CHARACTERIZATION OF HYDROXYAPATITE FROM CHANNA STRIATA AND SCOMBEROMORUS COMMERSON FISH BONE BY HEAT TREATMENT

Rista Mutia Anggraini[1*], Tika Restianingsih[1], Frastica Deswardani[1], Yoza Fendriani[1], Rodearni Ananda Putri Purba[1]

Department of Physics, Faculty of Science and Technology, Universitas Jambi, 36361, Indonesia

*email: ristamutia@unja.ac.id

ABSTRACT

Hydroxyapatite (HAp) biomaterials have been successfully obtained from Channa striata and Scomberomorus commerso fish bone. Heat treatment was chosen as the method to get it. Heat treatment was done at 400°C, 600°C, and 800°C. The X-ray diffractometer (XRD) was used to determine the crystalline phase and crystallography properties. Based on XRD data and JCPDS 09-0432, all samples showed the phase of HAp. However, the *β-TCP phase has been found in samples from Scomberomorus commerso, which calcined at 600°C and 800°C. The crystallite size of HAp from Channa striata fish bone increased as followed by higher temperature, and Scomberomorus commerso has the same crystallite size on samples that calcined at 600°C and 800°C. The microstrain of both samples has the same trend: the higher the temperature, the lower the microstrain. The temperature also affected the crystallinity sample; the higher the calcined temperature, the improved crystallinity. Fourier Transform Infrared (FTIR) data determined the functional groups of samples. The functional groups that appeared were OH^- and PO_4^{3-}. The mass of samples before calcined differs from after calcined, where the mass after calcined is smaller than before. The higher the temperature we used, the more decreased yield percentage we got.*

Keywords: *Hydroxyapatite; Fish Bone; Channa striata; Scomberomorus commerson; Heat treatment*

INTRODUCTION

Hydroxyapatite (HAp, $Ca_{10}(PO_4)_6OH_2$) is an inorganic mineral components containing calcium and phospate. This substance has a similar chemical structure to human bones and teeth. Through this similarity, HAp as a biomaterial and bioceramic is often used for biomedical applications, particularly in orthopedic, odontology, and as an implants of coating material. HAp is made up of 70% bone, 20% collagen, and the other 10% of water (Mohd Pu'ad, et al. 2020).

HAp can be obtained from natural materials, especially organic waste which contains high calcium such as eggshell (Mohd Pu'ad, et al., 2021), bovine bones, pig bones, fish bones and scales (Horta, et al. 2023). Other sources of calcium can also be obtained from pigeon bone (Sharifianjazi, F., et al, 2021) and Turkey femur-bone (Esmaeilkhanian, et al, 2019). Fish bones become interesting source of calcium for Hydroxyapatite as they are abundant and easy to obtain. Other research have been successfully extracted fish bones into hydroxyapatite, such as carp fish bones (Hammood, et al., 2019; Callob, et al., 2023), rabbitfish (Fendi, et al. 2023), sardinella longiceps (Surya, et al., 2021), and selayang fish (Afiqah et al., 2020).

A variety of methods have been used to get HAp, such as precipitation (Rizkayanti and Yusuf, 2019), microwave irradiation (Castro, et al. 2022), reflux method (Cahyanto, et al. 2017), heat treatment (Lolo, et.al. 2022), interfacial reaction with multiple emulsion, Solid-state syntesis (Khalid and Chaudhry, 2020) cationic surfactant template (Yusuf, et al. 2019), sol-gel (Charlena, et al. 2022), etc, Heat treatment known as the simplest and cheapest method because there is no need to mix additional sources to obtain HAp (Sunil & Jagannatham, 2016).

The variation of temperature has been used to obtain HAp with heat treatment. The HAp was successfully obtained in Turkey Femur-Bone Waste after heated at 850°C – 1150°C (Esmaeilkhanian, et al. 2019). The carp fish bones do the same after calcined at 900°C – 1100°C (Hammood, et al. 2019). However, lower temperatures enable the ability to obtain HAp from the fish bone. Characteristics of HAp (in major fraction) were observed when Sheelavati (roho labio) fish calcined starting at temperature 600^0, and so does the milkfish bone source (Sunil & Jagannatham, 2016; Lolo, et al. 2022). The lower temperature has been used to get HAp from Lates calcarifer fish bone. The calcined

started at 200°C and observed the characteristics of HAp (Pal, et al. 2017).

This work studied the potency of two kinds of fish bone - Channa striata and Scomberomorus commerson fish bones – as the natural sources of HAp. Heat treatment was used as the method. The temperature of heat treatment were 400°C, 600°C, and 800°C. All samples were heated in the same heater and had the same duration. The X-ray diffractometer (XRD) and The Fourier Transform Infrared (FTIR) were chosen to determine the crystalline phase, crystallography properties, and functional groups of the samples.

METHOD

Channa striata and Scomberomorus commerson fish bones used as biogenic material were taken in Jambi, Indonesia. The fish bones were washed with water first and left in boiling water at 100°C for 10 minutes. Then, the leftover meat was cleaned with the aid of a brush. After that, the cleaned fish bones were placed in acetone for 48 hours. To obtain the dried fish bones, they were dried in direct sunlight for three days and then dried in an oven at 100°C for 3 hours. Completely dried samples were blended in a blender and then crushed with mortar to obtain powder samples and smaller particle sizes. Further, samples were put in the furnace at 400°C, 600°C, and 800°C for 3 hours. In this research, Channa striata samples were given the symbol G, while Scomberomorus commerson samples were given the symbol T.

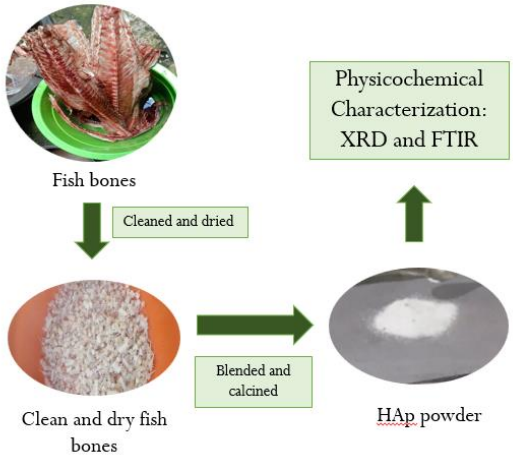

Figure 1. Schematic of methods to fabricate and characterize HAp

The samples were characterized using XRD (XPERT PRO PANalytical PW3040/60) to determine the crystalline phase and crystallography properties. The XRD data were recorded using CuKα radiation at λ =0,154 nm in the range 2θ: 10^0 – 100^0. The XRD data was identified with the help of the Joint Committee on Powder Diffraction Standards (JCPDS) 09-0432. Another characterization was done using FTIR (BRUKER Alpha II sample compartment RT-DLaTGS) to identify the functional groups of the samples, such as OH^- and PO_4^{3-}. The FTIR data was recorded in the range of 400 – 4000 cm^{-1}. The sample was measured directly from the powder. The detector of FTIR is DTGS, and the measurement mode is attenuated total reflectan (ATR).

RESULT AND DISCUSSION

The XRD determined the samples' crystalline phase and crystallography properties. Figure 2 shows the diffraction pattern resulting from the HAp samples. HAp phase has been found in all samples based on JCPDS 09-0432. According to Figure 2 (a), there is no other phase except the HAp phase in the HAp from Channa striata fish bones. However, another phase was found in samples from Scomberomorus commerson fish bones, specifically for samples heated at 600°C and 800°C. The phase of β-TCP appeared in samples from Scomberomorus commerson fish bone. Based on JCPDS 09-0169, the peak of β-TCP was formed at the position of the diffraction angle 31.2° and 34.7° with the diffraction plane (300) and (202) for calcined at 600°C. Then the peak of β-TCP was formed at 29.9°, 31.3°, and 34.6° with the diffraction plane (211), (300), and (202) for calcined at 800°C. The produced calcium phase might be deficient with calcium and calcined at high temperatures (beyond 650 °C). The calcium phosphate phase is transformed to β – tricalcium phosphate (β – TCP) phase if it heats at high temperatures for that source (Sunil & Jagannatham, 2016). This has not only happened in Scomberomorus commerson sources but also other kind of fish, such as Tylosurus crocodilus (Permatasari et al. 2019) and Sheelavati (Sunil & Jagannatham, 2016). Hence, no other phase was not detected on the HAp from the Channa striata fish bone. The HAp from the Channa striata fish bone was pure single phase.

Figure 2(a) shows that different temperatures affected the amount of HAp phase. Samples heated at 800°C had the greatest peak of HAp, with three main peaks: (211), (112), and (300). It means the higher the temperature we used, the more HAp phase formed. The peak became sharper as the temperature increased. The width of the lines becomes narrower, indicating an increase in the crystallinity degree (Ahmed, et al. 2015). The broader peak at samples

were heated at 400°C and 600°C, meaning their crystallinity degree was smaller than those at 800°C.

The crystallography properties - such as crystalline size, microstrain, crystallinity, and lattice

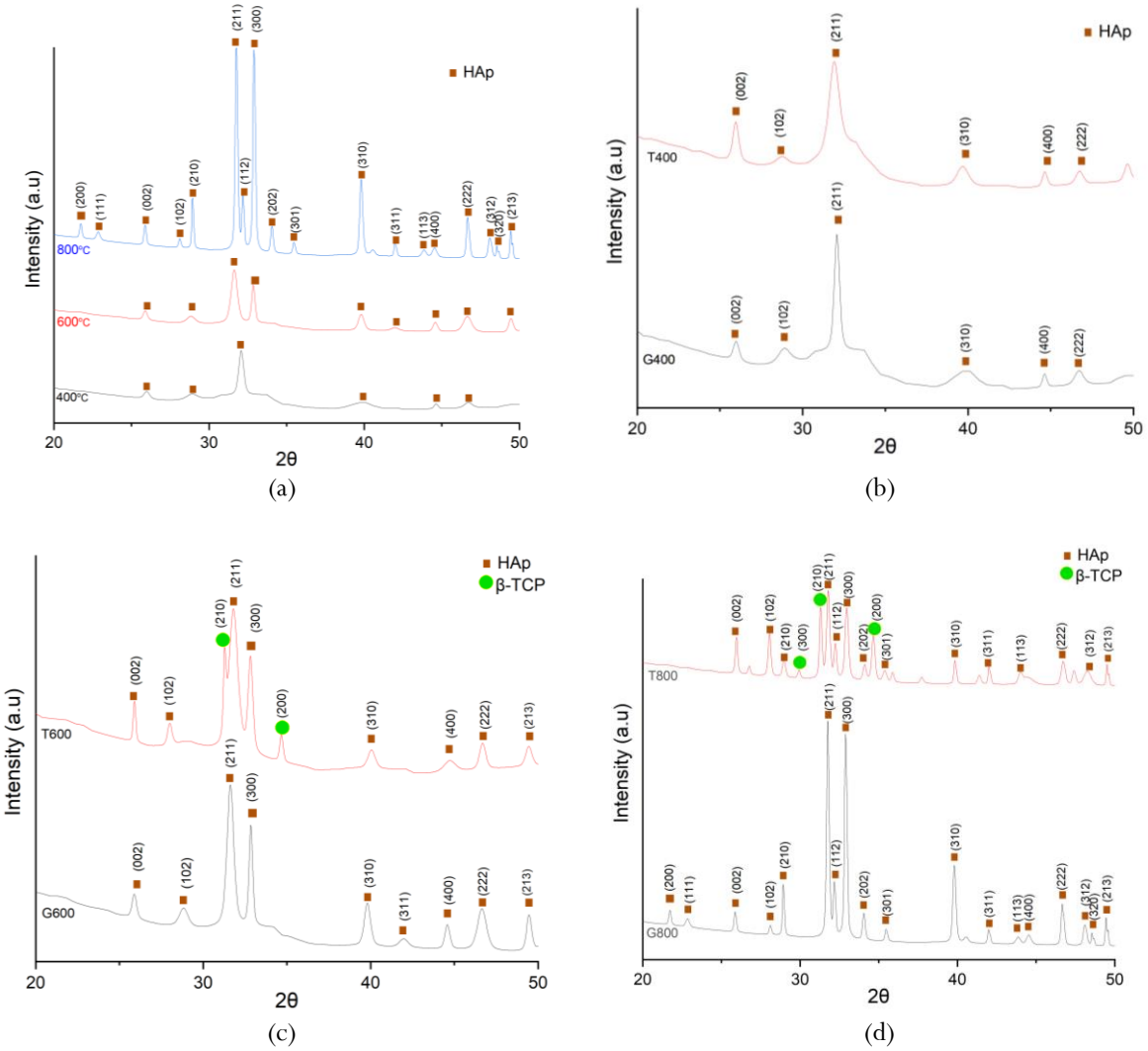

Figure 2. (a) XRD patterns of HAp powders from Channa striata fish bone; XRD pattern of different sources of HAp after heat treatment at temperatures: (b) 400°C; (c) 600°C; (d) 800°C.

Table 1. Crystallography properties of HAp based on XRD data

Samples	Crystallite size (nm)	Microstrain	Crystallinity (%)	Lattice parameter (Å)		
				a	c	c/a
G400	19.76	0.44	-	9.38	6.86	0.73
G600	26.35	0.33	59.5	9.39	6.89	0.73
G800	52.70	0.17	88.9	9.40	6.89	0.73
T400	19.76	0.44	-	9.38	6.87	0.73
T600	52.70	0.17	47.6	9.37	6.89	0.74
T800	52.70	0.17	75.3	9.41	6.87	0.73

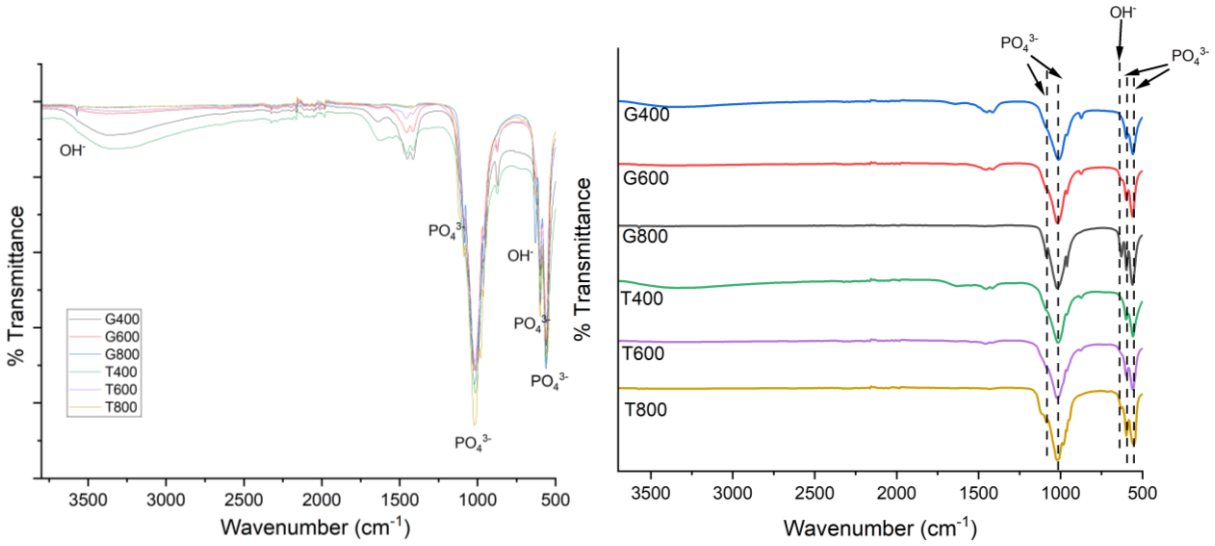

Figure 3. FTIR spectra of HAp powders from Channa striata and Scomberomorus commerson fish bones.

Table 2. Characteristic functional groups of HAp based on FTIR data

Samples	Wavenumber (cm^{-1})					
	(OH^-)		(PO_4^{3-})			
G400	-	-	560	595	1014	-
G600	632	3570	560	599	1020	1088
G800	632	3570	560	597	1026	1088
T400	-	-	558	599	1020	-
T600	636	3570	560	601	1020	1090
T800	632	3570	556	599	1022	1088

Table 3. Yield percentage of samples after heat treatment

Samples	Before heat treatment (g)	After heat treatment (g)	Yield Percentage (%)
G400	9.2	6.1	66.6
G600	24.0	15.1	63.0
G800	20.5	12.5	61.1
T400	57.5	26.6	46.3
T600	42.3	17.3	41.0
T800	42.3	18.3	43.3

crystal that appeared as a distortion or dislocation. The low microstrain showed a few deffect of crystal (Anggraini, et al. 2021).

HAp has parameter lattice a = 9.41 Å and c = 6.88 Å (based on JCPDS 09-0432). Lattice parameters a and c of both samples were almost the same. The different calcined temperatures did not affect the lattice parameter of HAp from fish bones. However, the temperature affected on crystallinity,

morphology, and crystal size of HAp (Sunil & Jagannatham, 2016).

Figure 3 presents the FTIR spectra of HAp after heat treatment in different temperatures and from different sources. The hydroxyl (OH^-) and phosphate (PO_4^{3-}) functional groups appeared on samples. As shown in Table 2, the bands at 632-636 cm^{-1} and 3570 cm^{-1} represent OH^- groups. Those bands were stretching vibration of low-intensity O-H groups (Shi, 2018). The samples exhibited transmittance of the functional groups of PO_4^{3-} at 556-560 cm^{-1}, 595-601 cm^{1}, and 1014-1026 cm^{-1}. The bands at 556-569 and 595-601 represent the bending mode of P-O groups (Sari and Yusuf, 2018; Pal, 2017), while 1014-1026 cm^{-1} and 1088-1090 cm^{-1} represents the stretching mode of P-O groups (Sari, et al. 2021; Siddiqi and Azhar, 2020

Table 3 shows the yield of samples after the calcined process. The samples from Channa striata fish bones had a higher percentage than Scomberomorus commerson fish bones. Also, the higher the temperature we used, the more decreased yield percentage we got. It happened not only in the sample from Channa striata but also from Scomberomorus commerson.

CONCLUSION

Hydroxyapatite (HAp) has succesfully produced from Channa striata and Scomberomorus commerson fish bones with heat treatment method. The characteristics of HAp, including crystalline phase, crystallographic properties, and functional groups, were influenced by the temperatures. The samples from Channa striata fish bones has pure HAp phase based on XRD data. However , a few $\beta - TCP$ phase has found in samples from Scomberomorus commerson fish bones. The heat treatment did not affect lattice parameters, but changed the crystallite size, the microstrain, and the crystallinity. The higher temperature, the higher crystallite size and crystalinity and the lower the microstrain. Based on FTIR data, the functional group HAp has found, such as hydroxyl (OH^-) and phosphate (PO_4^{3-}). The temperatures also affected the mass of sample after calcined.

ACKNOWLEDGEMENTS

The authors are immensely grateful to the PNPB funding of Universitas Jambi for its supports. The authors acknowledge the use of the facilities and the technical assistance of the Physics Laboratory and the staff at Sains and Technology Faculty Universitas Jambi, Indonesia.

REFERENCES

Ahmed, Y. M. Z., El-Sheikh, S. M., & Zaki, Z. I. (2015). Changes in hydroxyapatite powder properties via heat treatment. *Bulletin of Materials Science,* 38(7), 1807–1819.

Anggraini, R. M., Sari, M., Aminatun, T., Suciati, K., Dahlan, Yusuf, Y.2021. Nanostructure of carbonated hydroxyapatite precipitation extracted from pearl shells (Pinctada maxima) by pH treatment. *Digest Journal of Nanomaterials and Biostructures*. Vol. 16, No. 4, p. 1619-1625

Afiqah, I.Q., Aziz, N.A.N., Nurhaziqah, A.M.S., and Hasiah, S. 2020. Biocompatible Hydroxyapatite Derive from Selayang Fish Bone Via Mechanochemical Treatment. *Solis State Phenomena 307, 339-334.*

Cahyanto, A., Kosasih, E., Aripin, D., Hasratiningsih, Z. 2017. Fabrication of hydroxyapatite from fish bone waste using reflux method. *Materials Science and Engineering*. 172 012006

Castro,M. A. M., Portela, T.O., Correa, G.S., Olivera, M. M., Rangel, J.G., Rodrigues, S.F. Mercury, J.M.R. 2022. Synthesis of hydroxyapatite by hydrothermal and microwave irradiation methods from biogenic calcium source varying pH and synthesis time. *Boletín de la Sociedad Española de Cerámica y Vidrio*. Volume 61 Issue 1 Pages 35-41

Charlena, C., Maddu, A., Hidayat,T. 2022. Synthesis and Characterization of Hydroxyapatite from Green Mussel Shell with Sol-Gel Method. *Jurnal Kimia VALENSI.* Volume 8, No. 2

Challob, D.A., Saleh, A.T., Alshemary, A.Z., Muhammed, Y., and Cardakli, I.S. 2023. Characterization and in vitro bioactivity of hydroxyapatite extracted from carp fish bones for biomedical applications. *Ceramics-Silikaty (1), 81-86.*

Esmaeilkhanian, A., Sharifianjazi, F., Abouchenari, A., Rouhani, A., Parvin, N., and Irani, M. 2019. Synthesis and Characterization of Natural Nano-hydroxyapatite Derived from Turkey Femur-Bone Waste. Applied Biochemistry and Biotechnology

Fendi, F., Abdullah, B., Suryani, S., Raya, I., and Tahir, D. 2023. The use of waste bones of rabbitfish (Siganus sp.) for the synthesis of hydroxyapatite. *IOP Conference series*. 1230(1).

Hammood, A.S., Hassan, S.S., Alkhafagy, M.T., and Jaber, H.L. 2019. Effect of calcination

temperature on characterization of natural hydroxyapatite prepared from carp fish bones. *Springer Nature Applied Science* **1**(436),

Horta, M.K.D.S., Westin, C., Rocha, D.N.D., Campos, J.B.D., Souza, R.F.M.D., Aguilar, M.S., and Moura, F.J. 2023. Hydroxyapatite from biowaste for biomedical applications: obtainment, characterization and *in vitro* assays. *Materials Research*, *26*, e20220466.

Khalid, H., & Chaudhry, A. A. (2020). *Basics of hydroxyapatite—structure, synthesis, properties, and clinical applications. Handbook of Ionic Substituted Hydroxyapatites,* Woodhead Publishing Series in Biomaterials 85–115.

Lolo, J. A., Ambali, D.P.P., Jefrianto, W., Handayani, D., Afridah, W., Wikurendra, E. A., Amalia, R., Syafiuddin, A. 2022. Synthesis and Characterization of Hydroxyapatite Derived from Milkfish Bone by Simple Heat Treatments. *Biointerface research in applied chemistry*. Volume 12, Issue 2, 2440 – 2449.

Mohd Pu'ad, N.A.S., Haq, R.H.A., Noh, H.M., Abdullah, H.Z, Idris, M.I., and Lee, T.C. 2020. Synthesis method of hydroxyapatite: A review. *Materials Today: Proceedings 29(1), 233-239*.

Mohd Pu'ad, N.A.S., Alipal,J., Abdullah, H.Z., Idris, M.I., Lee, T.C. 2021. Synthesis of eggshell derived hydroxyapatite via chemical precipitation and calcination method. *Materials Today: Proceedings 42(1), 172-177,*

Pal, A., Paul, S., Choudhury, A. Y., Balla, V. K., Das, M., Sinha, A. 2017. Synthesis of hydroxyapatite from Lates calcarifer fish bone for biomedical applications. *Materials Letters.* Volume 203 Pages 89-92

Permatasari, H.A., Wati, R., Anggraini, R.M., Almukarramah, Yusuf, Y. 2019. Hydroxyapatite Extracted from Fish Bone Wastes by Heat Treatment. *Key Engineering Materials,* Vol. 840, pp 318-323.

Sari, M., & Yusuf, Y. 2018. Synthesis and Characterization of Hydroxyapatite based on Green Mussel Shells (*Perna viridis*) with Calcination Temperature Variation Using the Precipitation Method. *International Journal of Nanoelectronics and Materials.* Volume 11, No. 3 [357-370]

Sari, M., Hening, P., Chotimah, Ana, D.I., Yusuf, Y. 2021. Bioceramic hydroxyapatite-based scaffold with a porous structure using honeycomb as a natural polymeric Porogen for bone tissue engineering. *Biomaterials Research* (2021) 25:2

Sharifianjazi, F., Esmaeilkhanian, A., Moradi, M., Pakseresht, A., Asl, M.S., Maleh, H.K., Jang, H W., Shokouhimehr, M., and Varma, R.S. 2021. Biocompatibility and mechanical properties of pigeon bone waste extracted natural nano-hydroxyapatite for bone tissue engineering. *Materials Science and Engineering* B 264 .

Shi, P., Liu, M., Fan, F., Yu, C., Lu, W., Du, M.2018. Characterization of natural hydroxyapatite originated from fish bone and its biocompatibility with osteoblasts. *Materials Science & Engineering*. 706–712

Siddiqi, S. A., & Azhar, U. 2020. *Carbonate substituted hydroxyapatite. Handbook of Ionic Substituted Hydroxyapatites,* Woodhead Publishing Series in Biomaterials. 149–173.

Sunil, B.R. & Jagannatham, M. 2016. Producing hydroxyapatite from fish bones by heat treatment. *Materials Letters*. 185, 411–414

Surya, P., Nithin, A., Sundaramanickam, A., and Sathish, M. 2021. Synthesis and characterization of nano-hydroxyapatite from *Sardinella longiceps* fish bone and its effects on human osteoblast bone cells.*Journal of the Mechanical behavior of Biomedical Materials, 119.*

Rizkayanti, Y., and Yusuf, Y.2019. Optimization of the Temperature Synthesis of Hydroxyapatite from Indonesian Crab Shells. *International Journal of Nanoelectronics and Materials.* Volume 12, No. 1, [85-92]

Yusuf, Y., Khasanah, D.U., Syafaat, F.Y., Pawarangan, I., Sari, M., Mawuntu, V. J., Rizkayanti, Yazida. 2019. *Hidroksiapatit Berbahan Dasar Biogenik*. Yogyakarta: Gadjah Mada University Press.