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Effect Of Gamma Rays Irradiation Of Cesium-137 On Collagen Extraction Of Nile Fish Scales (*Oreochromis niloticus*)

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Abstract

Research on Cesium-137 gamma ray irradiation on collagen extraction from tilapia fish scales (*Oreocharomis Niloticus*). The aim of knowing the functional groups of collagen before and after irradiation and knowing the effect of the dose on the functional groups of collagen. This study used the extraction method with the addition of acid (maceration). Then irradiate the extraction results with a Cesium-137 source, with a constant (Source to Skin Distance) distance of 100 cm with varying doses of 10 mGy, 30 mGy and 50 mGy. The sample used in this study was Tilapia fish scales. The results showed that prior to irradiation during the FTIR test it produced several collagen functional groups, namely: amide A, amide I, amide II, and amide III. While the functional groups after irradiation were: amide A, amide I, and amide III at a dose of 50 mGy. Thus, radiation can cause changes in the structure and properties of collagen molecules and can trigger chemical reactions in functional groups.

INTRODUCTION

Indonesia is an archipelagic country where two-thirds of its territory consists of seas and beaches with a coastline of ± 80,791.42 km. With the expansion of Indonesia's territorial waters, the development of marine and fisheries potential has become one of the government's leading sectors. Based on information from the Ministry of Maritime Affairs and Fisheries and Indonesian Center for Law and Policy Studies (Barunastra, *et al*., 2019), aquaculture production has increased from year to year, reaching 1.12%. In 2015 it was 15.63 million tons, then in 2019 it was 16.33 million tons. Where freshwater fish dominate the increase in aquaculture production, including catfish, catfish, gourami fish and tilapia.

Collagen is an important biomaterial in medical applications because it is biodegradable. Collagen is widely used for various purposes including biomedical, food industry, pharmaceutical and cosmetic industries. Fishery waste can be an alternative source of collagen, so it is hoped that it can meet collagen needs and increase the added value of fishery waste. Skin, bones and scales are among the materials used to produce collagen (Andini, *et al*., 2021). Increasing fisheries production also affects the amount of waste from fish. By increasing waste products, large amounts of collagen can be obtained.

Abundant collagen production from fishery waste can be a source of collagen that can meet foreign and domestic collagen needs. Thus, fishery waste is no longer left aside but instead becomes a valuable product and has high economic value. Research conducted (Nurhayati and Peranginangin, 2009), states that fishery waste contains collagen which has high economic value. Apart from waste originating from fisheries, collagen is also used in industry from

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cows or pigs. Using ingredients from pork is prohibited for Muslims, on the other hand using beef can trigger fear of certain diseases such as mad cow and bovine spongiformisense folapati (BSF).

One of the weaknesses of collagen is that it has a relatively large molecular structure, making it difficult to absorb through the skin and difficult to dissolve in water. To overcome this, collagen is degraded using enzymes at quite high rates using different treatments. To overcome this, collagen should be broken down using gamma irradiation. Gamma radiation is an electromagnetic wave that carries energy in a dense form called a photon. Gamma radiation is used in health sterilization, food preservation and pasteurization of medicinal plants. The advantages of electron beams and gamma radiation are that the process is faster, no residue is left behind and the radiation dose can be adjusted according to needs (Dian, *et al*., 2012).

Based on the description above, the researcher intends to conduct research with the Effect Of Gamma Rays Irradiation Of Cesium-137 On Collagen Extraction Of Nile Fish Scales (*Oreochromis niloticus*)**.** The aim of knowing the collagen functional groups of Tilapia fish scales before and after irradiation and to determine the effect of radiation dose on the collagen functional groups of Tilapia fish scales. The novelty of previous research is that it used a different sample, namely Tilapia fish and the radiation source used was Cesium-137.

MATERIALS AND METHODS

The equipment used in this research includes by Fourier Transform Infra Red (FTIR) spectrophotometer, Cesium-137 radiation source, centrifuge refrigerator, centrifuge tube, irradiator, laser, CCTV monitor, Phantom Air, pH meter, analytical balance, measuring cup and measuring flask. The materials used in this research include by acetic acid solution (CH_3COOH), NaCl solution, NaOH solution, distilled water, polypropylene plastic and Whatman 42 paper. The process of extracting collagen from Tilapia fish scales uses the method of adding acid (maceration) which is carried out at room temperature (27°C). The collagen extraction process consists of several stages, namely soaking using NaOH which aims to remove fat, fishy odor, non-collagen proteins and dirt that is still on the fish scales. Then, neutralize the sample using distilled water. Next, soak the Tilapia fish scales using acetic acid (CH_3COOH) with a concentration of 0.7 M. Soaking using acetic acid aims to give the collagen a whiter color. After the soaking process with acetic acid solution is complete, then soak the sample again using sodium chloride (NaCl) with a concentration of 0.9 M for a period of 24 hours. Then a centrifuge is carried out to separate the collagen precipitate from the solution. The centrifuged collagen precipitate is then dried. This drying stage is to obtain collagen in dry powder form. Next, the dry collagen was tested for yield to determine the yield on the tilapia fish scales. Where the yield was 11.78%.

Collagen Extraction From Tilapia Fish

The scales produced from Tilapia fish are washed thoroughly using water. After that, soak the fish scales in 0.5 M NaOH solution for 12 hours, changing the dampening solution every 6 hours which is useful for removing fat and other dirt. Next, the fish scales are cleaned using distilled water which functions to remove the NaOH solution until the pH is neutral. Soak the fish scales in 0.7 M acetic acid solution for 48 hours. Filter the precipitate from the extraction using Whatman 42 paper. Next, the precipitate is soaked again in 0.9 M NaCl solution for 24 hours. Centrifuge at 3500 rpm at 4°C for 30 minutes. Dry at room temperature.

Irradiation of Collagen Solution

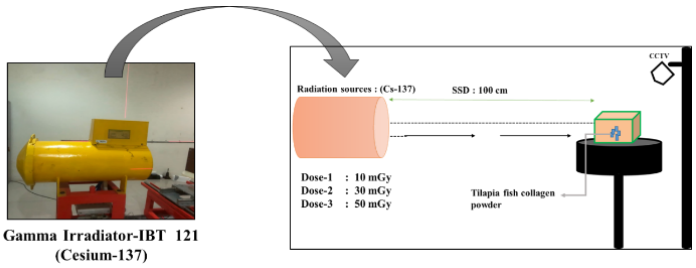

Figure 1. Method of irradiating Cesium-137 on samples

Prepare an irradiator with a calibrated Cesium-137 source. Place the extracted collagen that has been put into a polypropylene plastic bag amounting to ±5 grams. After that, irradiate using a Cesium-137 radiation source with doses of 10 mGy, 30 mGy and 50 mGy alternately. Monitor the sample irradiation process until the irradiation process is complete via CCTV monitor. After that, turn off the tool and take the sample that has been irradiated. Then the irradiated collagen solution was subjected to FTIR testing again to see whether there were changes in functional groups. Calculation of exposure time using Cs-137 using equation (1).

$$\text{Dose} = \text{Time x Hp}(10) \qquad (1)$$

Hp (10) is the KERMA rate (Kinetic Energy Released Per Unit Mass) of air. Kerma is the sum of the initial

kinetic energies of all charged particles liberated by uncharged ionizing radiation (neutrons and photons) in a sample of matter, divided by the mass of the sample. Cesium-137 radiation testing at the Balai Pengamanan Alat Fasilitas Kesehatan (BPAFK) or Health Facility Equipment Security Center in Makassar.

Table 1. The Hp (10) value for the KERMA decay rate of Cesium-137 air (BPAFK Makassar 2023).

SSD (cm)	Air Kerma (mGy/h)	Explanation
100	21.35	Without absorption

Chemical Composition Measurement

Collagen samples were characterized using an FTIR spectrophotometer with a wave number range of 4000 cm^{-1} to 400 cm^{-1}. The sample is placed on the surface of the ATR crystal. After that, the FTIR spectrum takes measurements. Observe the results of collagen extraction in Tilapia fish scales using an FTIR spectrophotometer for each dose treatment given to see the differences in functional groups obtained.

RESULTS AND DISCUSSION

The quality of collagen from Tilapia fish scales requires characterization of functional groups formed from Tilapia fish scales. FTIR spectrum of Tilapia fish scales irradiated at doses of 10 mGy, 30 mGy, and 50 mGy. The irradiation time can be seen in table 2. The parameters of time and distance from the radiation source have a significant influence on the radiation exposure dose, because the dose received decreases with increasing distance from the radiation source. The longer the exposure time, the higher the dose of radiation received (Yuliamdani, *et al*., 2020).

Table 2. Radiation exposure time of Cesium-137.

No.	Dose (mGy)	Exposure Time (hour)	Explanation
1	10	0.5	Without absorption
2	30	1.4	Without absorption
3	50	2.3	Without absorption

Changes in radiation absorbers and distances can calculate the dose and dose rate of gamma rays. During gamma irradiation, specific doses are given at intervals of minutes to hours. The thickness and volume of the product to be irradiated determine the time of dose administration. The amount of radiation energy absorbed by a substance is called the radiation dose. The energy produced by ionizing radiation absorbed by a substance per unit mass is measured in gray (Gy), the unit used. 1 joule/kg is equal to 1 gray (Khotimah, 2011). The dose, duration, and quantity of a substance exposed to gamma radiation all affect how it affects the substance. These elements working together can help determine the best combination to use to improve the quality of a particular product (Putra, 2018).

The yield value obtained was greater than research conducted (Senduk, *et al*., 2020) using black tilapia fish skin, where the yield obtained was 2.24%. The yield obtained from collagen processing is an important parameter in assessing the level of effectiveness of collagen production through several stages. It is very important to know the yield to get an idea of whether a product can be used properly or to find out the economic value of the product. The higher the yield value of a product, it can be said that the product has a high economic value (Pasaribu, *et al.*, 2021).

Identification of Collagen Functional Groups Before Irradiation

The results of the FTIR spectrum of collagen samples are shown in figure 2 and table 3.

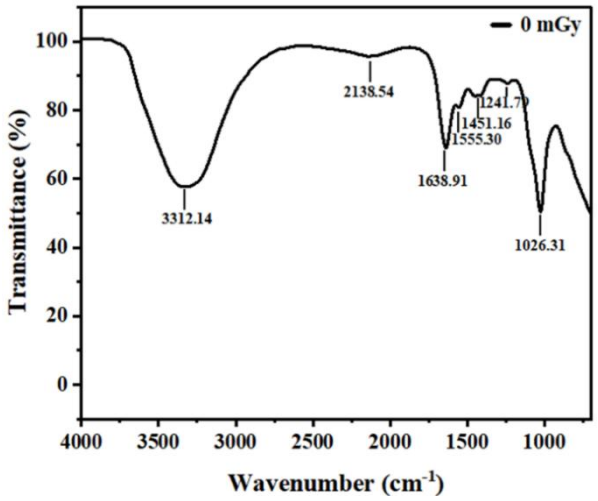

Figure 2. FTIR Spectrum of Collagen.

The FTIR spectrum of Tilapia fish scale collagen without irradiation treatment is presented in figure 2 and table 3, which has distinctive absorption peaks in several areas. It can be seen that the first peak spectrum, namely amide A, with an absorption area of 3300 cm^{-1} to 3340 cm^{-1} is seen at a wave number of 3312.14 cm^{-1}. The absorption area of amide A in collagen occurs due to NH stretching of the amide group with hydrogen bonds and the amino acid hydroxyproline. These absorption results are almost the same as the peak absorption spectrum of FTIR tuna collagen at a wave number of 3302 cm^{-1} (Hernawan, *et al*., 2016).

Table 3. Results of FTIR identification of Tilapia fish scale collagen

Compound	Absorption Area (cm^{-1}) (Suptijah, *et al*., 2018)	Absorption peak (cm^{-1})	Functional groups	Explanation
Amida A	3300 – 3340	3312.14	Vibrasi stretching NH	Hidroksiprolin
Amida B	2922 – 2940	-	Asimetrikal Stretching CH_2	-
Amida I	1600 – 1700	1638.91	Vibrasi stretching C=O	Glisin dan Prolin
Amida II	1480 – 1580	1555.30	CH stretching NH bending	Lisin
Amida III	1200 – 1300	1241.91	CH stretching NH bending	Tripel helix colagen

The second absorption peak is amide I where the maximum absorption peak is at a wave number of 1638.91 cm^{-1} where the absorption area is 1600 cm^{-1} to 1700 cm^{-1} which shows the C=O stretching vibration of the amide I group characterized by the presence of the amino acids glycine and proline on the collagen triple helix (Nurjanah, *et al*., 2021). Amide I is an important factor in understanding the secondary structure of collagen (Gustini, *et al.*, 2022). The amide I group also consists of four protein secondary structure components, namely α-helix (9%), β-sheet (35%), β-turn (18%), and random coil (20%) which overlap with each other. The α-helical component is shown in the absorption region between 1654 - 1658 cm^{-1}, β-sheet between 1624-1642 cm^{-1}, β-turn at 1666, 1672, 1680, 1688 cm^{-1}, and random coil at 1648 ± 2 cm^{-1} (Alhana, *et al*., 2015). Based on the absorption peak, amide I has a β-sheet structure. This absorption also appears in white sea bass collagen at a wave number of 1650 cm^{-1} (Dian, *et al*., 2012).

The third absorption peak is amide II which occurs in the absorption area 1480 cm^{-1} to 1580 cm^{-1} where the maximum absorption is seen at the wave number 1555.30 cm^{-1} which indicates CH stretching and NH bending vibrations. Almost the same absorption also appears in Tuna fish collagen at a wave number of 1552 cm^{-1} (Nurjanah, *et al*., 2021).

The fourth absorption peak is amide III which occurs in the absorption area of 1200 cm^{-1} to 1300 cm^{-1}. The maximum absorption peak in amide III absorption is 1241.91 cm^{-1} which indicates CH stretching and NH bending vibrations. This absorption also appears in the skin collagen of Patin fish at a wave number of 1239.46 cm^{-1}. The amide III absorption peak indicates the triple helix structure of collagen which is a characteristic of collagen (Safithri, *et al*., 2018).

To be said to be collagen, a material must contain a minimum of 3 functional groups out of the 5 functional groups that are required (Senduk, *et al*., 2020). This shows that the results of the research that has been carried out correctly contain collagen because it meets the standards for collagen functional groups. Undetectable amide groups can be caused by the chemical composition of Tilapia fish scales, the acid concentration used, the collagen extraction temperature, and differences in the acid used in collagen extraction(Nurjanah, *et al*., 2021).

Identification of Collagen Functional Groups After Irradiation

The FTIR spectrum of collagen samples after irradiation is shown in figure 3 and table 4. Figure 3 and table 4 explain the absorption values that meet the standards for the formation of functional groups in collagen after irradiation. The research results obtained show that the FTIR spectrum of Tilapia fish scales produced is amide A where the maximum absorption peaks for the three doses respectively are at wave numbers 3336.91 cm^{-1}, 3396.32 cm^{-1} and 3339.90 cm^{-1}. The amide A group does not show any significant differences and meets the standard range of absorption areas that have been determined. Amide A shows the NH stretching vibration of the amide group which is linked by hydrogen and OH bonds of hydrocyproline. The presence of amide A indicates the presence of a hydroxyproline component in the amino acid (Hernawan, *et al*., 2016).

The second absorption peak is amide I where the maximum absorption peak for the three doses respectively is at wave numbers 1640.81 cm^{-1}, 1639.76 cm^{-1}, and 1639.46 cm^{-1}. The amide I absorption peak indicates the C=O stretching vibration. The amide I group is characterized by the presence of the amino acids glycine and proline which are found in the triple helix structure of collagen (Nurjanah, et al., 2021). The amide I group also consists of four components of the protein secondary structure, namely α-helix, β-sheet, β-turn, and random coil which overlap with each other. The α-helical component is shown in the absorption region between 1654 - 1658 cm^{-1}, β-sheet between 1624-1642 cm^{-1}, β-turn at 1666, 1672, 1680, 1688 cm^{-1}, and random coil at 1648 ± 2 cm^{-1}. Based on the absorption peak, amide I for the three doses is found in the β-sheet structure (Alhana, *et al*., 2015).

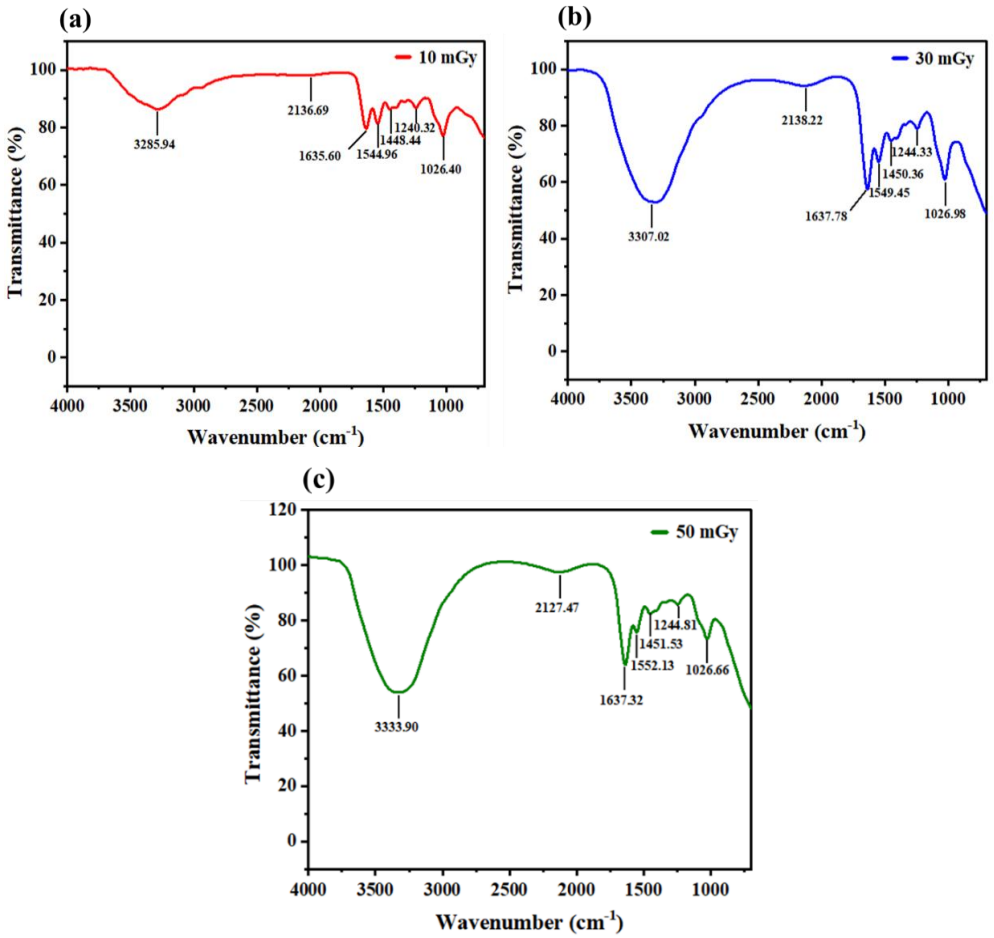

Figure 3. FTIR spectrum of collagen resulting from (a) 10 mGy, (b) 30 mGy and (c) 50 mGy irradiatio

Table 4. FTIR identification results after irradiation.

Compound	Absorption Peak (cm^{-1}) with Radiation			Absorption Area (cm^{-1}) (Riaz, *et al*., 2018)	Functional groups	Explanation
	10 mGy	30 mGy	50 mGy			
Amida A	3336.91	3396.32	3339.90	3300-3340	Vibrasi stretching NH	Hidroksiprolin
Amida B	-	-	-	2922-2940	Asimetrikal Stretching CH_2	-
Amida I	1640,81	1639,76	1639,46	1600-1700	Vibrasi stretching C=O	Glisin dan Prolin
Amida II	-	-	-	1480 – 1580	CH stretching NH bending	-
Amida III	-	-	1247,08	1200-1300	CH stretching NH bending	Tripel helix kolagen

The third absorption peak, namely amide III, it can be seen that the irradiation dose of 50 mGy meets the standard collagen absorption range, namely 1247.08 cm^{-1}. Amide III shows CH stretching and NH bending vibrations, and for doses of 10 mGy and 30 mGy it does not fill the absorption region.There are several functional groups that are not detected after irradiation. However, in terms of the durability of the collagen produced, collagen that undergoes the radiation process is much more durable than without radiation treatment. Irradiation aims to increase shelf life, improve quality and maintain the hygiene of a material. Preservation by irradiation requires paying attention to the dose used. Using an inappropriate dosage will cause the material to be damaged. So, it is hoped that irradiated materials can be stored longer than without irradiation and their quality can still be maintained (Putri, *et al*., 2015).

Changes in the structure and properties of collagen molecules can be triggered by chemical reactions in functional groups due to irradiation, as the radiation dose increases from 10 mGy to 30 mGy. A dose of 10 mGy to 30 mGy can be used to determine whether a material can still be used after irradiation (Hernawan, *et al*., 2016). In addition, low doses of 30 mGy gamma irradiation can be used to increase the overall proliferation potential of human fibroblasts, and gamma irradiation can inhibit the growth of primary prostate epithelial cells by inducing senescence, not apoptosis. When collagen

production by fibroblasts does not decrease, fibroblast cells can synthesize collagen by metabolically active cells (Chen, *et al*., 2019).

This indicates that with increasing radiation dose, collagen with extraction using acetic acid experiences denaturation. Dosing can affect functional groups, because dosage can result in modification of chemical reactions and degradation. Changes in the position of functional group intensity indicate that radiation treatment can reduce the absorption intensity which can cause damage to collagen.

CONCLUSION

Based on the research that has been carried out, a conclusion can be drawn, namely that the analysis of the functional groups of Tilapia fish scale collagen before and after irradiation was carried out using the FTIR spectrum which produced several functional groups before irradiation, namely: amide A, amide I, amide II, danamide III. Meanwhile, the functional groups after irradiation are: amide A, amide I, and amide III for a dose of 50 mGy. Radiation can cause changes in the structure and properties of collagen molecules and can also trigger chemical reactions in functional groups.

Dosing can have an influence on the functional groups and absorption spectrum which can trigger degradation and chemical modification of the functional groups. There are suggestions for future research if using Tilapia fish samples as a source of collagen, it is best not to use too high a dose when carrying out radiation because it can damage the structure of the collagen molecule. Add several tests such as: viscosity, UV-Vis spectrum to find out whether the collagen produced is really suitable for use or not.

REFERENCES

Andini, A. *et al.* (2021). Stimulasi Greenpreneur Melalui Webinar Cara Mudah Ekstraksi Kolagen dari Limbah. *Prosiding Penelitian Pendidikan dan Pengabdian 2021,* 1(1), 1423–1431. https://prosiding.rcipublisher.org/index.php/prosiding/article/view/308

Chen, Z. *et al*. (2019). Cellular Senescence in Ionizing Radiation (Review). *Oncology Reports*, 42, 883-894. https://doi.org/10.3892/or.2019.7209

Dian, P. P. *et al*. (2012). Isolasi dan Sintesis Gelatin dan Sisik Ikan Kakap Putih (*Lates Calcarifer*) Berikatan Silang dengan Teknik Induksi Iradiasi Gamma. *Jurnal Sains Materi Indonesia*, 14(1), 40-46. Doi: 10.17146/jsmi.2012.14.1.4639

Gustini, N. *et al*. (2022). Profil Asam Amino Kolagen Larut Asam Teripang Pasir (Holothuria Scabra). *Prosiding Sains Nasional dan Teknologi* 12(1), 72-77. https://doi.org/10.36499/psnst.v12i1.7094

Hernawan, H. *et al*. (2016). Pembuatan Dosimeter Termoluminesensi Dari Bahan Litium Fluorida dan Pengotor Titanium. *Jurnal Forum Nuklir (JFN)*, 10(1), 38-44. https://doi.org/10.17146/jfn.2016.10.1.3492

Putra, H. H. (2018). Pengaruh Radiasi Gamma Terhadap Kadar Protein, Lemak dan Radikal Bebas Daging Ikan Tenggiri (*Scomberomus Commerson*). Skripsi: Univresitas Islam Negeri Maulana Malik Ibrahim, 1-91. http://etheses.uin-malang.ac.id/13882/

Alhana, A. *et al*. (2015). Extraction and Characterization of Collagen from Sea Cucumber Flesh. *Jurnal Pengolahan Hasil Perikanan Indonesia* 18(2), 150–61. https://doi.org/10.17844/jphpi.2015.18.2.150

Khotimah, N. (2011). Penentuan Dosis Serapan Radiasi-Γ dari ^{60}Co Pada Radiotherapy Payudara Menggunakan Software MCNP5. Skripsi: Universitas Sebelas Maret. 1-44. https://digilib.uns.ac.id/dokumen/detail/19313/Penentuan-Dosis-Serapan-Radiasi-Dari-60co-pada-Radiotherapy-Payudara-Menggunakan-Software-Mcnp5

Barunastra, *et al*. (2019). The Legal Framework and Government Institutional Landscape of the Fisheries Sector in Indonesia. Jakarta, Indonesia.1-184. https://barunastra.org

Nurhayati, N. & Peranginangin, R. (2009). Prospek Pemanfaatan Limbah Perikanan Sebagai Sumber Kolagen. *Squalen Bulletin of Marine and Fisheries Postharvest and Biotechnology* 4 (3), 83. https://doi.org/10.15578/squalen.v4i3.157

Nurjanah, N. *et al*. (2021). Ekstraksi Kolagen Kulit Ikan Tuna Sirip Kuning (*Thunnus albacares*) menggunakan Enzim Pepsin dan Papain. *Jurnal Pengolahan Hasil Perikanan Indonesia* 24 (2), 174–87. https://doi.org/10.17844/jphpi.v24i2.35410

Pasaribu, P. *et al*. (2021). Randemen Kolagen yang di Ekstrak dari Cangkang Kijing (*Pilsbryoconcha*) dari Perairan Sungai dan Kolam. *Jurnal Online Mahasiswa-Universitas Riau*, 1-10. https://jom.unri..ac.id.

Putri, F, N, A. *et al*. (2015). Aplikasi Teknologi Iradiasi Gamma Dan Penyimpanan Beku Sebagai Upaya Penurunan Bakteri Patogen

Pada Seafood : Kajian Pustaka. *Jurnal Pangan dan Agroindustri*, 3(2), 345-352. https://jpa.ub.ac.id/index.php/jpa/article/view/150

Riaz, T. *et al*. (2018). FTIR Analysis of Natural and Synthetic Collagen. *Applied Spectroscopy Reviews* 53(9), 703–46. https://doi.org/10.1080/05704928.2018.1426595

Safithri, M. *et al*. (2018). Potensi Kolagen Teripang Emas Sebagai Inhibitor Tirosinase. *Jurnal Pengolahan Hasil Perikanan Indonesia* 21 (2), 296-303. https://doi.org/10.17844/jphpi.v21i2.23085

Senduk, T. W. *et al*. (2020). The rendement of boiled water extract of mature leaves of mangrove Sonneratia alba. *Jurnal Perikanan dan Kelautan Tropis,* 11(1), 9-15. https://doi.org/10.35800/jpkt.11.1.2020.28659

Suptijah, P. *et al*. (2018). Isolasi Dan Karakterisasi Kolagen Dari Kulit Ikan Patin (*Pangasius sp*.). *Jurnal Sains Natural*, 8(1), 8-23. https://doi.org/10.31938/jsn.v8i1.106

Yuliamdani, R. *et al*. (2020). Pengujian Paparan Radiasi Sinar-X Di Unit Radiologi Rsud Kota Makassar. *JFT : Jurnal Fisika dan Terapannya* 7 (1), 53-61. https://doi.org/10.24252/jft.v7i1.13867