and the collagen extracted is called Peptide Soluble Collagen (PSC). Both methods require a long time for collagen extraction (Singh *et al*., 2011). Based on several studies, collagen extraction using chemical and enzymatic methods have many drawbacks, namely a long time, the use of a lot of chemicals solution and expensive costs (Woo *et al*., 2008; Idrus *et al*., 2018), so a modification of the method is needed, one of which is the acid hydro extraction method. The hydroextraction method is an extraction method that involves water and temperature which will facilitate the extraction process and does not require a long time. This method produces several advantages, including short time, high yield of little waste and low production costs (Potaros *et al*., 2009; Sahubawa and Putra, 2011).

Research on fish collagen has been carried out, including collagen from the skin and scales of tilapia, collagen from yellow tail fish skin, tilapia skin collagen, and catfish skin collagen (Sahubawa and Putra, 2011). One of the fish ingredients that have the potential to be used as a source of collagen is red snapper. Based on data from the Ministry of Fisheries and Marine Affairs (KKP, 2019), red snapper production reached 8,117 tons in 2017. This figure increased dramatically from red snapper production in 2016 which was 7,890 tons. With the numbers above, it can be ascertained that the amount of waste generated from snapper production is very high. Based on the description above, it is necessary to research collagen derived from the skin of red snapper with hydroextraction method which has never been researched before.

METHODOLOGY

Place and Time

This research was carried out from April 2021 to July 2021 at the Chemistry Laboratory and Food Laboratory, Faculty of Fisheries and Marine, Universitas Airlangga Surabaya. Snapper skin obtained from PT. Alam Jaya, Surabaya, East Java. The data obtained were analyzed descriptively.

Research Materials

The tools used are Erlenmeyer (100 ml and 500 ml). beaker glass (500 ml), analytical balance, incubator shaker, tray, cutting board, knife, pH meter, spatula, freeze dryer, refrigerator, and freezer. The materials used are red snapper fish skin, aquades, NaOH and acetic acid (CH_3COOH).

Research Design

This study used an experimental method to determine the effect of using acetic acid (CH_3COOH) in the isolation of collagen from red snapper (*Lutjanus* sp.) skin. The research design used was a completely randomized design (CRD) with 3 treatments and each of them had 6 replications.

The concentration of the acetic acid solution (CH_3COOH) in the isolation of collagen from red snapper skin used three different concentrations (P1) Acetic acid with a concentration of 0,1 M; (P2) 0,15 M, and (P3) 0,2 M.

Cite this document as Prasetyo, R.R., Mubarak, A.S., Saputra, E. and Triastuti, J., 2023. The Characterization of Collagen Isolated from Red Snapper Fish Skin (*Lutjanus* sp.) by Hydroextraction Method with Different Concentration of Acid Solution. *Journal of Aquaculture and Fish Health*, *12*(2), pp.226-232.

Work Procedure

Collagen isolated from fish skin is freeze-dried to facilitate the characterization of collagen. Collagen that has been freeze-dried was then analyzed proximately to determine the protein and water content. To find out the yield value of collagen isolated from fish skin, weighing was conducted using an analytical balance. pH value of collagen is measured using a pH pen.

Data Analysis

The data obtained from the results of this study are in the form of parametric statistical data which will then be analyzed using ANOVA (Analysis of Variance) to determine the effect of the use of acetic acid concentration on the resulting collagen in the form of proximate values (protein content and water content), yield and acidity (pH). Then proceed with Duncan's test to find out the difference between one treatment and another (Kusriningrum, 2012).

RESULTS AND DISCUSSION

The protein content of collagen extracted was between 83.66 and 84.81% (Figure 1). Analysis of Variance (ANOVA) showed that the difference in the concentration of acetic acid solvent had a significant effect on the protein content of the collagen produced ($P<0.05$). Duncan's test indicated that the highest protein content was produced by 0,2 M acetic acid (P3) with a protein content of 84.81%. The lowest protein content was found in the treatment with a solvent concentration of 0,1 M acetic acid (P1) with a protein content of 83.66%. The protein content of collagen produced by 0,15 M (P2) acetic acid treatment was 84.38%.

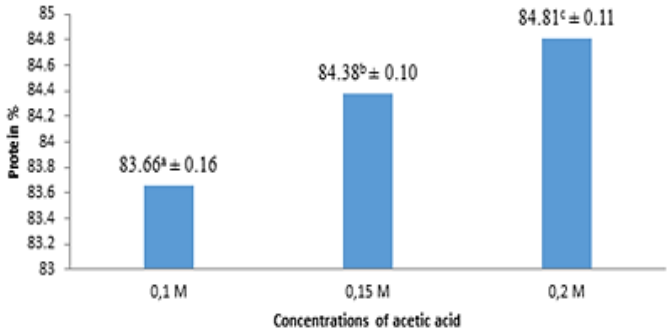

Figure 1. The average protein content of red snapper skin collagen. Values are average with a standard deviation of five-unit replicates. Different superscripts within a row indicate significantly different at $p<0.05$.

The yield of red snapper skin collagen (*Lutjanus* sp.) with different concentrations of the acetic acid solution resulted in a yield of 1.72 – 2.46% (Figure 2). Analysis of Variance (ANOVA) showed that the difference in the concentration of acetic acid solvent had a significant effect ($P<0.05$). Duncan's test, indicated that the highest yield of collagen was produced by 0,2 M acetic acid (P3) with a yield value of 2.46%, which was not significantly different from the solvent concentration of 0,15 M acetic acid (P2), namely by 2.33%. The lowest yield value was found in the treatment of 0,1 M acetic acid solvent concentration (P1) with a yield value of 1.72%.

Cite this document as Prasetyo, R.R., Mubarak, A.S., Saputra, E. and Triastuti, J., 2023. The Characterization of Collagen Isolated from Red Snapper Fish Skin (*Lutjanus* sp.) by Hydroextraction Method with Different Concentration of Acid Solution. *Journal of Aquaculture and Fish Health*, *12*(2), pp.226-232.

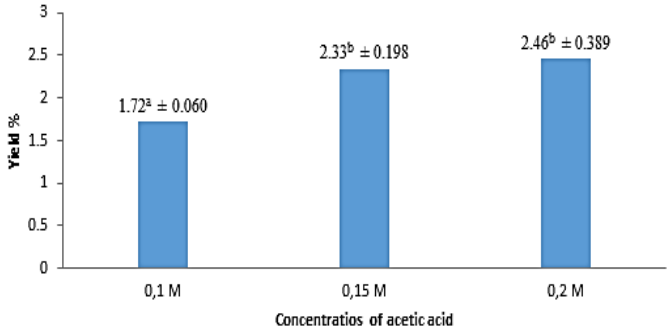

Figure 2. The yield of red snapper skin collagen. Values are average with a standard deviation of five-unit replicates. Different superscripts within a row indicate significantly different at $p<0.05$.

Collagen derived from the skin of red snapper (*Lutjanus* sp.) in this study had a pH value of 6.98 – 7.14 (Figure 3). The results of the Analysis of Variance (ANOVA) showed that the difference in solvent concentration of acetic acid had no significant effect ($P>0.05$) on the pH of the collagen produced.

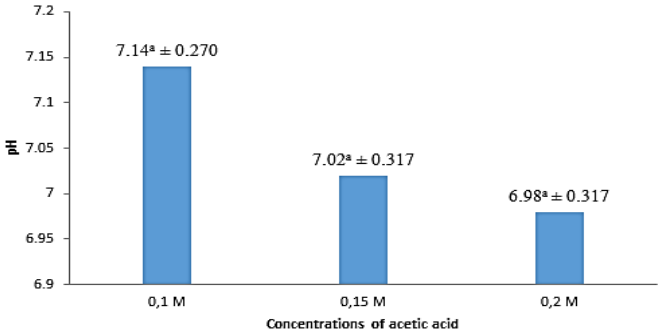

Figure 3. pH test average. Values are average with a standard deviation of five-unit replicates. Different superscripts within a row indicate significantly different at $p<0.05$.

The water content of collagen extracted was between 11.13 – 12.8% (Figure 4). Analysis of Variance (ANOVA) showed that the concentration of acetic acid solvent had a significant effect ($P<0.05$). Duncan's test indicated that the highest water content of collagen was produced by 0,2 M acetic acid (P3) with a water content of 12.8%. The lowest water content was found in the treatment with 0,1 M acetic acid (P1) with a water content of 11.13%, which was significantly different from 0,15 M acetic acid (P2) with a water content of 11.22%.

Cite this document as Prasetyo, R.R., Mubarak, A.S., Saputra, E. and Triastuti, J., 2023. The Characterization of Collagen Isolated from Red Snapper Fish Skin (*Lutjanus* sp.) by Hydroextraction Method with Different Concentration of Acid Solution. *Journal of Aquaculture and Fish Health*, *12*(2), pp.226-232.

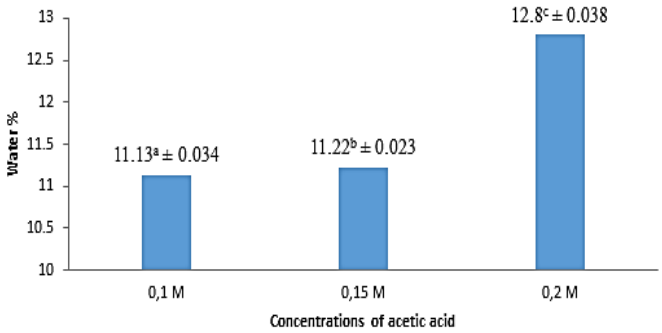

Figure 4. Average water content of red snapper skin collagen. Values are average with a standard deviation of five-unit replicates. Different superscripts within a row indicate significantly different at $p<0.05$.

Based on the results of the use of different concentrations of acetic acid (0,1M, 0,15M, 0,2M) in the isolation of red snapper skin collagen (hydro extraction method) it significantly affected the yield, water content and protein content but had no significant effect on the pH test. Collagen isolated using acetic acid (hydroextraction method) will increase H^+ ions, making it easier for water to enter and swelling (swelling) in the procollagen structure to facilitate extraction. Acetic acid has a great influence on the extraction yield (Mardina *et al*., 2014). During the hydroextraction stage, the hydrogen bonds were broken which caused a change in the structure of procollagen to become collagen so that it had an effect on protein content, yield and water content.

The pH value was not affected by the isolation of collagen using acetic acid (hydroextraction method) because during the neutralization process, it affected the final pH of the collagen. This process can reduce acid and base residues after deproteinate and hydrolysis processes. According to Zhou and Regenstein (2005), the correct neutralization process will result in the final pH of the product approaching a neutral value.

The protein content of red snapper skin collagen showed that the highest protein value was obtained in the use of acetic acid of 0,2 M with a protein value of 84.81%. These results show that the higher concentration of acetic acid used, the higher the protein obtained. Jamilah *et al.* (2013) stated that the difference in protein content could be caused by differences in the extraction method used and the large concentration of solvent used. These results have met the quality requirements of collagen SNI 8076:2014 which is 80-88% protein (Aberoumand, 2012).

Protein content in collagen is also influenced by other factors, namely the process of hydrolysis and extraction. The hydrolysis process with acetic acid will break the hydrogen bonds and open the collagen structure which occurs optimally so that the amount of protein obtained at a temperature of 40 degrees will increase (Aberoumand, 2012). Based on the results, the high protein content in collagen from snapper skin indicates that the collagen has good quality.

Yield shows the part of raw materials that can be utilized and becomes an important parameter to determine the economic value, as well as the effectiveness of raw materials to make a product. The higher the yield value of a treatment, the higher the level of effectiveness of the treatment (Sahubawa and Putra, 2011). Based on the results of the study, the highest red snapper skin collagen yield was found in the use of 0,2 M acetic acid (P3) with a value of 2.46%. This is due to the use of acetic acid according to Potaros *et al*. (2009), the difference in the yield value of the collagen produced can be caused by differences in the concentration of solvents that can remove non-collagen pro-

Cite this document as Prasetyo, R.R., Mubarak, A.S., Saputra, E. and Triastuti, J., 2023. The Characterization of Collagen Isolated from Red Snapper Fish Skin (*Lutjanus* sp.) by Hydroextraction Method with Different Concentration of Acid Solution. *Journal of Aquaculture and Fish Health*, *12*(2), pp.226-232.

teins during the collagen production process. The amount of collagen that is wasted during the deproteination and washing process can cause a decrease in the yield value. The hydroextraction process has a low reaction rate and the ability to penetrate tissues.

The pH value of red snapper skin collagen isolated using acetic acid solution (hydroextraction method) was neutral, in the range of 6.98 – 7.14. These results follow the collagen quality requirements of SNI 8076:2014, namely 6.5 – 8. This is because the process of isolating red snapper skin collagen through various washing processes and extraction using the hydroextraction method. Water itself functions as a pH neutralizer of collagen which has gone through a deproteinate process using bases and hydrolysis using acid then extraction using water at a temperature of 40 °C which causes the collagen pH to tend to be neutral. The combination of acetic acid and the hydroextraction process tends to produce a pH close to neutral (Zhou and Regenstein, 2005). This is because the isolation process uses water, where the water has a neutral pH and will reduce the remnants of acid and alkaline solutions in the neutralization process which will affect the final pH of the collagen product produced (Jamilah *et al.*, 2013).

Collagen molecules consist of protein and water. Soaking in an acid solution aims to remove non-collagen proteins, as well as other components. This process is carried out to obtain a high collagen protein content (Suptijah *et al.*, 2018). The chemical composition of red snapper skin collagen (*Lutjanus* sp.) is presented in Figure 4. Based on the results (Figure 4), the main component of collagen is protein and other components are water in small amounts.

The water content of red snapper skin collagen produced showed differences in each treatment. The highest water content was found in the use of acetic acid of 0,2 M with a value of 12.8%. The water content of a material is related to the effectiveness of storage. High water content exceeding the quality requirements of collagen causes ineffective storage and low shelf life (Jaswir *et al.*, 2011).

CONCLUSION

The use of the concentration of acetic acid solution to isolate collagen from the skin of red snapper (*Lutjanus* sp.) affects the collagen characteristics. These characteristics include yield, pH, water content, and protein content. The higher concentrations of acetic acid used the better characteristics of the collagen produced. In this study, the optimum concentration of acetic acid solution in the red snapper skin collagen isolated process using the hydroextraction method was 0,2 M which resulted in the highest yield of 2.46%, water content of 12.8% and protein content of 84.81%.

ACKNOWLEDGEMENT

The authors thank all parties who have aided in the completion of this research.

REFERENCES

Aberoumand, A., 2012. Comparative study between different methods of collagen extraction from fish and its properties. *World Applied Sciences Journal*, *16*(3), pp.316-319. https://idosi.org/wasj/wasj16(3)12/1.pdf

Gelse, K., Pöschl, E. and Aigner, T., 2003. Collagens—structure, function, and biosynthesis. *Advanced Drug Delivery Reviews*, *55*(12), pp.1531-1546. https://doi.org/10.1016/j.addr.2003.08.002

Gómez-Guillén, M.C., Giménez, B., López-Caballero, M.E. and Montero, M.P., 2011 Functional and bioactive properties of collagen and gelatin from alternative sources: A review. *Food Hydrocolloids*, *25*(8), pp.1813-1827. https://doi.org/10.1016/j.foodhyd.2011.02.007

Cite this document as Prasetyo, R.R., Mubarak, A.S., Saputra, E. and Triastuti, J., 2023. The Characterization of Collagen Isolated from Red Snapper Fish Skin (*Lutjanus* sp.) by Hydroextraction Method with Different Concentration of Acid Solution. *Journal of Aquaculture and Fish Health*, *12*(2), pp.226-232.

Gómez-Guillén, M.C., Turnay, J., Fernández-Díaz, M.D., Ulmo, N., Lizarbe, M.A. and Montero, P., 2002. Structural and physical properties of gelatin extracted from different marine species: a comparative study. *Food Hydrocolloids*, *16*(1), pp.25-34. https://doi.org/10.1016/S0268-005X(01)00035-2

Idrus, S., Hadinoto, S. and Kolanus, J., 2018. Collagen characterization from swim bladder of yellowfin tuna (*Thunnus albacares*) from Maluku using acid extraction. *BIOPROPAL Industri*, *9*(2), pp.87-94. http://bpkimi1.kemenperin.go.id/biopropal/article/view/4020

Jamilah, B., Hartina, M.R.U., Hashim, D.M. and Sazili, A.Q., 2013. Properties of collagen from barramundi (*Lates calcarifer*) skin. Int*ernational Food Research Journal*, *20*(2), pp.835-842. http://www.ifrj.upm.edu.my/volume-20-2013.html

Jaswir, I., Monsur, H.A. and Salleh, H.M., 2011. Nano-structural analysis of fish collagen extracts for new process development. *African Journal of Biotechnology*, *10*(81), pp.18847-18854. https://doi.org/10.5897/AJB11.2764

KKP: Kementerian Kelautan dan Perikanan, 2019. *Data Statistik Perikanan Tangkap 2019*. Jakarta, ID KKP.

Kusriningrum, R.S., 2012 *Perancangan Percobaan*. Airlangga University Press, Surabaya, pp. 43-98.

Mardina, P., Prathama, H.A. and Hayati, D.M., 2014. Pengaruh waktu hidrolisis dan konsentrasi katalisator asam sulfat terhadap sintesis furfural dari jerami padi. *Konversi*, *3*(2), pp.1-8. https://doi.org/10.20527/k.v3i2.158

Potaros, T., Raksakulthai, N., Runglerdkreangkrai, J. and Worawattanamateekul, W., 2009. Characteristics of collagen from nile tilapia (*Oreochromis niloticus*) skin isolated by two different methods. *Agriculture and Natural Resources*, *43*(3), pp.584-593. https://li01.tcithaijo.org/index.php/anres/article/view/244706

Sahubawa, L. and Putra, A.B.N., 2011. Pengaruh konsentrasi asam asetat dan waktu ekstraksi terhadap mutu kolagen kulit ikan nila hitam. *Jurnal Teknosains*, *1*(1), pp.16-25. https://doi.org/10.22146/teknosains.3987

Singh, P., Benjakul, S., Maqsood, S. and Kishimura, H., 2011. Isolation and characterization of collagen extracted from the skin of striped catfish (*Pangasianodon hypophtalmus*). *Food Chemistry*, *124*(1), pp.97-105. https://doi.org/10.1016/j.foodchem.2010.05.111

Suptijah, P.D., Indriani, D. and Wardoyo, S.E., 2018. Isolation and Characterization of Collagen from the Skin of Catfish (*Pangasius* sp.). *Jurnal Sains Natural*, *8*(1), pp.8-23. https://doi.org/10.31938/jsn.v8i1.106

Woo, J.W., Yu, S.J., Cho, S.M., Lee, Y.B. and Kim, S.B., 2008. Extraction optimization and properties of collagen from yellowfin tuna (*Thunnus albacares*) dorsal skin. *Food Hydrocolloids*, *22*(5), pp.879-887. https://doi.org/10.1016/j.foodhyd.2007.04.015

Zhou, P. and Regenstein, J.M., 2005. Effects of alkaline and acid pretreatments on Alaska pollock skin gelatin extraction. *Journal Food Science*, *70*(6), pp.c392-c396. https://doi.org/10.1111/j.1365-2621.2005.tb11435.x

Cite this document as Prasetyo, R.R., Mubarak, A.S., Saputra, E. and Triastuti, J., 2023. The Characterization of Collagen Isolated from Red Snapper Fish Skin (*Lutjanus* sp.) by Hydroextraction Method with Different Concentration of Acid Solution. *Journal of Aquaculture and Fish Health*, *12*(2), pp.226-232.