Ceramics-Silikáty 69 (1), 132-137 (2025)
www.ceramics-silikaty.cz doi: 10.13168/cs.2024.0072

# ENHANCING THE POTENTIAL OF PLECO FISH BONES (HYPOSTOMUS PLECOSTOMUS) WITH GRADUAL HEATING AND BALL MILLING METHODS FOR BIOMEDICAL APPLICATIONS

NURFITRI RAHMI SARI*, #GUNAWARMAN GUNAWARMAN*, RIZA VERDIAN*, ADE INDRA**, IS PRIMA NANDA*, RISKA ASTIN FITRIA***

*Mechanical Engineering Department, Engineering Faculty, Universitas Andalas, Limau Manis 25166 Padang, West Sumatera, Indonesia
**Mechanical Engineering Department, Engineering Faculty, Institut Teknologi Padang, Kp Olo 25143 Padang, West Sumatera, Indonesia
***Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia

#E-mail: gunawarman@eng.unand.ac.id

Submitted October 15, 2024, accepted November 18, 2024

**Keywords:** Ball Milling, Biomedical Applications, Hydroxyapatite, Gradual Heating, Pleco Fish Bones

*Fish bones are a rich source of naturally occurring calcium phosphate that can be processed to obtain hydroxyapatite (HA). HA was produced by the gradual heating and ball milling of pleco bones to 900 °C for 5 hours. HA was characterised using scanning electron microscopy (SEM), energy dispersive x-ray (EDX), x-ray diffraction (XRD), and Fourier transform infrared spectroscopy (FTIR) analyses. The SEM images showed HA with different morphologies before and after the gradual heating and ball milling process. The EDX analysis indicated the ratio of calcium and phosphate with an atomic ratio of Ca/P of 2.10. The FTIR showed a characteristic wavenumber of the functional groups of HA ($OH^-$ and $PO_4^{3}$). The HA phase was identified through the XRD analysis. HA obtained from pleco fish bones (Hypostomus plecostomus) has the potential to be used in bone repair and biomedical applications.*

## INTRODUCTION

A biomaterial refers to any natural or synthetic material that can be replaced another organ or structure in the human body [1]. Biomedical uses of calcium phosphate (CaP) are attributed to their resemblance to the mineral constituents found in bone tissue [2]. Nowadays, the preparation of CaP from natural sources has been intensively studied, including the costs, the efficiency, and preparation methods. Hydroxyapatite (HA), which has the chemical formula $Ca_{10}(PO_4)_6(OH)_2$, constitutes a significant mineral component in the bones and teeth of vertebrates [3-6].

HA is also a major inorganic component found in the hard tissues of bones having approximately 60 – 65 % of the mineral phase in human bones. HA is one of the most important biomaterials possessing all distinguishing features, such as being bioactive, biodegradable, non-inflammatory, non-toxic, biocompatible, osteoconductive, non-immunogenic and having anti-bacterial properties [7-16]. HA has garnered significant attention, especially in the field of biomedical applications like tissue engineering [17-20], coating metal implants [21--25], a replacement for damaged bones [8, 26, 27] and as a coating dental implant [28].

The utilisation of HA products challenges researchers to develop various synthesis methods capable of providing the material characteristics required for each application. HA can be extracted from natural sources, such as bovine bones [29-31], pig bones [32], fish bones [26, 27, 33-37], cockle shells [38], and egg shells [39-41]. Cockle shells and egg shells can be used as a precursor, which are different than fish bones and pig bones, however, fish bones can be processed as HA because there are calcium and phosphate in the bones.

The HA products are mostly prepared from synthetic reagent-grade materials through combinations of chemical, thermal, and mechanical procedures. Various typical approaches can be used to obtain HA such as calcination [10, 20, 42-47], alkaline hydrolysis [48], hydrothermal methods [39, 49], precipitation methods [37, 50] and a combination of these techniques [6].

Fish bones are one of the natural sources that can produce HA. Fish bones constitute a primary waste

product in fish processing and are often discarded. Some types of fish used as the raw material for HA production include barramundi [51], tilapia [14] and carp [36], using temperatures ranging from 650 – 900 °C. The obtained Ca/P ratio from barramundi fish bones at 650 °C is 1.848 [51], while the synthesis of HA using tilapia fish bones at 900 °C results in a Ca/P ratio of 1.66 [14]. The calcination of fish bones is a simple, efficient, and low-cost method that allows one to obtain good HA yields without using precursors in the production of aggressive residues [14].

The growing research is focused on the utilisation and development of biological resources, especially by utilising organic materials for the manufacture of HA, considering the vast potential of waste processing that can be processed and produce something useful. In this research, HA was extracted from a natural source (fish bone), the simple and cost-effective process was followed by gradual heating and ball milling methods.

## EXPERIMENTAL

### Material

Pleco fish (*Hypostomus plecostomus*) bones totalling 20 kg were collected by researchers in June in the Padang West Sumatera River. Distilled water was used to boil the fish bones. 99 % acetone was used to soak the bones. 96 % alcohol was used to clean the containers or equipment used during the research.

### Pre-treatment of the fish bones

The bones were separated from the meat. The bones were washed with running water. The bones were boiled in distilled water for 4 hours to remove the meat and fat attached to the bones [52]. The bones were soaked in acetone for 3 hours to remove any lingering proteins fats, oils, and organic impurities [36]. The bones were washed in distilled water and dried for 2 days [34].

### Ball mill process stage 1 (before the gradual heating)

The fish bones were crushed with a pestle until they become a coarse powder. After that, the bone powder was ball milled using a Pulverisette 5 classic line planetary mill with a jar volume (250 ml) and zirconia balls with a diameter of 5 mm. The ball-to-powder ratio was 10:1. The applied rotation speed was 150 rpm for a duration of 3 hours.

### Gradual heating process

Heating was carried out using an IFU Muffle Furnace. The gradual heating process was carried out from room temperature to 900 °C for 5 hours with a heating rate of 5 °C·min$^{-1}$, and then cooled in the furnace until it reached room temperature.

### Ball mill process stage 2 (after gradual heating)

The fish bones powder that had been heated gradually was then carried out with a ball milling process using the same method as the ball mill process in stage 1.

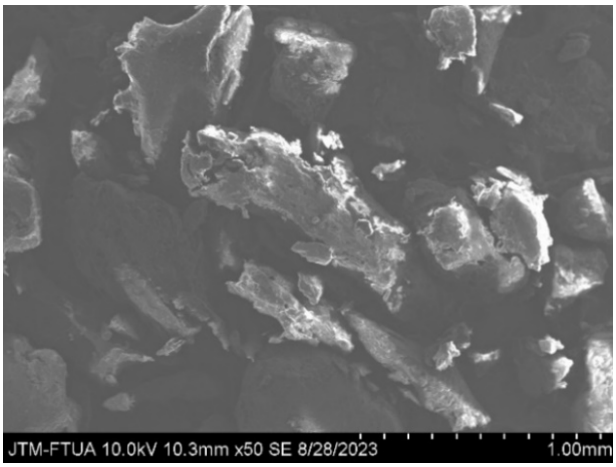
### Characterisation of the fish bone

The characterisation of the fish bone powder was undertaken using a Hitachi S-3400N scanning electron microscopy (SEM) to determine the morphology of the fish bone powder and EDX was used to determine the composition of the fish bone powder. X-ray diffraction (XRD) (PANalytical, Type PW3040/60, the Netherlands) was used to identify the phase composition of the fish bone powder. The HA functional groups were monitored with FTIR. The size distribution of the HA powder was tested using a particle size analyser (PSA).

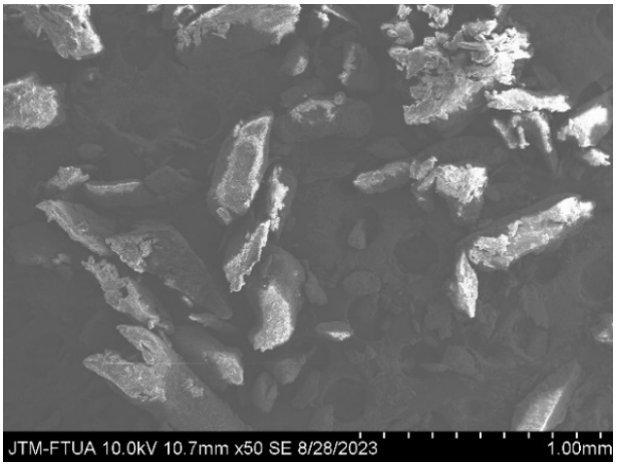
## RESULTS AND DISCUSSION

### SEM and EDX

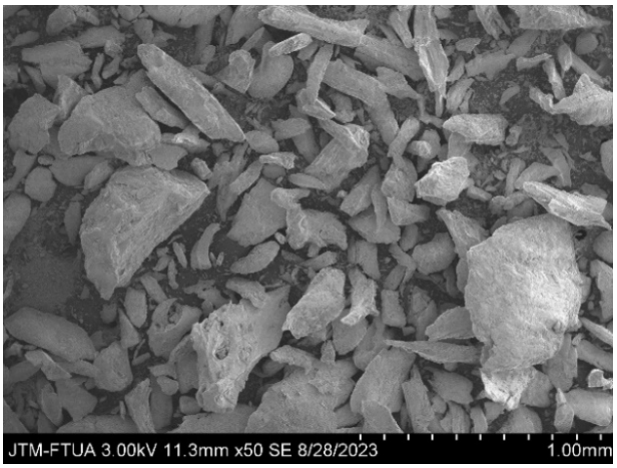
Based on the SEM results in Figure 1a, the raw fish bone (RFB) shows that the powder on the fish bones before the ball mill is still rough and the raw fish bone, in Figure 1b, with ball mill 1 (RFB BM1) shows that the powder is smaller than the powder before the ball mill process stage 1. Figure 1c shows the gradual heating to 900 °C, which was carried out for 5 hours and produced a much finer powder than before the gradual heating. It can also be seen that the powder, after this gradual heating, has become small parts unlike the lumps in figures a and b next, in Figure 1d) 900 °C



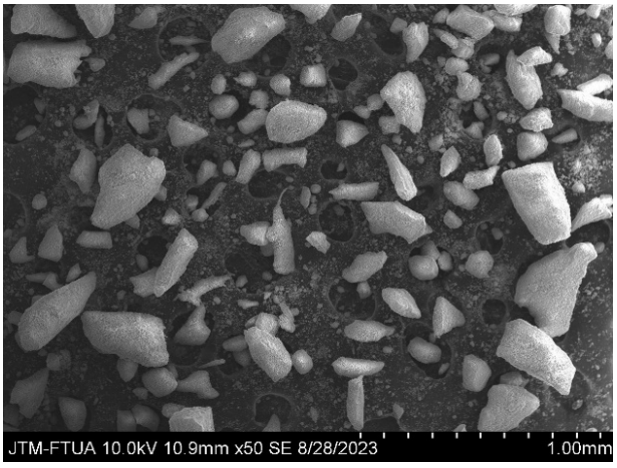

a) RFB




b) RFB BM 1




c) 900 °C




d) 900 °C BM 2

Figure 1. SEM.

ball milling 2 (BM2). The second ball milling process was carried out after 900 °C gradual heating, the results showed that the resulting powder was finer than before the second ball milling process. This shows that the ball milling process can reduce the powder size. Based on the SEM and EDX analyses, the calcium-to-phosphorus (Ca/P) ratios were determined at various stages of the process. The initial Ca/P ratio of the raw fish bones (RFB) was 1.50. After the first ball milling stage, the ratio increased to 1.84. The subsequent calcination resulted in a Ca/P ratio of 2.03, which further increased to 2.10 following the second ball milling stage.

## Particle size analyser (PSA)

The results of the PSA measurements showed that there were significant changes in the particle size before and after ball milling and gradual heating. The obtained particle sizes are 611.7 µm, 486.3 µm, 162.6 µm and 1.903 µm for RFB, RFB BM1, 900 °C, and 900 °C BM2, respectively.

## XRD

The XRD characterisation can be used to identify the phases formed from the synthesised HA. Based on the XRD results on the RFB before and after the ball milling, the HA phase was formed at 2θ = 26.01° and 31.95°. The results of the gradual heating 2d and 2e are in accordance with ICSD standard no. 154781 which indicates that 2a hydroxyapatite phase has been formed. The main peak of HA is at around 2θ = 31°. Figure 2d showed the fish bone powder heated at 900 °C formed HA phases at 2θ = 25.87°, 28.91°, 31.52°, 32.39°, 32.97°, 34.13°, 39.35°, 46.88°, 49.63°. Figures 2d and 2e do not show other 2 phases formed as a result of the grinding using a ball mill.

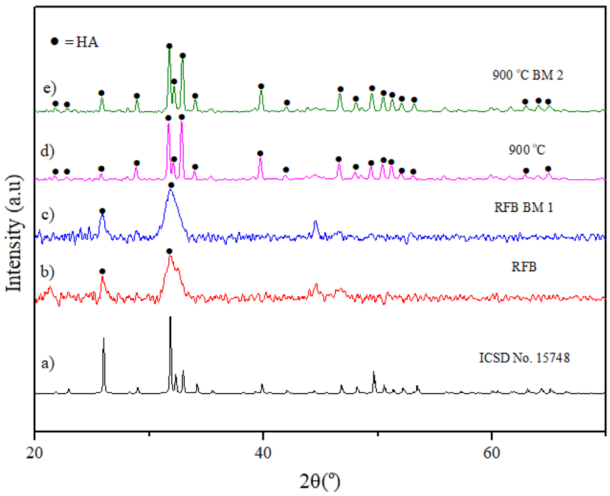



Figure 2. XRD spectrum of a) ICSD Standard No. 157481, b) RFB, c) RFB BM 1, d) 900 °C, e) 900 °C BM 2.

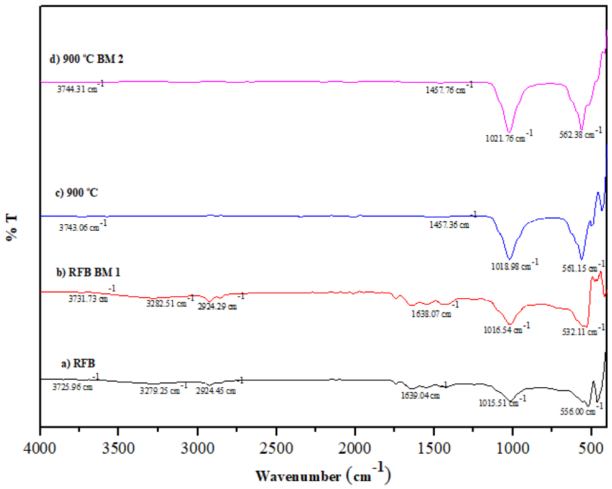



Figure 3. FTIR spectra for a) RFB, b) RFB BM 1, c) 900 °C, d) 900 °C BM 2.

## FTIR

An FTIR characterisation was carried out by identifying the functional groups contained in the fish bone powder. The FTIR spectrum of HA a) RFB, b) RFB BM 1, c) 900 °C, d) 900 °C BM 2 can be seen in the Figure 3. The FTIR spectrum in this study is in the wavenumber range of 400 – 4000 $cm^{-1}$.

Figures 3a, b, c and d show that there is a stretching vibration of the P-O bond from $PO_4^{3-}$ at a wavenumber around 500 $cm^{-1}$ with sharp peaks and a wavenumber around 1000 $cm^{-1}$. Based on the FTIR result, the wavenumbers 1000 – 1100 $cm^{-1}$ and 560 – 609 $cm^{-1}$ were identified as the phosphate groups [53]. Based on Figures 3a, b, c and d, there are OH vibrations around the wavenumber 3700 $cm^{-1}$. The absorption bands within 3700 $cm^{-1}$ are caused by the ion O-H. In the FTIR spectra, there were no intensive peaks which indicated that the organic components in the fish bones were lost during the gradual heating process.

## CONCLUSIONS

The methods of gradual heating and ball milling in this study were used to synthesise HA from fish bones because the process is simple, the reproducibility is easy with a low cost, and has environmental advantages. The HA powder is derived from natural sources that are readily applicable for bone repair and can be used for various other biomedical applications.

### Acknowledgments

*The authors would like to thank the Ministry of Education and Culture for providing PMDSU Scholarship and research budgets from DRTPM Dikti in 2023 and also publication support from the engineering Faculty of Andalas University.*

## REFERENCES

1. Arokiasamy P., Abdullah M.M.A.B., Abd Rahim S.Z. et al. (2022): Synthesis methods of hydroxyapatite from natural sources: A review. *Ceramics International, 48*(11), 14959-14979. doi: 10.1016/j.ceramint.2022.03.064
2. Maidaniuc A., Miculescu F., Ciocoiu R.C. et al. (2020): Effect of the processing parameters on surface, physico-chemical and mechanical features of bioceramics synthesized from abundant carp fish bones. *Ceramics International, 46*(8), 10159-10171. doi:10.1016/j.ceramint.2020.01.007
3. Renda C.G., Ruellas T.M.D.O., Malafatti J.O.D. et al. (2023): A "Zero-Cost" Adsorbing Hydroxyapatite-Based Material from Amazon Fishery Waste for Water Remediation and Nutrient Release for Agriculture. *Physchem, 3*(1), 34-60. doi: 10.3390/physchem3010004
4. Agbeboh N.I., Oladele I.O., Daramola O.O. et al. (2020): Environmentally sustainable processes for the synthesis of hydroxyapatite. *Heliyon, 6*(4), e03765. doi: 10.1016/j.heliyon.2020.e03765
5. Hernández-Ruiz K.L., López-Cervantes J., Sánchez-Machado D.I. et al. (2022): Hydroxyapatite recovery from fish byproducts for biomedical applications. *Sustainable Chemistry and Pharmacy, 28*, 100726. doi: 10.1016/j.scp.2022.100726
6. Hussin M.S.F., Abdullah H.Z., Idris M.I., Wahap M.A.A. (2022): Extraction of natural hydroxyapatite for biomedical applications - A review. *Heliyon, 8*(8), e10356. doi: 10.1016/j.heliyon.2022.e10356
7. Surya P., Nithin A., Sundaramanickam A., 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,* 104501. doi: 10.1016/j.jmbbm.2021.104501
8. Khamkongkaeo A., Jiamprasertboon A., Jinakul N., Srabua P., Tantavisut S., Wongrakpanich A. (2023): Antibiotic-loaded hydroxyapatite scaffolds fabricated from Nile tilapia bones for orthopaedics. I*nternational Journal of Pharmaceutics: X, 5*, 100169. doi: 10.1016/j.ijpx.2023.100169
9. Mohammadi M., Tulliani J.M., Montanaro L., Palmero P. (2021): Gelcasting and sintering of hydroxyapatite materials: Effect of particle size and Ca/P ratio on microstructural, mechanical and biological properties. *Journal of the European Ceramic Society, 41*(14), 7301-7310. doi: 10.1016/j.jeurceramsoc.2021.07.025
10. Daupor H., Wijaya A.R., Amornpitoksuk P., Chelong I.A., Suksuwan A. (2022): Synthesis of Hydroxyapatite from Budu Waste by Calcination Method. *Iran Journal of Chemistry and Chemical Engineering, 41*(12), 4203-4212.
11. Afriani F., Amelia R., Hudatwi M., Tiandho Y. (2020). Hydroxyapatite from natural sources: methods and its characteristics. In *IOP Conference Series: Earth and Environmental Science* (Vol. 599, No. 1, p. 012055). IOP Publishing. doi: 10.1088/1755-1315/599/1/012055
12. Ideia P., Degli Esposti L., Miguel C.C., Adamiano A., Iafisco M., Castilho P.C. (2021): Extraction and characterization of hydroxyapatite-based materials from grey triggerfish skin and black scabbardfish bones. *International Journal of Applied Ceramic Technology, 18*(1), 235-243. doi: 10.1111/ijac.13625
13. Abulyazied D.E., Alturki A.M., Youness R.A., Abomostafa

H.M. (2021): Synthesis, structural and biomedical characterization of hydroxyapatite/borosilicate bioactive glass nanocomposites. *Journal of Inorganic and Organometallic Polymers and Materials, 31*, 4077-4092. doi: 10.1007/s10904-021-02070-6

14. Da Cruz J.A., Weinand W.R., Neto A.M. et al. (2020): Low-cost hydroxyapatite powders from tilapia fish. *Jom, 72*, 1435-1442. doi: 10.1007/s11837-019-03998-4
15. Adamiano A., Scialla S., Carella F. et al. (2023): Simultaneous extraction of calcium phosphates and proteins from fish bones. Innovative valorisation of food by-products. *Journal of Cleaner Production, 385*, 135656. doi: 10.1016/j.jclepro.2022.135656
16. Sathiyavimal S., Vasantharaj S., Shanmugavel M. et al. (2020): Facile synthesis and characterization of hydroxyapatite from fish bones: Photocatalytic degradation of industrial dyes (crystal violet and Congo red). *Progress in Organic Coatings, 148*, 105890. doi: 10.1016/j.porgcoat.2020.105890
17. Granito R.N., Renno A.C.M., Yamamura H., et al. (2018): Hydroxyapatite from fish for bone tissue engineering: A promising approach. *International Journal of Molecular and Cellular Medicine, 7*(2), 80. doi: 10.22088/IJMCM.BUMS.7.2.80
18. Zakaria S.M., Sharif Zein S.H., Othman M.R., Yang F., Jansen J.A. (2013): Nanophase hydroxyapatite as a biomaterial in advanced hard tissue engineering: a review. *Tissue Engineering Part B: Reviews, 19*(5), 431-441. doi: 10.1089/ten.teb.2012.0624
19. Adamski R., Siuta D. (2021): Mechanical, structural, and biological properties of chitosan/hydroxyapatite/silica composites for bone tissue engineering. *Molecules, 26*(7), 1976. doi: 10.3390/molecules26071976
20. Bahraminasab M., Doostmohammadi N., Alizadeh A. (2021): Low-cost synthesis of nano-hydroxyapatite from carp bone waste: effect of calcination time and temperature. *International Journal of Applied Ceramic Technology, 18*(3), 573-582. doi: 10.1111/ijac.13678
21. Nuswantoro N.F., Budiman I., Septiawarman A., Tjong D.H., Manjas M. (2019). Effect of applied voltage and coating time on nano hydroxyapatite coating on titanium alloy Ti6Al4V using electrophoretic deposition for orthopaedic implant application. In *IOP Conference Series: Materials Science and Engineering* (Vol. 547, No. 1, p. 012004). IOP Publishing. doi: 10.1088/1757-899X/547/1/012004
22. Nuswantoro N.F., Maulana I., Tjong D.H., Manjas M., Gunawarman G. (2018): Hydroxyapatite Coating on New Type Titanium, TNTZ, Using Electrophoretic Deposition. *Journal of Ocean, Mechanical and Aerospace-science and Engineering, 56*(1), 1-4.
23. Fajri H., Ramadhan F., Nuswantoro N.F. et al. (2020): Electrophoretic deposition (EPD) of natural hydroxyapatite coatings on titanium Ti-29Nb-13Ta-4.6 Zr substrates for implant material. In *Materials Science Forum* (Vol. 1000, pp. 123-131). Trans Tech Publications Ltd. doi: 10.4028/www.scientific.net/MSF.1000.123
24. Nuswantoro N.F., Manjas M., Suharti N. et al. (2021): Hydroxyapatite coating on titanium alloy TNTZ for increasing osseointegration and reducing inflammatory response in vivo on Rattus norvegicus Wistar rats. *Ceramics International, 47*(11), 16094-16100. doi: 10.1016/j.ceramint.2021.02.184
25. Juliadmi D., Nuswantoro N.F., Fajri H. et al. (2020): The coating of bovine-source hydroxyapatite on titanium alloy (Ti-6Al-4V ELI) using electrophoretic deposition for biomedical application. In *Materials Science Forum* (Vol. 1000, pp. 97-106. Trans Tech Publications Ltd. doi: 10.4028/www.scientific.net/MSF.1000.97
26. Hooi M.T., Phang S.W., Yow H.Y., David E., Kim N.X., Choo H.L. (2021): FTIR spectroscopy characterization and critical comparison of poly (vinyl) alcohol and natural hydroxyapatite derived from fish bone composite for bone-scaffold. In *Journal of Physics: Conference Series* (Vol. 2120, No. 1, p. 012004). IOP Publishing. doi: 10.1088/1742-6596/2120/1/012004
27. Kim S.C., Heo S.Y., Oh G.W., Yi M., Jung W.K. (2022): A 3D-printed polycaprolactone/marine collagen scaffold reinforced with carbonated hydroxyapatite from fish bones for bone regeneration. *Marine Drugs, 20*(6), 344. doi: 10.3390/md20060344
28. Dorcioman G., Grumezescu V., Stan G.E., et al. (2023): Hydroxyapatite thin films of marine origin as sustainable candidates for dental implants. *Pharmaceutics, 15*(4), 1294.doi: 10.3390/pharmaceutics15041294
29. Odusote J.K., Danyuo Y., Baruwa A.D., Azeez A.A. (2019): Synthesis and characterization of hydroxyapatite from bovine bone for production of dental implants. *Journal of Applied Biomaterials & Functional Materials, 17*(2), 2280800019836829. doi: 10.1177/2280800019836829
30. Catalgol Z. (2019): Sintering effect on borosilicate glass-bovine hydroxyapatite composites. *Journal of the Australian Ceramic Society, 55*(4), 1075-1079. doi: 10.1007/s41779-019-00320-y
31. Gani M.A., Budiatin A.S., Lestari M.L.A.D., et al. (2022): Fabrication and characterization of submicron-scale bovine hydroxyapatite: A top-down approach for a natural biomaterial. *Materials, 15*(6), 2324. doi: 10.3390/ma15062324
32. Ofudje E.A., Rajendran A., Adeogun A.I., Idowu M.A., Kareem S.O., Pattanayak D.K. (2018): Synthesis of organic derived hydroxyapatite scaffold from pig bone waste for tissue engineering applications. *Advanced Powder Technology, 29*(1), 1-8. doi: 10.1016/j.apt.2017.09.008
33. Aydin G., Terzioğlu P., Öğüt H., Kalemtas A. (2021): Production, characterization, and cytotoxicity of calcium phosphate ceramics derived from the bone of meagre fish, Argyrosomus regius. *Journal of the Australian Ceramic Society, 57*, 37-46. doi: 10.1007/s41779-020-00513-w
34. Abdulkadhim A.A., Abdulameer N. (2021): Experimental and numerical study to prepare hydroxyapatite powder from fish bones. *Transactions on Electrical and Electronic Materials, 22*(4), 481-488. doi: 10.1007/s42341-020-00254-4
35. Anggresani L., Sari Y.N., Rahmadevi R. (2021): Hydroxyapatite (HAp) from tenggiri fish bones as abrasive material in toothpaste formula. *Jurnal Kimia Valensi, 7*(1), 1-9. doi: 10.15408/jkv.v7i1.19165
36. Challob D.A., Saleh A.T., Alshemary A.Z., Muhammed Y., Cardakli I.S. (2023): Characterization and in vitro bioactivity of hydroxyapatite extracted from carp fish bones for biomedical applications. *Ceramics-Silikaty, 1*, 81-86. doi: 10.13168/cs.2023.0005
37. Wardani S.C., Sujuti H., Mustamsir E., Hapsari D.N. (2020). Synthesis and potential of skipjack tuna bone hydroxyapatite as bone tissue engineering biomaterial. In *Journal of Physics: Conference Series* (Vol. 1665,

No. 1, p. 012032). IOP Publishing. doi: 10.1088/1742-6596/1665/1/012032
38. Razali N.A.I.M., Pramanik S., Osman, N.A.A., Radzi Z., Pingguan-Murphy B. (2016): Conversion of calcite from cockle shells to bioactive nanorod hydroxyapatite for biomedical applications. *Journal of Ceramic Processing Research, 17*(7), 699-706.
39. Kusuma H.H., Sifah L., Anggita S.S. (2021). The characterization of hydroxyapatite from blood clam shells and eggs shells: Shyntesis by hydrothermal method. In *Journal of Physics: Conference Series* (Vol. 1918, No. 2, p. 022040). IOP Publishing. doi: 10.1088/1742-6596/1918/2/022040
40. Sabry A.A.A., Salih N.A. (2020): Synthetic properties of hydroxyapatite powder prepared from natural eggs shell. *Journal of Green Engineering, 10*(7), 3498-3507.
41. Apalangya V., Rangari V., Jeelani S., Dankyi E., Yaya A., Darko S. (2018): Rapid microwave synthesis of needle-liked hydroxyapatite nanoparticles via template directing ball-milled spindle-shaped eggshell particles. *Ceramics International, 44*(6), 7165-7171. doi: 10.1016/j.ceramint.2018.01.161
42. Malau N.D., Faradiba F., Bintang H.C.D., Vita, N. (2022): Calcination of CaO compounds from cow bone waste and tuna fish bones. *International Journal of Progressive Sciences and Technologies, 32*(1), 67-72.
43. Wu S.C., Hsu H.C., Hsu S.K., Tseng C.P., Ho W.F. (2019): Effects of calcination on synthesis of hydroxyapatite derived from oyster shell powders. *Journal of the Australian Ceramic Society, 55*, 1051-1058.doi: 10.1007/s41779-019-00317-7
44. Desmond S., Kurniawan D., Nor F.M. (2020). Synthesis of hydroxyapatite from seashells via calcination at various temperature using microwave and furnace. In *AIP Conference Proceedings* (Vol. 2217, No. 1). AIP Publishing. doi: 10.1063/5.0000902.
45. Dermawan S.K., Ismail Z.M.M., Jaffri M.Z., Abdullah H.Z. (2022). Effect of the calcination temperature on the properties of hydroxyapatite from black tilapia fish bone. In *Journal of Physics: Conference Series* (Vol. 2169, No. 1, p. 012034). IOP Publishing. doi: 10.1088/1742-6596/2169/1/012034
46. Abdullah N.H., Mohamed Noor A.A., Mat Rasat M.S., et al. (2020). Preparation and characterization of calcium hydroxyphosphate (Hydroxyapatite) from tilapia fish bones and scales via calcination method. In *Materials Science Forum* (Vol. 1010, pp. 596-601). Trans Tech Publications Ltd.doi: 10.4028/www.scientific.net/MSF.1010.596
47. Hasan M.R., Ghazali M.S. M., Mohtar N.F. (2021): Correlation of heating profile with calcination temperature for the extraction of nano hydroxyapatite (Nano-HAp) derived from bone. *Journal of Mechanical Engineering and Sciences, 15*(1), 7792-7806. doi: 10.15282/jmes.15.1.2021.15.0615
48. Le Ho K.H., Dao V.H., Pham X.K., et al. (2022): Physicochemical properties, acute and subchronic toxicity of nano-hydroxyapatite obtained from Lates calcarifer fish bone. *Regional Studies in Marine Science, 55*, 102560. doi: 10.11648/j.ajcbe.20210501.15
49. Hussain S., Sabiruddin K. (2021): Effect of heat treatment on the synthesis of hydroxyapatite from Indian clam seashell by hydrothermal method. *Ceramics International, 47*(21), 29660-29669. doi: 10.1016/j.ceramint.2021.07.137
50. Sinurat E., Dewi F.R., Fransiska D., Nurbayasari R. (2022). Synthesis and characterization of hydroxyapatite of cockle shells (anadara granosa) originated from Indonesia through precipitation method. In *IOP Conference Series: Earth and Environmental Science* (Vol. 1118, No. 1, p. 012035). IOP Publishing. doi: 10.1088/1755-1315/1118/1/012035
51. Le Ho K.H., Dao V.H., Pham X.K., et al. (2022): Physicochemical properties, acute and subchronic toxicity of nano-hydroxyapatite obtained from Lates calcarifer fish bone. *Regional Studies in Marine Science, 55*, 102560. doi: 10.1016/j.rsma.2022.102560
52. Latif A.F.A., Mohd Pu'ad N.A.S., Ramli N.A.A. et al. (2020) Extraction of biological hydroxyapatite from tuna fish bone for biomedical applications. In *Materials Science Forum* (Vol. 1010, pp. 584-589). Trans Tech Publications Ltd. doi: 10.4028/www.scientific.net/MSF.1010.584
53. Ur Rehman M., Qureshi A., & Baloch M.M. (2021): Extraction of hydroxyapatite from caprine bones and its anti-bacterial study. *Mehran University Research Journal Of Engineering & Technology, 40*(4), 867-873. doi: 10.22581/muet1982.2104.16