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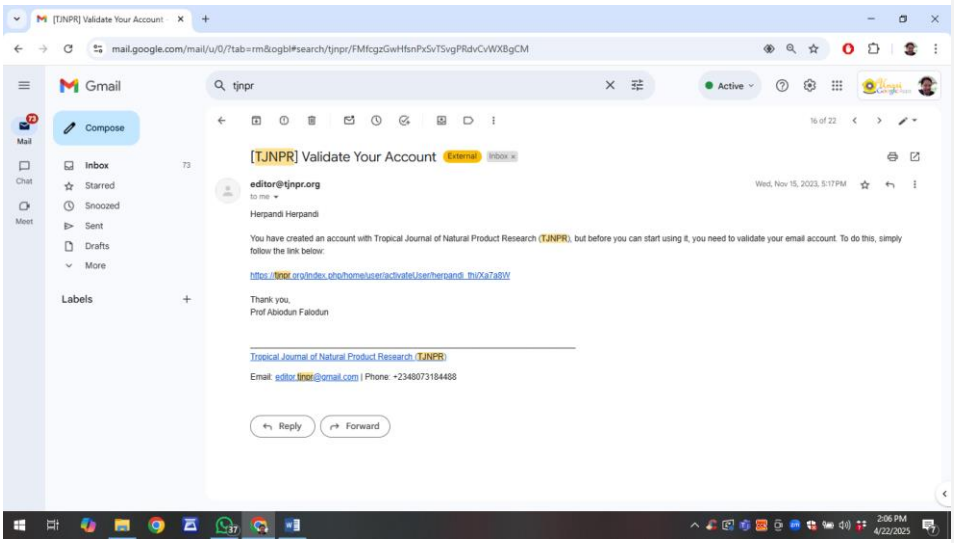

Manuscript

Hydroxyapatite Characteristics from Snakehead Fish (*Channa striata*) Bone via Alkali Treatment followed by Calcination Method

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Abstract

Snakehead fish (*Channa striata*) is commonly used as a raw material in traditional foods in South Sumatra. However, some parts of this fish, such as bone, skin, and viscera, are not used during this food processing. This study aimed to determine the characterization of hydroxyapatite snakehead fish bone with different extraction times using ultrasound-assisted extraction followed by the calcination method. The hydroxyapatite was extracted using sodium hydroxide (NaOH) with three different extraction times (20, 40, and 60 minutes) before continuing with calcination. The extraction yield is about 16.03%-19.99%. The smallest particle size is found in 40 minutes of extraction time (63.90 nm). The ash content of the hydroxyapatite is about 98.09% - 99.04%, calcium is about 17.86% - 18.12%, and the phosphorus is about 10.23% - 10.74%. The non-stoichiometric form is found in the hydroxyapatite from snakehead fish bone with a Ca/P ratio of about 1.69 - 1.72. Analysis of the hydroxyapatite functional groups of snakehead fish bone showed the presence of phosphate groups (PO_4^{3-}), carbonate groups (CO_3^{2-}), and hydroxyl groups (OH^-). These data indicated that the hydroxyapatite was successfully extracted from snakehead fish bone in nanoparticle form. Therefore, snakehead fish bone can be used as a source of hydroxyapatite.

Keywords: Calcination; *Channa striata*; extraction; hydroxyapatite; phosphorus

Introduction

Consumption of fish products has increased significantly since they were identified as essential to a healthy, balanced diet and way of life. As a result, there has been a sharp rise in the global production of fish waste [1]. Fish waste is a potential source of minerals or trace metals, essential fatty acids, amino acids, peptides, and protein of high biological value as well as vitamins [2]. Therefore, fish waste thus offers enormous untapped potential for value addition. The fish waste includes fish bones, scales, and fins. Whereas, the fish bone waste is around 9-15% [3, 4].

The fish bone waste composed of various mineral such as calcium, phosphorus, zinc, and magnesium that can be used a source of hydroxyapatite [5, 6].

Hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] is a common of the calcium phosphates that has compositions similar to natural bone [6, 7]. Due to its bioactive qualities and osteoconductive properties, hydroxyapatite has garnered interest for use in bone grafting procedures. Additionally, other medical applications of hydroxyapatite have also been studied because hydroxyapatite is non-toxic, bioactive, biocompatible, and non-inflammatory [2]. Natural hydroxyapatite has recently been extracted from various biowastes, including fish waste such as fish bone [6, 8, 9]. Various methods have been used to hydroxyapatite extraction from the natural sources, including calcination, alkali treatment, and the combination methods [10]. A previous study reported that calcination method was used for hydroxyapatite extraction [11, 12]. NaOH treatment also was reported for the extraction hydroxyapatite from bovine bone [13]. Additionally, hydroxyapatite successfully extracted from tilapia fish scale using combination method (NaOH treatment and calcination) [14].

Snakehead fish (*Channa striata*) is a major source material for a traditional fish cake dish from South Sumatra, Indonesia, called "pempek". This product is made from snakehead fish meal; therefore, some parts of the fish become waste, including the bones. However, the study about the utilization of fish bone waste from this snakehead fish has not yet been reported. Therefore, this study aimed to determine the characteristics of hydroxyapatite from snakehead fish bone using a combination of alkali (NaOH) treatment and calcination process.

Materials and Methods

Sample preparation

The snakehead fish bone preparation was performed according to the previous methods [6, 15]. Briefly, the fish bones were washed and boiled at 80°C for 30 minutes. After that, it was cleaned and mixed with lime water, and then it was boiled for 3 hours. The fish bone was cooled at room temperature for 15 minutes and dried in an oven at 80°C for 12 hours. The dried fish bone was then ground to powder (80 mesh) then continued to the calcination process.

Hydroxyapatite extraction and calcination process

The hydroxyapatite extraction and calcination processes from fish bones were performed according to the previous methods [6, 16]. Briefly, 10 g of the fish bone powder was mixed with 1 N NaOH and then extracted using an ultrasonic bath at 50°C for different extraction times (20, 40, and 60 minutes). After the extraction times, it was cooled, filtrated using filter paper (Whatman No. 42), and neutralized using distilled water. Then, it was oven-dried at 105°C for 3 hours before the calcination process. The calcination process was performed using a muffle furnace at 600°C for 5 hours. After that, it was cooled to room temperature and kept for further analysis.

Hydroxyapatite characterizations

The ash content analysis was determined according to the AOAC (2000). Briefly, the hydroxyapatite powder was completely incinerated in a muffle furnace at 550°C (AOAC method 930.05). Whereas the element composition was analyzed using energy dispersive X-ray spectroscopy (EDS) and the functional group of the hydroxyapatite powder was analyzed using a Fourier transform infrared spectrometer (FTIR, Brucker Tensor 27) according to the previous method [2]. The particle size of the hydroxyapatite powder was analyzed using a particle size analyzer (Malvern Zetasizer Nano ZS Ver. 7.01) and the hydroxyapatite powder was also viewed by scanning electron microscopy (SEM, JEOL JSM-7000F FE-SEM, Japan) according to the previous methods [2, 17].

Statistical analysis

The data on yield, ash content, and particle size were expressed as the mean ± standard deviation (SD). It was analyzed using Duncan's multiple range test ($p<0.05$) using SPSS software (ver. 22.0; IBM Corporation, Armonk, NY, USA). Whereas, the mineral composition, functional groups and scanning electron microscope image were analyzed using descriptive analysis.

Results and Discussion

Yield of the hydroxyapatite

The yield of the snakehead fish bone hydroxyapatite is shown in **Figure 1.** The yields of the hydroxyapatite are about 16.03% - 19.99%. The highest yield of the hydroxyapatite is found in 60 minutes of extraction time and the lowest in 20 minutes. Whereas, there is no significant ($p>0.05$) difference between the yield of 40 minutes and other treatments. However, the yield of 20 minutes was significantly ($p<0.05$) different from 60 minutes of extraction time. A previous study reported that hydroxyapatite yield from bigeye snapper (*Priancanthus tayenus*) bones is about 13.4% [18]. Generally, there are two main methods for obtaining hydroxyapatite: chemical synthesis and biological resource extraction. Compared to synthetic forms, animal bone hydroxyapatite has a few advantages in terms of chemical composition and structure, which are identical to human bone, reduced manufacturing costs, and enhanced biological response [12]. The organic content in the bone is normally removed using an alkaline solution, such as NaOH. The organic component of the bone is hydrolyzed by the NaOH solution, and the remaining calcium phosphate is then washed and filtered. Whereas, calcination was used to completely remove the organic content from the bone [10].

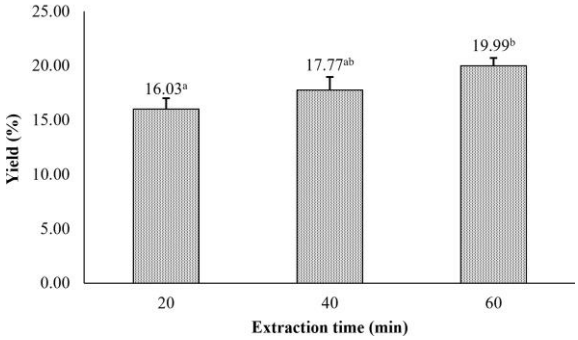

Figure 1. The yield of snakehead fish (*C. striata*) bone hydroxyapatite with different extraction time. Data are shown as mean ± SD (*n*=3).

Ash content

The ash content of the snakehead fish bone hydroxyapatite powder is shown in **Figure 2.** The ash contents of the hydroxyapatite from snakehead fish bone are about 98.09% - 99.04%. The ash content for 40 minutes of extraction shows a significant ($p<0.05$) difference with the other treatments. Whereas, the ash content of 20 minutes was no significant ($p>0.05$) different with 60 minutes of extraction time. This condition indicated that the hydroxyapatite powder was composed of high-level minerals. A previous study reported that hydroxyapatite from the sardinella (*Amblygaster sirm*) is composed of 98.24% of ash [19]. Additionally, 99.75% of the ash content is observed in calcined bone from skipjack tuna [20].

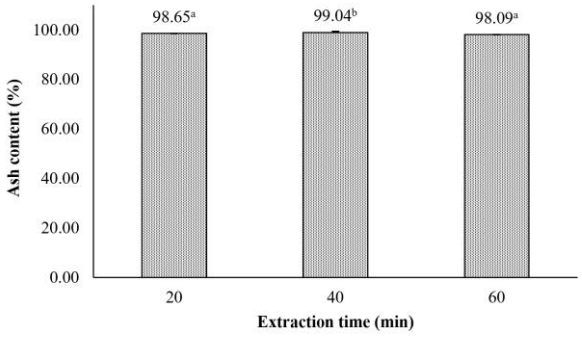

Figure 2. The ash content of the snakehead fish (*Channa striata*) bone hydroxyapatite powder. Data are shown as mean ± SD (*n*=3).

Mineral compositions of the hydroxyapatite

The mineral composition of the hydroxyapatite powder according to energy dispersive X-ray spectroscopy (EDS) is shown in **Figure 3** and **Table 1**. The main minerals of the hydroxyapatite from snakehead fish bone are calcium (Ca) and phosphorus (P), followed by a small amount of other minerals such as magnesium (Mg) and natrium (Na). According to **Table 1**, the calcium value is about 17.86% - 18.12% and the phosphorus value is about 10.23% - 10.74%. These data indicated that the non-stoichiometric form is found in the hydroxyapatite from snakehead fish bone, with a Ca/P molar ratio of about 1.69 - 1.72. Generally, stoichiometric hydroxyapatite is composed of a Ca/P ratio of about 1.67 [21]. Biological sources such as the scale of fish and bones of animals are the sources of non-stoichiometric hydroxyapatite [22]. A previous study reported that the Ca/P ratio of bovine bone hydroxyapatite was about 1.86 [23]. The Ca/P ratio of hydroxyapatite from black tilapia fish bone is about 1.56 - 1.65 and it was also reported that calcination temperature affected the Ca/P ratio [24]. Additionally, calcium and phosphorus are the main elements in hydroxyapatite powder [25].

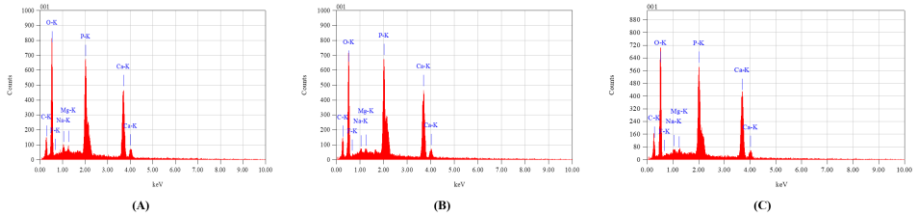

Figure 3. The mineral composition of hydroxyapatite from snakehead fish bones as analyzed by Energy Dispersive X-ray Spectroscopy (EDS).

Table 1. Mineral compositions of the hydroxyapatite snakehead fish bone to the energy dispersive X-ray spectroscopy.

Minerals	Extraction times (min)					
	20		40		60	
	Energy (keV)	Atom (%)	Energy (keV)	Atom (%)	Energy (keV)	Atom (%)
Ca	3.69	17.95	3.69	18.12	3.69	17.86
P	2.01	10.23	2.01	10.74	2.01	10.36
Mg	1.25	0.52	1.25	0.49	1.25	0.40
Na	1.04	0.78	1.04	0.43	1.04	0.44
O	0.53	55.76	0.53	53.23	0.53	53.90

Functional groups of the hydroxyapatite

The Fourier-transform infrared (FT-IR) spectra and functional groups of the hydroxyapatite are shown in **Figure 4** and **Table 2**. The functional group of the FT-IR spectra was analyzed according to the previous studies [2, 26]. The results showed that asymmetric bending vibrations of phosphate (v_3 PO_4^{3-}) were detected at 571.03 cm^{-1} (20 min), 568.36 cm^{-1} (40 min), and 567.78 cm^{-1} (60 min). The symmetric stretching of PO_4^3 (v_1 PO_4^{3-}) is detected at 962.54 cm^{-1} (20 min), 962.47 cm^{-1} (40 min), and 962.19 cm^{-1} (60 min). Also, the asymmetric stretching of PO_4^3 (v_2 PO_4^{3-}

) is detected at 1034.40 cm^{-1} (20 min), 1034.26 cm^{-1} (40 min), and 1035.01 cm^{-1} (60 min). A previous study reported that hydroxyapatite from fish scale indicated the presence of asymmetric bending vibrations of the phosphate group at 563 cm^{-1}, symmetric stretching of PO_4^{3-} at 957 cm^{-1}, and asymmetric stretching of PO_4^{3-} at 1030 cm^{-1} [2]. A previous study also reported that the phosphate groups of natural hydroxyapatites from veal bone were also detected at 560 cm^{-1} and 1000 – 1100 cm^{-1} [26].

In this present study, the carbonate (CO_3^{2-}) groups were also detected in the hydroxyapatite powder. The out-of-plane bending modes of CO_3^{2-} (v_1 CO_3^{2-}) were detected at 875.47 cm^{-1} (20 min), 874.87 cm^{-1} (40 min), and 874.12 cm^{-1}. Whereas, the asymmetric stretching of CO_3^{2-} (v_2 CO_3^{2-}) was detected at 1458.85 cm^{-1} (20 min), 1456.74 cm^{-1} (40 min), and 1467.00 cm^{-1} (60 min). The hydroxyapatite powder also indicated the presence of hydroxyl (OH^-) groups at 3571.83 cm^{-1} (20 min), 3565.20 cm^{-1} (40 min), and 3443.75 cm^{-1} (60 min). A previous study reported that hydroxyapatite powder from fish scale indicated the presence of out-of-plane bending mode of CO_3^{2-} at 876 cm^{-1} and asymmetric stretching of CO_3^{2-} at 1412-1547 cm^{-1} [2]. Also, CO_3^{2-} natural hydroxyapatite powder from veal bone was detected at 1460 – 1530 cm^{-1} [26]. According to these data, the hydroxyapatite was successfully extracted from snakehead fish bone. These data also support the EDS analysis that confirm the presence of calcium and phosphorus in the hydroxyapatite powder.

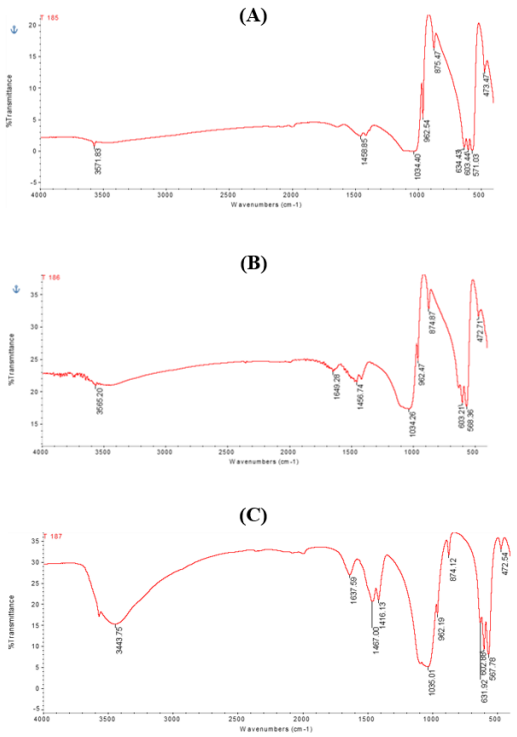

Figure 4. The FT-IR spectra of the snakehead fish bone hydroxyapatite with different extraction times. (A) 20 minutes; (B) 40 minutes; and (C) 60 minutes.

Table 2. The functional groups of the hydroxyapatite from snakehead fish bone.

Functional groups	Extraction times (min)					
	20		40		60	
	cm^{-1}	%T	cm^{-1}	%T	cm^{-1}	%T
v_1 PO_4^{3-}	962.54	55.25	962.47	24.80	962.19	17.60
v_2 PO_4^{3-}	1034.40	0.01	1034.26	17.37	1035.01	5.19
v_3 PO_4^{3-}	571.03	0.12	569.36	17.79	567.78	8.12
v_1 CO_3^{2-}	875.47	16.33	874.87	32.82	874.12	31.74
v_2 CO_3^{2-}	1458.85	2.38	1456.74	21.53	1467.00	20.72
OH^-	3571.83	0.84	3565.20	22.09	3443.75	15.99

Particle size of the hydroxyapatite

The particle size of the snakehead fish (*C. striata*) bone hydroxyapatite powder is shown in **Figure 5**. The particle size of the hydroxyapatite is about 63.9 nm - 138.2 nm. The smaller size of the hydroxyapatite is found in 40 minutes of extraction time. The value was significantly (p<0.05)

different from other treatments. Whereas, there is no significant (p>0.05) difference in particle size between 20 minutes and 60 minutes of extraction time. The small particle size in 40 minutes of extraction time was also confirmed by scanning electron microscopy (SEM) as shown in **Figure 6**. The irregular form is observed in the hydroxyapatite powder. A previous study also reported hydroxyapatite extracted from fish bone alkali treatment and calcination, resulting in an irregular form of hydroxyapatite [27, 28].

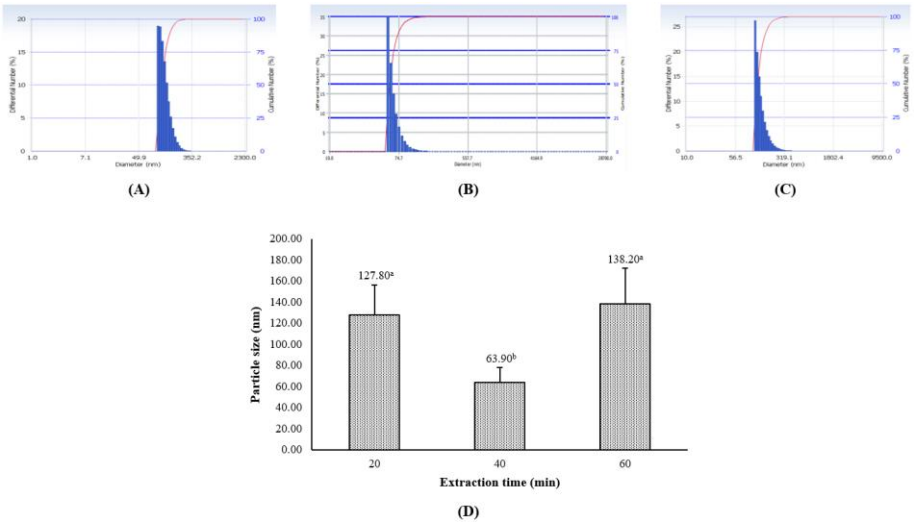

Figure 5. The particle size of the snakehead fish (*C. striata*) bone hydroxyapatite with different extraction times. (A) 20 minutes of extraction time; (B) 40 minutes of extraction time; (C) 60 minutes of extraction time; and (D) the mean ± SD of the hydroxyapatite particle size (n=3).

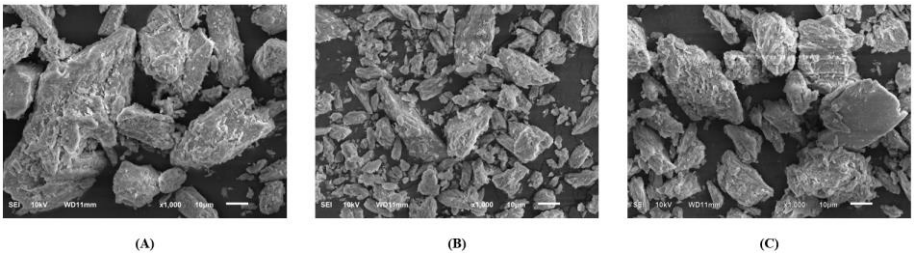

Figure 6. The representative of hydroxyapatite powder morphology by scanning electron microscopy (SEM) with different extraction times. (A) 20 minutes; (B) 40 minutes; and (C) 60 minutes.

Conclusion

In this present study, the hydroxyapatite was successfully extracted from snakehead fish (*Channa striata*) bone. The extraction yield is about 16.03%-19.99%. The smallest particle size is found in 40 minutes of extraction time (63.90 nm). The ash content of the hydroxyapatite is about 98.09% - 99.04%, calcium is about 17.86% - 18.12%, and the phosphorus is about 10.23% - 10.74%. The non-stoichiometric form is found in this hydroxyapatite with a Ca/P ratio of about 1.69 - 1.72. Analysis of the hydroxyapatite functional groups of snakehead fish bone showed the presence of phosphate groups (PO_4^{3-}), carbonate groups (CO_3^{2-}), and hydroxyl groups (OH^-). These data indicated that the hydroxyapatite was successfully extracted from snakehead fish bone in nanoparticle form. Therefore, snakehead fish bone can be used as a source of nanohydroxyapatite.

Acknowledgment

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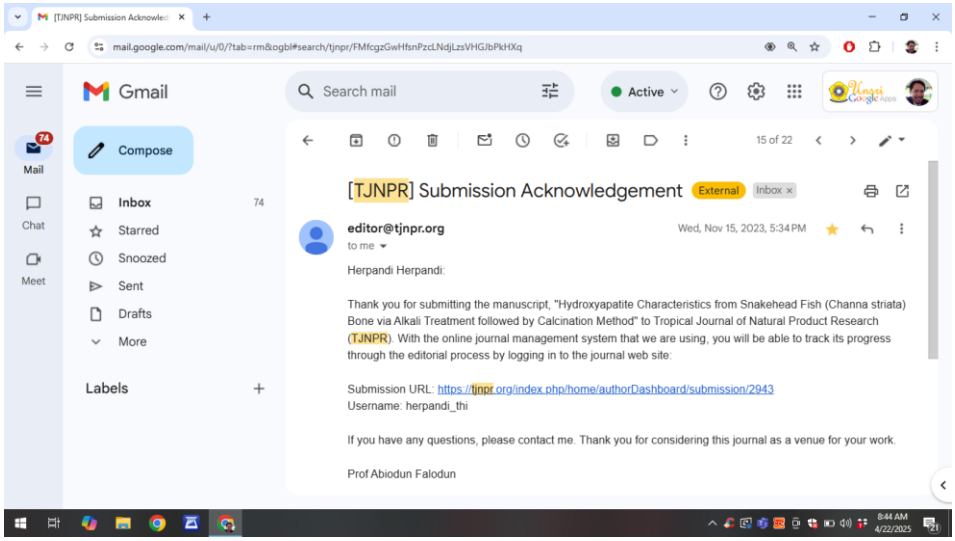
References

1. Coppola, D., et al., *Fish Waste: From Problem to Valuable Resource.* Marine Drugs, 2021. **19**(2).
2. Huang, Y.-C., P.-C. Hsiao, and H.-J. Chai, *Hydroxyapatite extracted from fish scale: Effects on MG63 osteoblast-like cells.* Ceramics International, 2011. **37**(6): p. 1825-1831.
3. Maktoof, A.A., R.J. Elherarlla, and S. Ethaib, *Identifying the nutritional composition of fish waste, bones, scales, and fins.* IOP Conference Series: Materials Science and Engineering, 2020. **871**(1).
4. Boronat, Ò., et al., *Development of added-value culinary ingredients from fish waste: Fish bones and fish scales.* International Journal of Gastronomy and Food Science, 2023. **31**.
5. Lall, S.P. and S.J. Kaushik, *Nutrition and Metabolism of Minerals in Fish.* Animals, 2021. **11**(9).
6. Marliana, A., et al., *Synthesis and characterization of hydroxyapatite from fish bone waste*. 2015.
7. Jaffri, M.Z., et al., *Sample Preparation of the Natural Source Hydroxyapatite (HAp) derived from Black Tilapia Fish Scales.* Journal of Physics: Conference Series, 2022. **2169**(1).
8. Hernández-Ruiz, K.L., et al., *Hydroxyapatite recovery from fish byproducts for biomedical applications.* Sustainable Chemistry and Pharmacy, 2022. **28**.
9. Granito, R.N., et al., *Hydroxyapatite from Fish for Bone Tissue Engineering: A Promising Approach.* Int J Mol Cell Med, 2018. **7**(2): p. 80-90.

10. Pu'ad, M.N.A.S., et al., *Syntheses of hydroxyapatite from natural sources.* Heliyon, 2019. **5**(5).
11. Sobczak-Kupiec, A. and Z. Wzorek, *The influence of calcination parameters on free calcium oxide content in natural hydroxyapatite.* Ceramics International, 2012. **38**(1): p. 641-647.
12. Dabiri, S.M.H., et al., *Extraction of Hydroxyapatite from Fish Bones and Its Application in Nickel Adsorption.* BioNanoScience, 2018. **8**(3): p. 823-834.
13. Brzezińska-Miecznik, J., et al., *Hydroxyapatite from animal bones – Extraction and properties.* Ceramics International, 2015. **41**(3): p. 4841-4846.
14. Zainol, I., et al., *Extraction of natural hydroxyapatite from tilapia fish scales using alkaline treatment.* Materials Today: Proceedings, 2019. **16**: p. 1942-1948.
15. Putranto, F., H., N.A. Andi, and K. Indrati, *Properties of belida (Chitala sp.) fish bone powder as calcium source based on protein hydrolysis method.* Ziraa'ah, 2015. **40**(1): p. 11 - 20.
16. Mustafa, N., et al., *Hydroxyapatite Extracted from Waste Fish Bones and Scales via Calcination Method.* Applied Mechanics and Materials, 2015. **773-774**: p. 287-290.
17. Barre, M.S., et al., *Potential food additive of Boswellia carterii essential oil encapsulated within gum arabic: a particle size distribution and zeta potential analysis.* Food Research, 2020. **4**(S2): p. 19-23.
18. Jindapon, N., et al., *Preparation, Characterization, and Biological Properties of Hydroxyapatite from Bigeye Snapper (Priancanthus tayenus) Bone.* International Journal of Molecular Sciences, 2023. **24**(3).
19. Hasan, M.R., et al., *Proximate and Morphological Characteristics of Nano Hydroxyapatite (Nano Hap) Extracted from Fish Bone.* Journal of Sustainability Science and Management, 2020. **15**(8): p. 9-21.
20. Benjakul, S., et al., *Biocalcium powder from precooked skipjack tuna bone: Production and its characteristics.* Journal of Food Biochemistry, 2017. **41**(6).
21. Beaufils, S., et al., *Synthesis of calcium-deficient hydroxyapatite nanowires and nanotubes performed by template-assisted electrodeposition.* Materials Science and Engineering: C, 2019. **98**: p. 333-346.
22. Dorozhkin, V.S., *Amorphous Calcium Orthophosphates: Nature, Chemistry and Biomedical Applications.* International Journal of Materials and Chemistry, 2012. **2**(1): p. 19-46.
23. Barakat, N.A.M., et al., *Extraction of pure natural hydroxyapatite from the bovine bones bio waste by three different methods.* Journal of Materials Processing Technology, 2009. **209**(7): p. 3408-3415.
24. Dermawan, S.K., et al., *Effect of the Calcination Temperature on the Properties of Hydroxyapatite from Black Tilapia Fish Bone.* Journal of Physics: Conference Series, 2022. **2169**(1).
25. Saeed, G.K., A.F. Essa, and S.A.-A. Said, *Preparation and characterization of hydroxyapatite powder and study of hydroxyapatite - alumina Composite.* Journal of Physics: Conference Series, 2020. **1591**(1).
26. Gheisari, H., E. Karamian, and M. Abdellahi, *A novel hydroxyapatite –Hardystonite nanocomposite ceramic.* Ceramics International, 2015. **41**(4): p. 5967-5975.
27. Pal, A., et al., *Synthesis of hydroxyapatite from Lates calcarifer fish bone for biomedical applications.* Materials Letters, 2017. **203**: p. 89-92.

28. Venkatesan, J., et al., *A comparative study of thermal calcination and an alkaline hydrolysis method in the isolation of hydroxyapatite from Thunnus obesus bone.* Biomedical Materials, 2011. **6**(3).

Submission Acknoledgement



2. Pre-Review Editor (16 November 2023)

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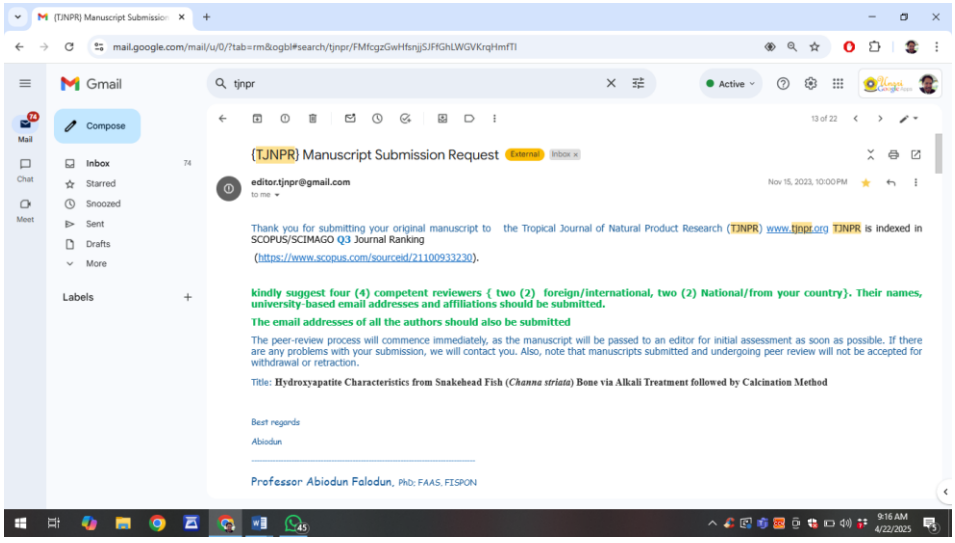
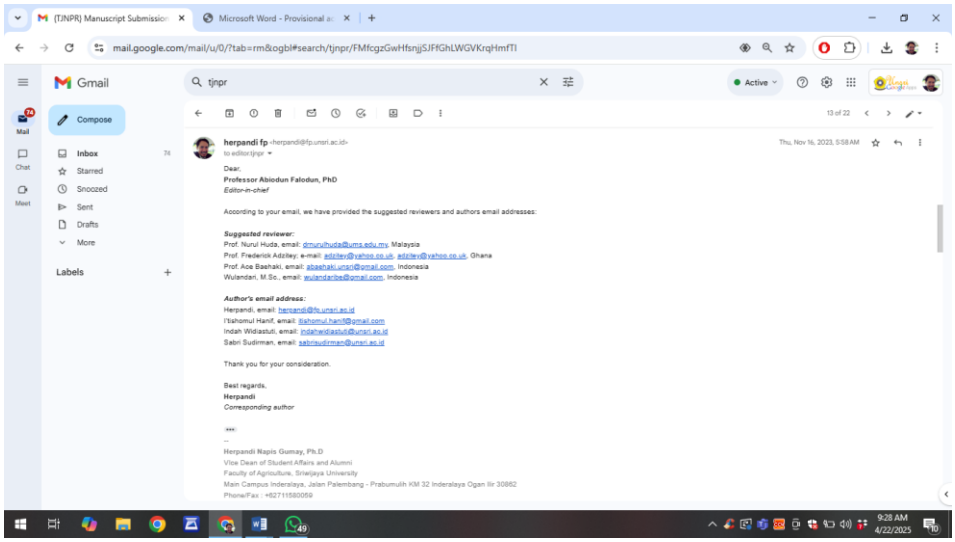


Provided the Suggested reviewers (16 November 2023)



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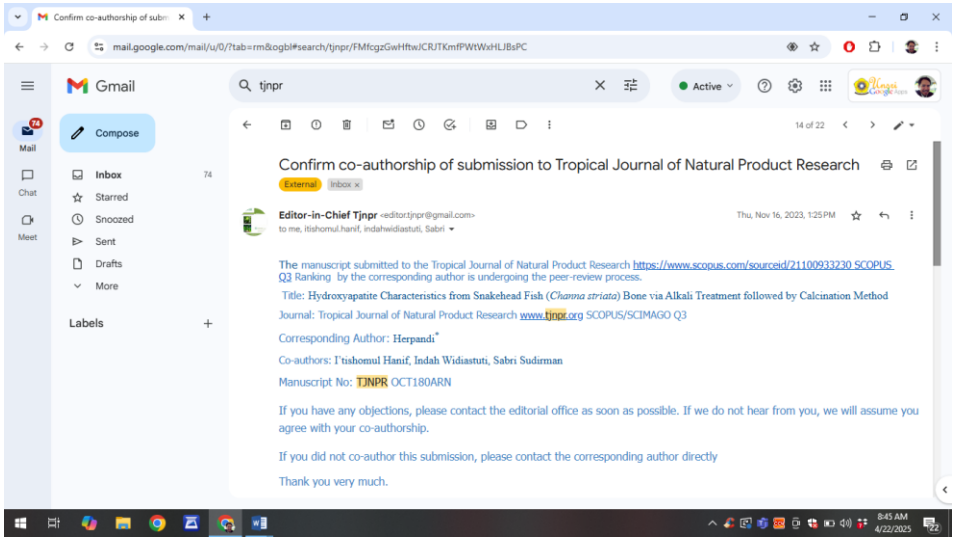

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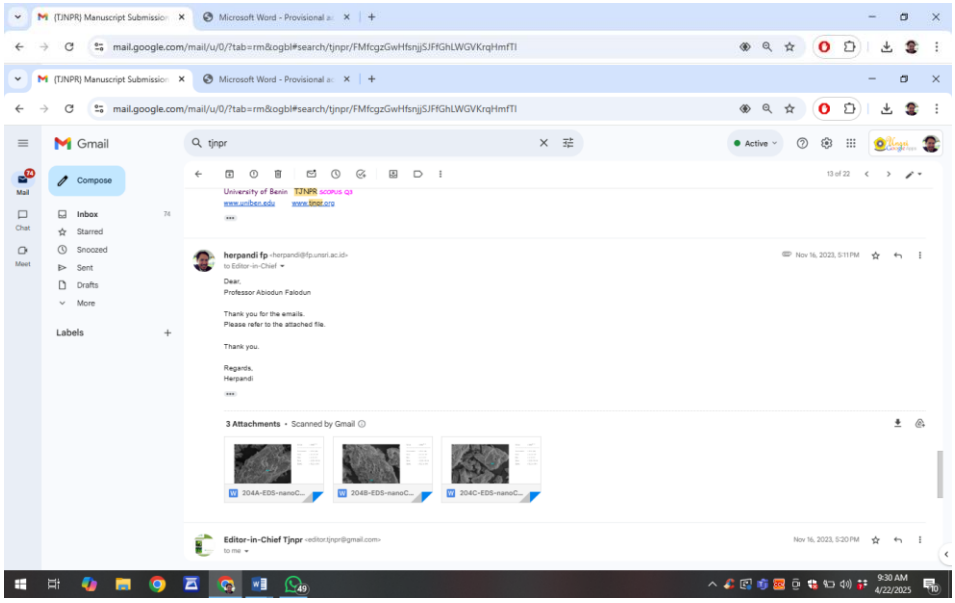
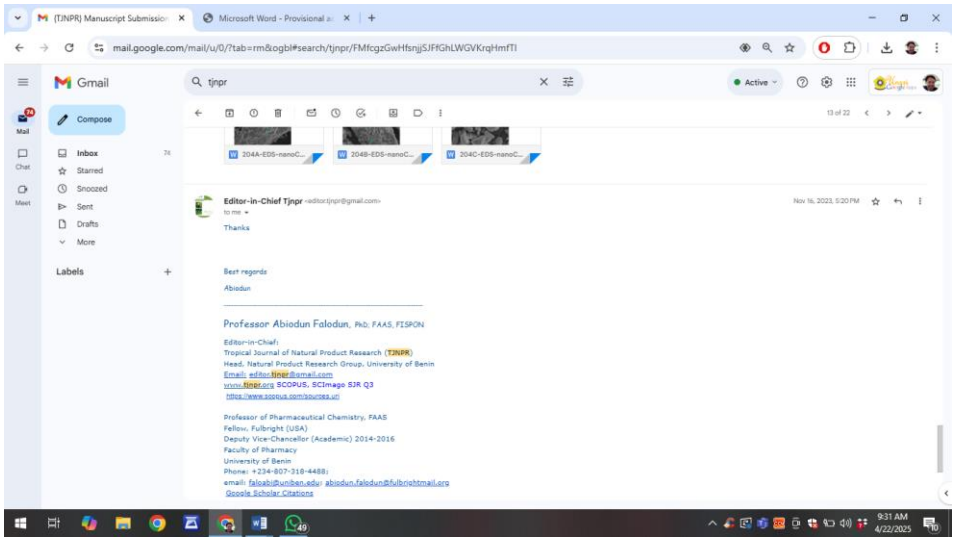
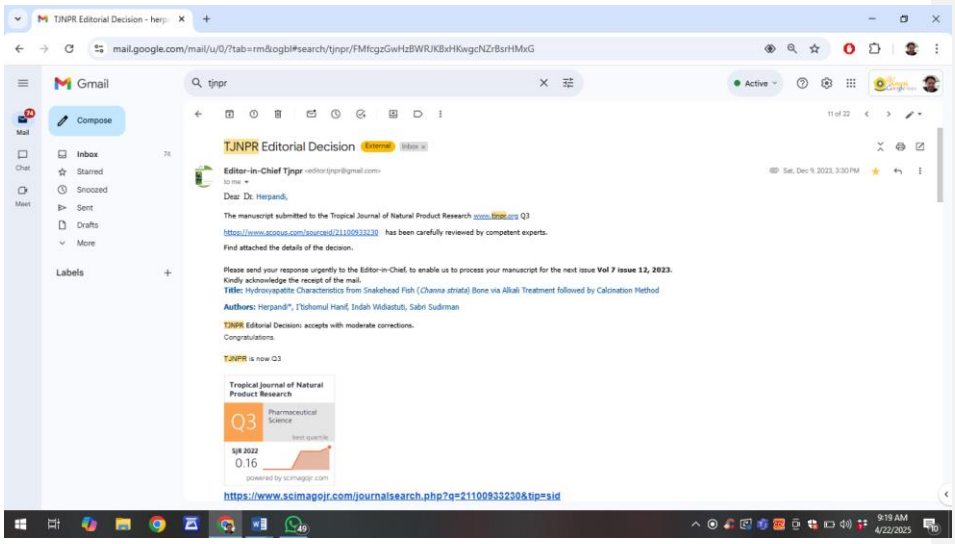




3. Editor Decision (9 Desember 2023)



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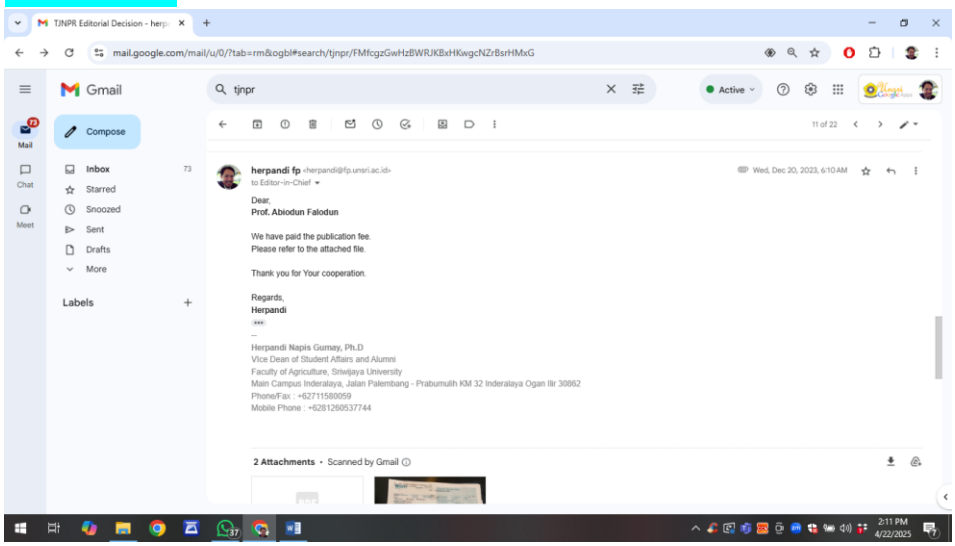
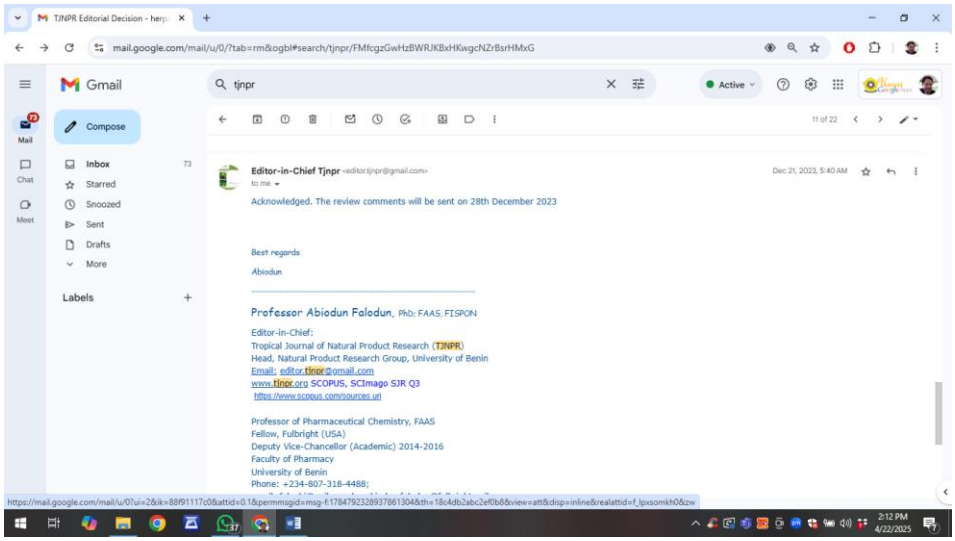
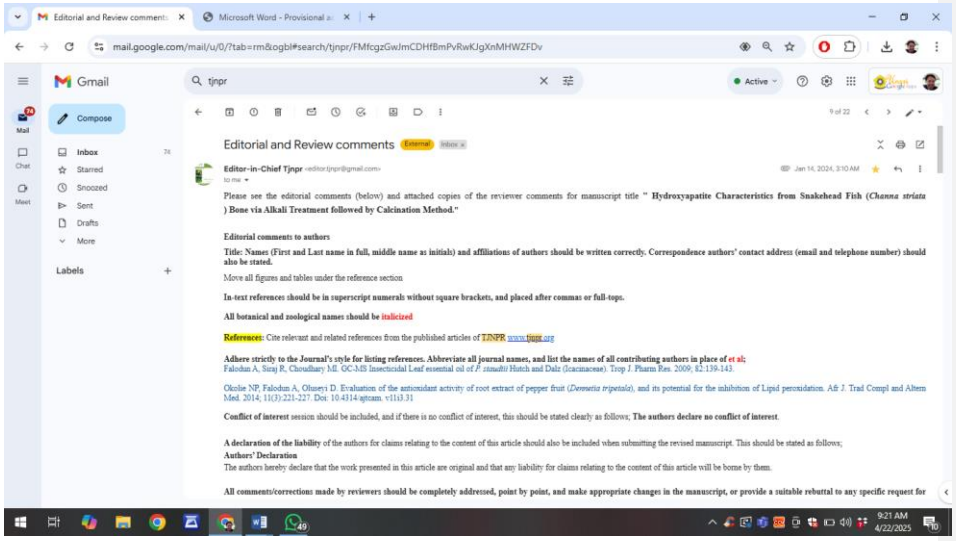


4. Peer Review (28 Desember 2023)



4. Editor dan Reviewer Comment (14 Januari 2024)



Editorial and Review comments

Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com> Jan 14, 2024, 3:10 AM

Please see the editorial comments (below) and attached copies of the reviewer comments for manuscript title " **Hydroxyapatite Characteristics from Snakehead Fish (*Channa striata*) Bone via Alkali Treatment followed by Calcination Method.**"

Editorial comments to authors

Title: Names (First and Last name in full, middle name as initials) and affiliations of authors should be written correctly. Correspondence authors' contact address (email and telephone number) should also be stated.

Move all figures and tables under the reference section

In-text references should be in superscript numerals without square brackets, and placed after commas or full-tops.

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Falodun A, Siraj R, Choudhary MI. GC-MS Insecticidal Leaf essential oil of *P. stenadtii* Hutch and Dalz (Icacinaceae). Trop J. Pharm Res. 2009; 82:139-143.

Okolie NP, Falodun A, Oluseyi D. Evaluation of the antioxidant activity of root extract of pepper fruit (*Dennettia tripetala*), and its potential for the inhibition of Lipid peroxidation. Afr J. Trad Compl and Altern Med. 2014; 11(3):221-227. Doi: 10.4314/ajtcam. v11i3.31

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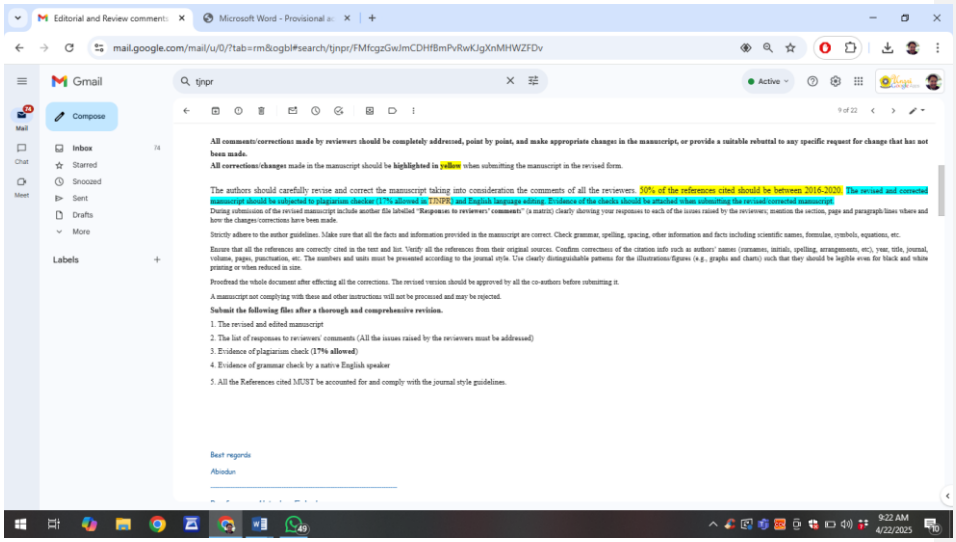

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Reviewer Comment (1)

REVIEW FORM

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A. MANUSCRIPT

Journal	**Tropical Journal of Natural Product Research**
Manuscript Number	TJNPR 180 R
Type of paper	Research article
Title of paper	Hydroxyapatite Characteristics from Snakehead Fish (*Channa striata*) Bone via Alkali Treatment followed by Calcination Method
Name of Authors	

B. REVIEWER'S SPECIFIC COMMENTS PER SECTION OF MANUSCRIPT

Title	The title is mostly appropriate
Abstract	The abstract word count is < 250. All grammatical, spelling and punctuation errors should be corrected. Authors should ensure that they correct all grammatical syntax errors/ wrong sentences • All grammatical syntax errors should be corrected
Keywords	Keywords should be separated by commas
Introduction	The authors should use the journal citation style. All grammatical, spelling and punctuation errors should be corrected. The aim and objectives of the research is obvious. The authors should include a statement of research novelty. The relevance of the adopted methods to the research should be discussed comparatively. • The introduction should be revised as recommended
Methodology	All instruments and equipment used should be mentioned alongside their model, manufacturer and country. All chemicals and reagents used should be mentioned alongside their manufacturer, grade, percentage purity, conc., and specificities. All grammatical syntax errors should be corrected. The methods for the estimation of the percentage yield was not given – authors should include the method for the estimation of the % yield of the hydroxyapatite. • The methods used are mostly appropriate for the research
Results	All images should be of a very high quality. Supplementary data should be supplied where necessary for validations. Results should be presented based on the journal guideline • The results obtained are mostly sufficient for the methods used
Discussion	The discussion is concise. The discussion was comparative and statistically done. • The discussion is appropriate
Conclusion	Authors should include the future prospects of their research
References	Authors should strictly follow journal guideline in referencing. Also, authors should ensure that the number of cited references tally with those at the reference section
Figures	All figures were captured and discussed in the manuscript. Authors should present all figures based on the journal guideline
Tables	All Tables were captured and discussed in the manuscript. Authors should present all Tables based on the journal guideline

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C. REVIEWER'S GENERAL COMMENTS AND REMARKS

The authors studied "Hydroxyapatite Characteristics from Snakehead Fish (*Channa striata*) Bone via Alkali Treatment followed by Calcination Method". All grammatical syntax errors should be corrected, Keywords should be separated by commas. The introduction should be revised as recommended. The methods used are mostly appropriate for the research. The results obtained are mostly sufficient for the methods used. The discussion is appropriate. Authors should include the future prospects of their research. Authors should strictly follow journal guideline in referencing. Also, authors should ensure that the number of cited references tally with those at the reference section. Manuscript contained a lot of grammatical syntax errors therefore, authors are to seek the assistance of a professional manuscript editing service provider/personnel to help polish the manuscript. The manuscript should be presented based on the journal guideline. Authors should include the methods for the estimation of the % yield of the hydroxyapatite Accept with moderate revision

D. REVIEWER'S RECOMMENDATION
Please mark with "**X**" one of the options.

You state the article should:

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Accept with minor revisions (editor will check), specific comments to the editor below	
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Reviewer Comment (2)

Hydroxyapatite Characteristics from Snakehead Fish (*Channa striata*) Bone via Alkali Treatment followed by Calcination Method

Abstract

Snakehead fish (*Channa striata*) is commonly used as a raw material in traditional foods in South Sumatra. However, some parts of this fish, such as bone, skin, and viscera, are not used during this food processing. This study aimed to determine the characterization of hydroxyapatite snakehead fish bone with different extraction times using ultrasound-assisted extraction followed by the calcination method. The hydroxyapatite was extracted using sodium hydroxide (NaOH) with three different extraction times (20, 40, and 60 minutes) before continuing with calcination. The extraction yield is about 16.03%-19.99%. The smallest particle size is found in 40 minutes of extraction time (63.90 nm). The ash content of the hydroxyapatite is about 98.09% - 99.04%, calcium is about 17.86% - 18.12%, and the phosphorus is about 10.23% - 10.74%. The non-stoichiometric form is found in the hydroxyapatite from snakehead fish bone with a Ca/P ratio of about 1.69 - 1.72. Analysis of the hydroxyapatite functional groups of snakehead fish bone showed the presence of phosphate groups (PO_4^{3-}), carbonate groups (CO_3^{2-}), and hydroxyl groups (OH^-). These data indicated that the hydroxyapatite was successfully extracted from snakehead fish bone in nanoparticle form. Therefore, snakehead fish bone can be used as a source of hydroxyapatite.

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Keywords: Calcination; *Channa striata*; extraction; hydroxyapatite; phosphorus

Commented [II2]: Keywords should be separated by commas

Introduction

Consumption of fish products has increased significantly since they were identified as essential to a healthy, balanced diet and way of life. As a result, there has been a sharp rise in the global production of fish waste [1]. Fish waste is a potential source of minerals or trace metals, essential fatty acids, amino acids, peptides, and protein of high biological value as well as vitamins [2]. Therefore, fish waste thus offers enormous untapped potential for value addition. The fish waste includes fish bones, scales, and fins. Whereas, the fish bone waste is around 9-15% [3, 4].

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The fish bone waste composed of various mineral such as calcium, phosphorus, zinc, and magnesium that can be used a source of hydroxyapatite [5, 6].

Hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] is a common of the calcium phosphates that has compositions similar to natural bone [6, 7]. Due to its bioactive qualities and osteoconductive properties, hydroxyapatite has garnered interest for use in bone grafting procedures. Additionally, other medical applications of hydroxyapatite have also been studied because hydroxyapatite is non-toxic, bioactive, biocompatible, and non-inflammatory [2]. Natural hydroxyapatite has recently been extracted from various biowastes, including fish waste such as fish bone [6, 8, 9]. Various methods have been used to hydroxyapatite extraction from the natural sources, including calcination, alkali treatment, and the combination methods [10]. A previous study reported that calcination method was used for hydroxyapatite extraction [11, 12]. NaOH treatment also was reported for the extraction hydroxyapatite from bovine bone [13]. Additionally, hydroxyapatite successfully extracted from tilapia fish scale using combination method (NaOH treatment and calcination) [14].

Snakehead fish (*Channa striata*) is a major source material for a traditional fish cake dish from South Sumatra, Indonesia, called "pempek". This product is made from snakehead fish meal; therefore, some parts of the fish become waste, including the bones. However, the study about the utilization of fish bone waste from this snakehead fish has not yet been reported. Therefore, this study aimed to determine the characteristics of hydroxyapatite from snakehead fish bone using a combination of alkali (NaOH) treatment and calcination process.

Materials and Methods

Sample preparation

The snakehead fish bone preparation was performed according to the previous methods [6, 15]. Briefly, the fish bones were washed and boiled at 80°C for 30 minutes. After that, it was cleaned and mixed with lime water, and then it was boiled for 3 hours. The fish bone was cooled at room temperature for 15 minutes and dried in an oven at 80°C for 12 hours. The dried fish bone was then ground to powder (80 mesh) then continued to the calcination process.

Hydroxyapatite extraction and calcination process

The hydroxyapatite extraction and calcination processes from fish bones were performed according to the previous methods [6, 16]. Briefly, 10 g of the fish bone powder was mixed with 1 N NaOH and then extracted using an ultrasonic bath at 50°C for different extraction times (20, 40, and 60 minutes). After the extraction times, it was cooled, filtrated using filter paper (Whatman No. 42), and neutralized using distilled water. Then, it was oven-dried at 105°C for 3 hours before the calcination process. The calcination process was performed using a muffle furnace at 600°C for 5 hours. After that, it was cooled to room temperature and kept for further analysis.

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Hydroxyapatite characterizations

The ash content analysis was determined according to the AOAC (2000). Briefly, the hydroxyapatite powder was completely incinerated in a muffle furnace at 550°C (AOAC method 930.05). Whereas the element composition was analyzed using energy dispersive X-ray spectroscopy (EDS) and the functional group of the hydroxyapatite powder was analyzed using a Fourier transform infrared spectrometer (FTIR, Brucker Tensor 27) according to the previous method [2]. The particle size of the hydroxyapatite powder was analyzed using a particle size analyzer (Malvern Zetasizer Nano ZS Ver. 7.01) and the hydroxyapatite powder was also viewed by scanning electron microscopy (SEM, JEOL JSM-7000F FE-SEM, Japan) according to the previous methods [2, 17].

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Statistical analysis

The data on yield, ash content, and particle size were expressed as the mean ± standard deviation (SD). It was analyzed using Duncan's multiple range test ($p<0.05$) using SPSS software (ver. 22.0; IBM Corporation, Armonk, NY, USA). Whereas, the mineral composition, functional groups and scanning electron microscope image were analyzed using descriptive analysis.

Results and Discussion

Yield of the hydroxyapatite

The yield of the snakehead fish bone hydroxyapatite is shown in **Figure 1.** The yields of the hydroxyapatite are about 16.03% - 19.99%. The highest yield of the hydroxyapatite is found in 60 minutes of extraction time and the lowest in 20 minutes. Whereas, there is no significant ($p>0.05$) difference between the yield of 40 minutes and other treatments. However, the yield of 20 minutes was significantly ($p<0.05$) different from 60 minutes of extraction time. A previous study reported that hydroxyapatite yield from bigeye snapper (*Priancanthus tayenus*) bones is about 13.4% [18]. Generally, there are two main methods for obtaining hydroxyapatite: chemical synthesis and biological resource extraction. Compared to synthetic forms, animal bone hydroxyapatite has a few advantages in terms of chemical composition and structure, which are identical to human bone, reduced manufacturing costs, and enhanced biological response [12]. The organic content in the bone is normally removed using an alkaline solution, such as NaOH. The organic component of the bone is hydrolyzed by the NaOH solution, and the remaining calcium phosphate is then washed and filtered. Whereas, calcination was used to completely remove the organic content from the bone [10].

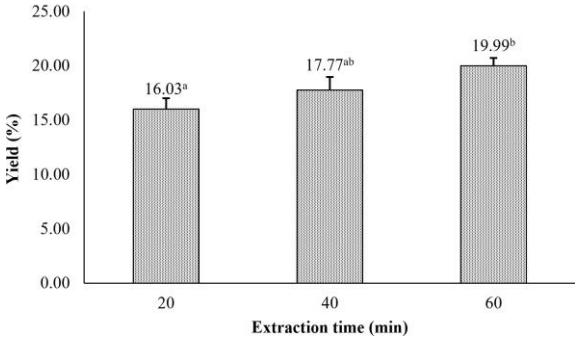

Figure 1. The yield of snakehead fish (*C. striata*) bone hydroxyapatite with different extraction time. Data are shown as mean ± SD (*n*=3).

Ash content

The ash content of the snakehead fish bone hydroxyapatite powder is shown in **Figure 2.** The ash contents of the hydroxyapatite from snakehead fish bone are about 98.09% - 99.04%. The ash content for 40 minutes of extraction shows a significant ($p<0.05$) difference with the other treatments. Whereas, the ash content of 20 minutes was no significant ($p>0.05$) different with 60 minutes of extraction time. This condition indicated that the hydroxyapatite powder was composed of high-level minerals. A previous study reported that hydroxyapatite from the sardinella (*Amblygaster sirm*) is composed of 98.24% of ash [19]. Additionally, 99.75% of the ash content is observed in calcined bone from skipjack tuna [20].

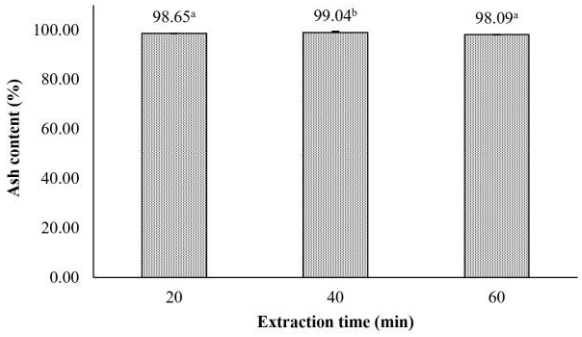

Figure 2. The ash content of the snakehead fish (*Channa striata*) bone hydroxyapatite powder. Data are shown as mean ± SD (*n*=3).

Mineral compositions of the hydroxyapatite

The mineral composition of the hydroxyapatite powder according to energy dispersive X-ray spectroscopy (EDS) is shown in **Figure 3** and **Table 1**. The main minerals of the hydroxyapatite from snakehead fish bone are calcium (Ca) and phosphorus (P), followed by a small amount of other minerals such as magnesium (Mg) and natrium (Na). According to **Table 1**, the calcium value is about 17.86% - 18.12% and the phosphorus value is about 10.23% - 10.74%. These data indicated that the non-stoichiometric form is found in the hydroxyapatite from snakehead fish bone, with a Ca/P molar ratio of about 1.69 - 1.72. Generally, stoichiometric hydroxyapatite is composed of a Ca/P ratio of about 1.67 [21]. Biological sources such as the scale of fish and bones of animals are the sources of non-stoichiometric hydroxyapatite [22]. A previous study reported that the Ca/P ratio of bovine bone hydroxyapatite was about 1.86 [23]. The Ca/P ratio of hydroxyapatite from black tilapia fish bone is about 1.56 - 1.65 and it was also reported that calcination temperature affected the Ca/P ratio [24]. Additionally, calcium and phosphorus are the main elements in hydroxyapatite powder [25].

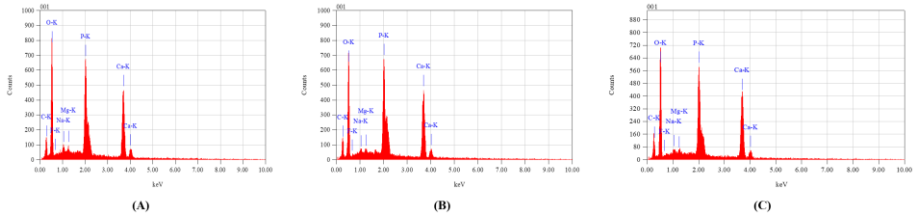

Figure 3. The mineral composition of hydroxyapatite from snakehead fish bone as analyzed by Energy Dispersive X-ray Spectroscopy (EDS).

Table 1. Mineral compositions of the hydroxyapatite snakehead fish bone to the energy dispersive X-ray spectroscopy.

Minerals	Extraction times (min)					
	20		40		60	
	Energy (keV)	Atom (%)	Energy (keV)	Atom (%)	Energy (keV)	Atom (%)
Ca	3.69	17.95	3.69	18.12	3.69	17.86
P	2.01	10.23	2.01	10.74	2.01	10.36
Mg	1.25	0.52	1.25	0.49	1.25	0.40
Na	1.04	0.78	1.04	0.43	1.04	0.44
O	0.53	55.76	0.53	53.23	0.53	53.90

Functional groups of the hydroxyapatite

The Fourier-transform infrared (FT-IR) spectra and functional groups of the hydroxyapatite are shown in **Figure 4** and **Table 2**. The functional group of the FT-IR spectra was analyzed according to the previous studies [2, 26]. The results showed that asymmetric bending vibrations of phosphate (v_3 PO_4^{3-}) were detected at 571.03 cm^{-1} (20 min), 568.36 cm^{-1} (40 min), and 567.78 cm^{-1} (60 min). The symmetric stretching of PO_4^3 (v_1 PO_4^{3-}) is detected at 962.54 cm^{-1} (20 min), 962.47 cm^{-1} (40 min), and 962.19 cm^{-1} (60 min). Also, the asymmetric stretching of PO_4^3 (v_2 PO_4^{3-}

) is detected at 1034.40 cm^{-1} (20 min), 1034.26 cm^{-1} (40 min), and 1035.01 cm^{-1} (60 min). A previous study reported that hydroxyapatite from fish scale indicated the presence of asymmetric bending vibrations of the phosphate group at 563 cm^{-1}, symmetric stretching of PO_4^{3-} at 957 cm^{-1}, and asymmetric stretching of PO_4^{3-} at 1030 cm^{-1} [2]. A previous study also reported that the phosphate groups of natural hydroxyapatites from veal bone were also detected at 560 cm^{-1} and 1000 – 1100 cm^{-1} [26].

In this present study, the carbonate (CO_3^{2-}) groups were also detected in the hydroxyapatite powder. The out-of-plane bending modes of CO_3^{2-} (v_1 CO_3^{2-}) were detected at 875.47 cm^{-1} (20 min), 874.87 cm^{-1} (40 min), and 874.12 cm^{-1}. Whereas, the asymmetric stretching of CO_3^{2-} (v_2 CO_3^{2-}) was detected at 1458.85 cm^{-1} (20 min), 1456.74 cm^{-1} (40 min), and 1467.00 cm^{-1} (60 min). The hydroxyapatite powder also indicated the presence of hydroxyl (OH^-) groups at 3571.83 cm^{-1} (20 min), 3565.20 cm^{-1} (40 min), and 3443.75 cm^{-1} (60 min). A previous study reported that hydroxyapatite powder from fish scale indicated the presence of out-of-plane bending mode of CO_3^{2-} at 876 cm^{-1} and asymmetric stretching of CO_3^{2-} at 1412-1547 cm^{-1} [2]. Also, CO_3^{2-} natural hydroxyapatite powder from veal bone was detected at 1460 – 1530 cm^{-1} [26]. According to these data, the hydroxyapatite was successfully extracted from snakehead fish bone. These data also support the EDS analysis that confirm the presence of calcium and phosphorus in the hydroxyapatite powder.

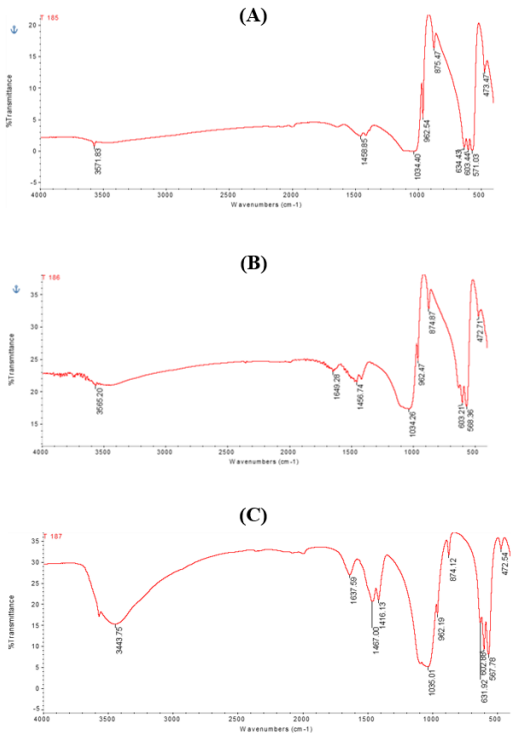

Figure 4. The FT-IR spectra of the snakehead fish bone hydroxyapatite with different extraction times. (A) 20 minutes; (B) 40 minutes; and (C) 60 minutes.

Table 2. The functional groups of the hydroxyapatite from snakehead fish bone.

Functional groups	Extraction times (min)					
	20		40		60	
	cm^{-1}	%T	cm^{-1}	%T	cm^{-1}	%T
v_1 PO_4^{3-}	962.54	55.25	962.47	24.80	962.19	17.60
v_2 PO_4^{3-}	1034.40	0.01	1034.26	17.37	1035.01	5.19
v_3 PO_4^{3-}	571.03	0.12	569.36	17.79	567.78	8.12
v_1 CO_3^{2-}	875.47	16.33	874.87	32.82	874.12	31.74
v_2 CO_3^{2-}	1458.85	2.38	1456.74	21.53	1467.00	20.72
OH^-	3571.83	0.84	3565.20	22.09	3443.75	15.99

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Particle size of the hydroxyapatite

The particle size of the snakehead fish (*C. striata*) bone hydroxyapatite powder is shown in **Figure 5**. The particle size of the hydroxyapatite is about 63.9 nm - 138.2 nm. The smaller size of the hydroxyapatite is found in 40 minutes of extraction time. The value was significantly (p<0.05)

different from other treatments. Whereas, there is no significant (p>0.05) difference in particle size between 20 minutes and 60 minutes of extraction time. The small particle size in 40 minutes of extraction time was also confirmed by scanning electron microscopy (SEM) as shown in **Figure 6**. The irregular form is observed in the hydroxyapatite powder. A previous study also reported hydroxyapatite extracted from fish bone alkali treatment and calcination, resulting in an irregular form of hydroxyapatite [27, 28].

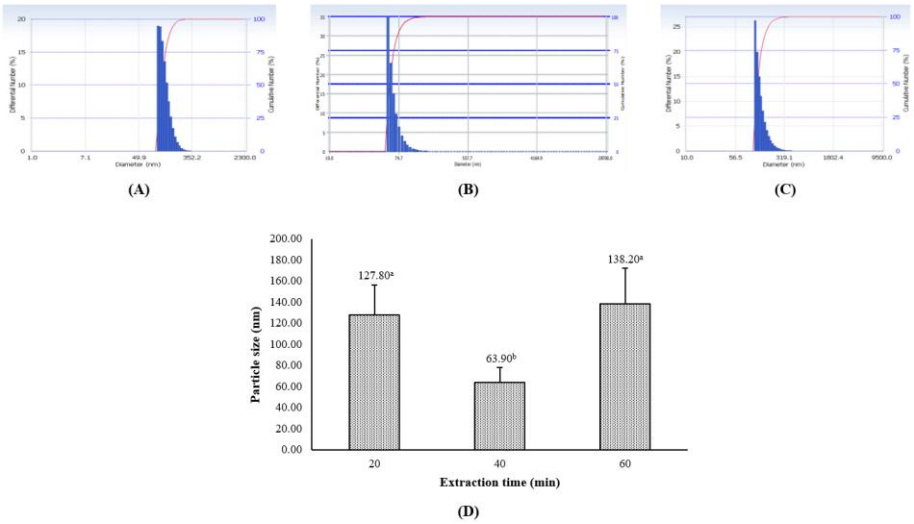

Figure 5. The particle size of the snakehead fish (*C. striata*) bone hydroxyapatite with different extraction times. (A) 20 minutes of extraction time; (B) 40 minutes of extraction time; (C) 60 minutes of extraction time; and (D) the mean ± SD of the hydroxyapatite particle size (n=3).

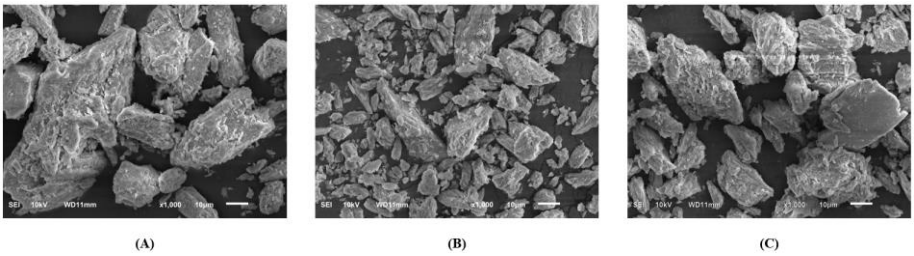

Figure 6. The representative of hydroxyapatite powder morphology by scanning electron microscopy (SEM) with different extraction times. (A) 20 minutes; (B) 40 minutes; and (C) 60 minutes.

Conclusion

In this present study, the hydroxyapatite was successfully extracted from snakehead fish (*Channa striata*) bone. The extraction yield is about 16.03%-19.99%. The smallest particle size is found in 40 minutes of extraction time (63.90 nm). The ash content of the hydroxyapatite is about 98.09% - 99.04%, calcium is about 17.86% - 18.12%, and the phosphorus is about 10.23% - 10.74%. The non-stoichiometric form is found in this hydroxyapatite with a Ca/P ratio of about 1.69 - 1.72. Analysis of the hydroxyapatite functional groups of snakehead fish bone showed the presence of phosphate groups (PO_4^{3-}), carbonate groups (CO_3^{2-}), and hydroxyl groups (OH^-). These data indicated that the hydroxyapatite was successfully extracted from snakehead fish bone in nanoparticle form. Therefore, snakehead fish bone can be used as a source of nanohydroxyapatite.

Acknowledgment

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References

1. Coppola, D., et al., *Fish Waste: From Problem to Valuable Resource.* Marine Drugs, 2021. **19**(2).
2. Huang, Y.-C., P.-C. Hsiao, and H.-J. Chai, *Hydroxyapatite extracted from fish scale: Effects on MG63 osteoblast-like cells.* Ceramics International, 2011. **37**(6): p. 1825-1831.
3. Maktoof, A.A., R.J. Elherarlla, and S. Ethaib, *Identifying the nutritional composition of fish waste, bones, scales, and fins.* IOP Conference Series: Materials Science and Engineering, 2020. **871**(1).
4. Boronat, Ò., et al., *Development of added-value culinary ingredients from fish waste: Fish bones and fish scales.* International Journal of Gastronomy and Food Science, 2023. **31**.
5. Lall, S.P. and S.J. Kaushik, *Nutrition and Metabolism of Minerals in Fish.* Animals, 2021. **11**(9).
6. Marliana, A., et al., *Synthesis and characterization of hydroxyapatite from fish bone waste*. 2015.
7. Jaffri, M.Z., et al., *Sample Preparation of the Natural Source Hydroxyapatite (HAp) derived from Black Tilapia Fish Scales.* Journal of Physics: Conference Series, 2022. **2169**(1).
8. Hernández-Ruiz, K.L., et al., *Hydroxyapatite recovery from fish byproducts for biomedical applications.* Sustainable Chemistry and Pharmacy, 2022. **28**.
9. Granito, R.N., et al., *Hydroxyapatite from Fish for Bone Tissue Engineering: A Promising Approach.* Int J Mol Cell Med, 2018. **7**(2): p. 80-90.

10. Pu'ad, M.N.A.S., et al., *Syntheses of hydroxyapatite from natural sources.* Heliyon, 2019. **5**(5).
11. Sobczak-Kupiec, A. and Z. Wzorek, *The influence of calcination parameters on free calcium oxide content in natural hydroxyapatite.* Ceramics International, 2012. **38**(1): p. 641-647.
12. Dabiri, S.M.H., et al., *Extraction of Hydroxyapatite from Fish Bones and Its Application in Nickel Adsorption.* BioNanoScience, 2018. **8**(3): p. 823-834.
13. Brzezińska-Miecznik, J., et al., *Hydroxyapatite from animal bones – Extraction and properties.* Ceramics International, 2015. **41**(3): p. 4841-4846.
14. Zainol, I., et al., *Extraction of natural hydroxyapatite from tilapia fish scales using alkaline treatment.* Materials Today: Proceedings, 2019. **16**: p. 1942-1948.
15. Putranto, F., H., N.A. Andi, and K. Indrati, *Properties of belida (Chitala sp.) fish bone powder as calcium source based on protein hydrolysis method.* Ziraa'ah, 2015. **40**(1): p. 11 - 20.
16. Mustafa, N., et al., *Hydroxyapatite Extracted from Waste Fish Bones and Scales via Calcination Method.* Applied Mechanics and Materials, 2015. **773-774**: p. 287-290.
17. Barre, M.S., et al., *Potential food additive of Boswellia carterii essential oil encapsulated within gum arabic: a particle size distribution and zeta potential analysis.* Food Research, 2020. **4**(S2): p. 19-23.
18. Jindapon, N., et al., *Preparation, Characterization, and Biological Properties of Hydroxyapatite from Bigeye Snapper (Priancanthus tayenus) Bone.* International Journal of Molecular Sciences, 2023. **24**(3).
19. Hasan, M.R., et al., *Proximate and Morphological Characteristics of Nano Hydroxyapatite (Nano Hap) Extracted from Fish Bone.* Journal of Sustainability Science and Management, 2020. **15**(8): p. 9-21.
20. Benjakul, S., et al., *Biocalcium powder from precooked skipjack tuna bone: Production and its characteristics.* Journal of Food Biochemistry, 2017. **41**(6).
21. Beaufils, S., et al., *Synthesis of calcium-deficient hydroxyapatite nanowires and nanotubes performed by template-assisted electrodeposition.* Materials Science and Engineering: C, 2019. **98**: p. 333-346.
22. Dorozhkin, V.S., *Amorphous Calcium Orthophosphates: Nature, Chemistry and Biomedical Applications.* International Journal of Materials and Chemistry, 2012. **2**(1): p. 19-46.
23. Barakat, N.A.M., et al., *Extraction of pure natural hydroxyapatite from the bovine bones bio waste by three different methods.* Journal of Materials Processing Technology, 2009. **209**(7): p. 3408-3415.
24. Dermawan, S.K., et al., *Effect of the Calcination Temperature on the Properties of Hydroxyapatite from Black Tilapia Fish Bone.* Journal of Physics: Conference Series, 2022. **2169**(1).
25. Saeed, G.K., A.F. Essa, and S.A.-A. Said, *Preparation and characterization of hydroxyapatite powder and study of hydroxyapatite - alumina Composite.* Journal of Physics: Conference Series, 2020. **1591**(1).
26. Gheisari, H., E. Karamian, and M. Abdellahi, *A novel hydroxyapatite –Hardystonite nanocomposite ceramic.* Ceramics International, 2015. **41**(4): p. 5967-5975.
27. Pal, A., et al., *Synthesis of hydroxyapatite from Lates calcarifer fish bone for biomedical applications.* Materials Letters, 2017. **203**: p. 89-92.

28. Venkatesan, J., et al., *A comparative study of thermal calcination and an alkaline hydrolysis method in the isolation of hydroxyapatite from Thunnus obesus bone.* Biomedical Materials, 2011. **6**(3).

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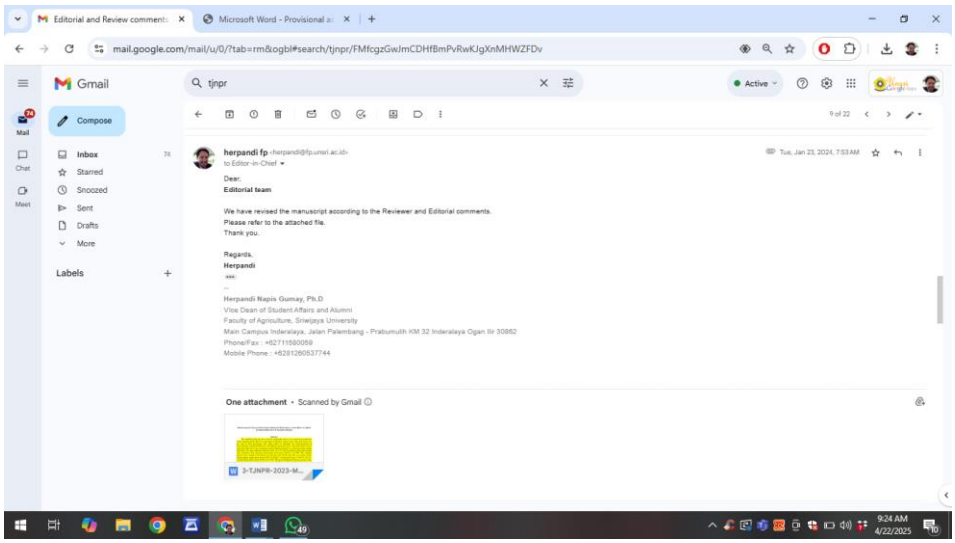

Hydroxyapatite Characteristics from Snakehead Fish (*Channa striata*) Bone via Alkali Treatment followed by Calcination Method

Abstract

The snakehead fish (*Channa striata*) is commonly used as a raw material in traditional South Sumatran foods. However, some parts of this fish, such as bone, skin, and viscera, are not used in food processing. This study aimed to determine the characterization of hydroxyapatite snakehead fish bone with different extraction times using ultrasound-assisted extraction followed by the calcination method. Hydroxyapatite was extracted using sodium hydroxide with three different extraction times (20, 40, and 60 minutes) before proceeding with calcination. The extraction yield ranges from about 16.03% to 19.99%. The smallest particle size is found at 40 minutes of extraction time (63.90 nm). The ash content of the hydroxyapatite ranges from about 98.09% to 99.04%, calcium from about 17.86% to 18.12%, and phosphorus from about 10.23% to 10.74%. The non-stoichiometric form is present in the hydroxyapatite from snakehead fish bone, with a Ca/P ratio of about 1.69 to 1.72. Analysis of the hydroxyapatite functional groups in snakehead fish bone showed the presence of phosphate groups, carbonate groups, and hydroxyl groups. This

data indicates that hydroxyapatite was successfully extracted from snakehead fish bone in nanoparticle form. Therefore, snakehead fish bone can be used as a source of hydroxyapatite.

Keywords: Calcination, *Channa striata*, extraction, hydroxyapatite, phosphorus

Introduction

Consumption of fish products has increased significantly since they were identified as essential to a healthy, balanced diet and way of life. As a result, there has been a sharp rise in the global production of fish waste.[1] Fish waste is a potential source of minerals or trace metals, essential fatty acids, amino acids, peptides, and protein of high biological value as well as vitamins.[2] Therefore, fish waste thus offers enormous untapped potential for value addition. The fish waste includes fish bones, scales, and fins. Whereas, the fish bone waste is around 9-15%.[3, 4] The fish bone waste composed of various mineral, such as calcium, phosphorus, zinc, and magnesium that can be used a source of hydroxyapatite.[5, 6]

Hydroxyapatite, with the molecular formula $Ca_{10}(PO_4)_6(OH)_2$, commonly referred to as HA, is one of the most common calcium phosphates that has compositions similar to those of natural bone.[6, 7] Due to its bioactive qualities and osteoconductive properties, hydroxyapatite has garnered interest for use in bone grafting procedures. Additionally, other medical applications of hydroxyapatite have also been studied because hydroxyapatite is non-toxic, bioactive, biocompatible, and non-inflammatory.[2] Natural hydroxyapatite has recently been extracted from various biowastes, including fish waste such as fish bone.[6, 8, 9] Various methods have been used for hydroxyapatite extraction from natural sources, including calcination, alkali treatment, and the combination methods.[10] A previous study reported that calcination method was used for hydroxyapatite extraction.[11, 12] NaOH treatment also was reported for the extraction hydroxyapatite from bovine bone.[13] Additionally, hydroxyapatite successfully extracted from tilapia fish scale using combination method (NaOH treatment and calcination).[14]

Snakehead fish (*Channa striata*) is the primary ingredient used in a traditional fishcake dish from South Sumatra, Indonesia, called "pempek." Generally, this product is made from snakehead fish meat; therefore, some parts of this fish become waste, including the bones. However, the study about the utilization of fishbone waste from "pempek" production has not yet been reported. Therefore, this study aimed to determine the characteristics of hydroxyapatite from snakehead fish bone using a combination of alkali (NaOH) treatment and calcination processes.

Materials and Methods

Sample preparation

The snakehead fish bone preparation was performed according to the previous methods.[6, 15] Briefly, the fish bones were washed and boiled at 80°C for 30 minutes. After that, it was cleaned

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and mixed with lime water, and then it was boiled for 3 hours. The fish bone was cooled at room temperature for 15 minutes and dried in an oven (Memmert Universal Oven UN55, Germany) at 80°C for 12 hours. The dried fish bone was then ground to powder (80 mesh) then continued to the calcination process.

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Hydroxyapatite extraction and calcination process

The hydroxyapatite extraction and calcination processes from fish bones were performed according to the previous methods.[6, 16] Briefly, 10 g of the fish bone powder was mixed with 1 N NaOH and then extracted using an ultrasonic bath at 50°C for different extraction times (20, 40, and 60 minutes). After the extraction times, it was cooled, filtered using filter paper (Whatman No. 42), and neutralized using distilled water. Then, it was oven-dried at 105°C for 3 hours before the calcination process. The calcination process was performed using a muffle furnace (Thermo Scientific FB1410M-33, USA) , at 600°C for 5 hours. After that, it was cooled to room temperature and kept for further analysis.

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Hydroxyapatite characterizations

The ash content analysis was determined according to the AOAC (2000). Briefly, the hydroxyapatite powder was completely incinerated in a muffle furnace at 550°C (AOAC method 930.05). Whereas the element composition was analyzed using energy dispersive X-ray spectroscopy integrated into scanning electron microscopy (SEM, JEOL JSM-7000F FE-SEM, Japan) and the functional group of the hydroxyapatite powder was analyzed using a Fourier transform infrared spectrometer (FTIR, Brucker Tensor 27) according to the previous method.[2] The particle size of the hydroxyapatite powder was analyzed using a particle size analyzer (Malvern Zetasizer Nano ZS Ver. 7.01) and the hydroxyapatite powder was also viewed by scanning electron microscopy according to the previous methods.[2, 17]

Statistical analysis

The data on yield, ash content, and particle size were expressed as the mean ± standard deviation (SD). It was analyzed using Duncan's multiple range test (p<0.05) using SPSS software (ver. 22.0; IBM Corporation, USA). Whereas, the mineral composition, functional groups and scanning electron microscope image were analyzed using descriptive analysis.

Results and Discussion

Yield of the hydroxyapatite

The yield of the snakehead fish bone hydroxyapatite is shown in **Figure 1.** The yields of the hydroxyapatite are about 16.03% - 19.99%. The highest yield of the hydroxyapatite is found in 60

minutes of extraction time and the lowest in 20 minutes. Whereas, there is no significant (p>0.05) difference between the yield of 40 minutes and other treatments. However, the yield of 20 minutes was significantly (p<0.05) different from 60 minutes of extraction time. A previous study reported that hydroxyapatite yield from bigeye snapper (*Priancanthus tayenus*) bones is about 13.4%.[18] Generally, there are two main methods for obtaining hydroxyapatite: chemical synthesis and biological resource extraction. Compared to synthetic forms, animal bone hydroxyapatite has a few advantages in terms of chemical composition and structure, which are identical to human bone, reduced manufacturing costs, and enhanced biological response.[12] The organic content in the bone is normally removed using an alkaline solution, such as NaOH. The organic component of the bone is hydrolyzed by the NaOH solution, and the remaining calcium phosphate is then washed and filtered. Whereas, calcination was used to completely remove the organic content from the bone.[10]

Ash content

The ash content of the snakehead fish bone hydroxyapatite powder is shown in **Figure 2.** The ash contents of the hydroxyapatite from snakehead fish bone are about 98.09% - 99.04%. The ash content for 40 minutes of extraction shows a significant (p<0.05) difference with the other treatments. Whereas, the ash content of 20 minutes was no significant (p>0.05) different with 60 minutes of extraction time. This condition indicated that the hydroxyapatite powder was composed of high-level minerals. A previous study reported that hydroxyapatite from the sardinella (*Amblygaster sirm*) is composed of 98.24% of ash.[19] Additionally, 99.75% of the ash content is observed in calcined bone from skipjack tuna.[20]

Mineral compositions of the hydroxyapatite

The mineral composition of the hydroxyapatite powder according to energy dispersive X-ray spectroscopy is shown in **Figure 3** and **Table 1**. The main minerals of the hydroxyapatite from snakehead fish bone are calcium (Ca) and phosphorus (P), followed by a small amount of other minerals such as magnesium (Mg) and natrium (Na). According to **Table 1**, the calcium value is about 17.86% - 18.12% and the phosphorus value is about 10.23% - 10.74%. These data indicated that the non-stoichiometric form is found in the hydroxyapatite from snakehead fish bone, with a Ca/P molar ratio of about 1.69 - 1.72. Generally, stoichiometric hydroxyapatite is composed of a Ca/P ratio of about 1.67.[21] Biological sources such as the scale of fish and bones of animals are the sources of non-stoichiometric hydroxyapatite.[22] A previous study reported that the Ca/P ratio of bovine bone hydroxyapatite was about 1.86.[23] The Ca/P ratio of hydroxyapatite from black tilapia fish bone is about 1.56 - 1.65 and it was also reported that calcination temperature affected the Ca/P ratio.[24] Additionally, calcium and phosphorus are the main elements in hydroxyapatite powder.[25]

Functional groups of the hydroxyapatite

The Fourier-transform infrared (FT-IR) spectra and functional groups of the hydroxyapatite are shown in **Figure 4** and **Table 2**. The functional group of the FT-IR spectra was analyzed according to the previous studies.[2, 26] The results showed that asymmetric bending vibrations of phosphate (v_3 PO_4^{3-}) were detected at 571.03 cm^{-1} (20 min), 568.36 cm^{-1} (40 min), and 567.78 cm^{-1} (60 min). The symmetric stretching of PO_4^3 (v_1 PO_4^{3-}) is detected at 962.54 cm^{-1} (20 min), 962.47 cm^{-1} (40 min), and 962.19 cm^{-1} (60 min). Also, the asymmetric stretching of PO_4^3 (v_2 PO_4^{3-}) is detected at 1034.40 cm^{-1} (20 min), 1034.26 cm^{-1} (40 min), and 1035.01 cm^{-1} (60 min). A previous study reported that hydroxyapatite from fish scale indicated the presence of asymmetric bending vibrations of the phosphate group at 563 cm^{-1}, symmetric stretching of PO_4^{3-} at 957 cm^{-1}, and asymmetric stretching of PO_4^{3-} at 1030 cm^{-1}.[2] A previous study also reported that the phosphate groups of natural hydroxyapatites from veal bone were also detected at 560 cm^{-1} and 1000 – 1100 cm^{-1}.[26]

In this present study, the carbonate (CO_3^{2-}) groups were also detected in the hydroxyapatite powder. The out-of-plane bending modes of CO_3^{2-} (v_1 CO_3^{2-}) were detected at 875.47 cm^{-1} (20 min), 874.87 cm^{-1} (40 min), and 874.12 cm^{-1}. Whereas, the asymmetric stretching of CO_3^{2-} (v_2 CO_3^{2-}) was detected at 1458.85 cm^{-1} (20 min), 1456.74 cm^{-1} (40 min), and 1467.00 cm^{-1} (60 min). The hydroxyapatite powder also indicated the presence of hydroxyl (OH^-) groups at 3571.83 cm^{-1} (20 min), 3565.20 cm^{-1} (40 min), and 3443.75 cm^{-1} (60 min). A previous study reported that hydroxyapatite powder from fish scale indicated the presence of out-of-plane bending mode of CO_3^{2-} at 876 cm^{-1} and asymmetric stretching of CO_3^{2-} at 1412-1547 cm^{-1}.[2] Also, CO_3^{2-} natural hydroxyapatite powder from veal bone was detected at 1460 – 1530 cm^{-1}.[26] According to these data, the hydroxyapatite was successfully extracted from snakehead fish bone. These data also support the energy dispersive X-ray spectroscopy (EDS) analysis that confirm the presence of calcium and phosphorus in the hydroxyapatite powder.

Particle size of the hydroxyapatite

The particle size of the snakehead fish (*C. striata*) bone hydroxyapatite powder is shown in **Figure 5**. The particle size of the hydroxyapatite is about 63.9 nm - 138.2 nm. The smaller size of the hydroxyapatite is found in 40 minutes of extraction time. The value was significantly (p<0.05) different from other treatments. Whereas, there is no significant (p>0.05) difference in particle size between 20 minutes and 60 minutes of extraction time. The small particle size in 40 minutes of extraction time was also confirmed by scanning electron microscopy (SEM) as shown in **Figure 6**. The irregular form is observed in the hydroxyapatite powder. A previous study also reported hydroxyapatite extracted from fish bone alkali treatment and calcination, resulting in an irregular form of hydroxyapatite.[27, 28]

Conclusion

In this present study, the hydroxyapatite was successfully extracted from snakehead fish (*Channa striata*) bone. The extraction yield is about 16.03%-19.99%. The smallest particle size is

found in 40 minutes of extraction time (63.90 nm). The ash content of the hydroxyapatite is about 98.09% - 99.04%, calcium is about 17.86% - 18.12%, and the phosphorus is about 10.23% - 10.74%. The non-stoichiometric form is found in this hydroxyapatite with a Ca/P ratio of about 1.69 - 1.72. Analysis of the hydroxyapatite functional groups of snakehead fish bone showed the presence of phosphate groups (PO_4^{3-}), carbonate groups (CO_3^{2-}), and hydroxyl groups (OH^-). These data indicated that the hydroxyapatite was successfully extracted from snakehead fish bone in nanoparticle form. Therefore, snakehead fish bone can be used as a source of nanohydroxyapatite.

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Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Coppola D, Lauritano D, Esposito FP, Riccio G, Rizzo C, de Pascale D. Fish waste: From problem to valuable resource. Marine Drugs. 2021; 19:116.
2. Huang YC, Hsiao PC, Chai HJ. Hydroxyapatite extracted from fish scale: Effects on MG63 osteoblast-like cells. Ceram. Int. 2011; 37:1825-1831.
3. Maktoof AA, Elherarlla RJ, Ethaib S. Identifying the nutritional composition of fish waste, bones, scales, and fins. IOP Conf. Ser.: Mater. Sci. Eng. 2020; 871:012013.
4. Boronat O, Sintes P, Celis F, Díez M, Ortiz J, Aguayo IA, Gómez HM. Development of added-value culinary ingredients from fish waste: Fish bones and fish scales. Int. J. Gastron. Food Sci. 2023, 31:100657.
5. Lall SP, Kaushik SJ. Nutrition and metabolism of minerals in fish. Animals. 2021; 11: 2711.
6. Marliana A, Fitriani E, Ramadhan F, Suhandono S, Yuliani K, Windarti T. Synthesis and characterization of hydroxyapatite from fish bone waste. AIP Conf. Proc. 2015; 1699: 040006.
7. Jaffri MZ, Ismail ZMM, Dermawan SK, Abdullah HZ. Sample preparation of the natural source hydroxyapatite (HAp) derived from black tilapia fish scales. J. Phys. Conf. Ser. 2022; 2169:012033.
8. Ruiz KLH, Cervantes JL, Machado DIS, Macias MdRM, Murrieta MAC, Silva AS. Hydroxyapatite recovery from fish byproducts for biomedical applications. Sustain. Chem. Pharm. 2022; 28:100726.

9. Granito RN, Renno ACM, Yamamura H, Almeida MCd, Ruiz PLM, Ribeiro DA. Hydroxyapatite from fish for bone tissue engineering: A promising approach. Int. J. Mol. Cell Med. 2018; 7:80-90.
10. Pu'ad MNAS, Koshy P, Abdullah HZ, Idris MI, Lee TC. Syntheses of hydroxyapatite from natural sources. Heliyon. 2019; 5:e01588.
11. Kupiec AS, Wzorek Z. The influence of calcination parameters on free calcium oxide content in natural hydroxyapatite. Ceram. Int. 2012; 38:641-647.
12. Dabiri SMH, Rezaie AA, Moghimi M, Rezaie H. Extraction of hydroxyapatite from fish bones and its application in nickel adsorption. BioNanoScience. 2018; 8:823-834.
13. Miecznik JB, Haberko K, Sitarz M, Bućko MM, Macherzyńska B. Hydroxyapatite from animal bones – Extraction and properties. Ceram. Int. 2015; 41:4841-4846.
14. Zainol I, Adenan NH, Rahim NA, Jaafar CAN. Extraction of natural hydroxyapatite from tilapia fish scales using alkaline treatment. Mater. Today: Proc. 2019; 16:1942-1948.
15. Putranto FH, Andi NA, Indrati K. Properties of belida (*Chitala* sp.) fish bone powder as calcium source based on protein hydrolysis method. Ziraa'ah. 2015; 40:11-20.
16. Mustafa N, Ibrahim MHI, Asmawi R, Amin AM. Hydroxyapatite extracted from waste fish bones and scales via calcination method. Appl. Mech. Mater. 2015; 773-774:287-290.
17. Barre MS, Ali FB, Mirghani MES, Hazri NF, Anuar H, Nasir NAM. Potential food additive of *Boswellia carterii* essential oil encapsulated within gum arabic: A particle size distribution and zeta potential analysis. Food Res. 2020; 4:19-23.
18. Jindapon N, Klinmalai P, Surayot U, Tanadchangsaeng N, Pichaiaukrit W, Phimolsiripol Y, Vichasilp C, Wangtueai S. Preparation, characterization, and biological properties of hydroxyapatite from bigeye snapper (*Priancanthus tayenus*) bone. Int. J. Mol. Sci. 2023; 24:2776.
19. Hasan MR, Yasin NSM, Ghazali MSM, Mohtar NF. Proximate and morphological characteristics of nano hydroxyapatite (Nano HAp) extracted from fish bone. J. Sustain. Sci. Manag. 2020; 15:9-21.
20. Benjakul S, Ali SM, Senphan T, Sookchoo P. Biocalcium powder from precooked skipjack tuna bone: Production and its characteristics. J. Food Biochem. 2017; 41:e12412.
21. Beaufils S, Rouillon T, Millet P, Bideau JL, Weiss P, Chopart JP, Daltin AL. Synthesis of Calcium-deficient hydroxyapatite nanowires and nanotubes performed by template-assisted electrodeposition. Mater. Sci. Eng. 2019; 98:333-346.
22. Dorozhkin VS. Amorphous Calcium orthophosphates: Nature, chemistry and biomedical applications. Int. J. Mater. Chem. 2012; 2:19-46.
23. Barakat NAM, Khil MS, Omran AM, Sheikh FA, Kim HY. Extraction of pure natural hydroxyapatite from the bovine bones bio waste by three different methods. J. Mater. Process. Technol. 2009; 209:3408-3415.
24. Dermawan SK, Ismail ZMM, Jaffri MZ, Abdullah HZ. Effect of the Calcination temperature on the properties of hydroxyapatite from black tilapia fish bone. J. Phys. Conf. Ser. 2022; 2169:012034.
25. Saeed GK, Essa AF, Said SAA. Preparation and characterization of hydroxyapatite powder and study of hydroxyapatite - alumina composite. J. Phys. Conf. Ser. 2020; 1591:012006.
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27. Pal A, Paul S, Choudhury AR, Balla VK, Das M, Sinha A. Synthesis of hydroxyapatite from *Lates calcarifer* fish bone for biomedical applications. Mater. Lett. 2017; 203:89-92.
28. Venkatesan J, Qian ZJ, Ryu B, Thomas NV, Kim SK. A comparative study of thermal calcination and an alkaline hydrolysis method in the isolation of hydroxyapatite from *Thunnus obesus* bone. Biomed. Mater. 2011; 6:035003.

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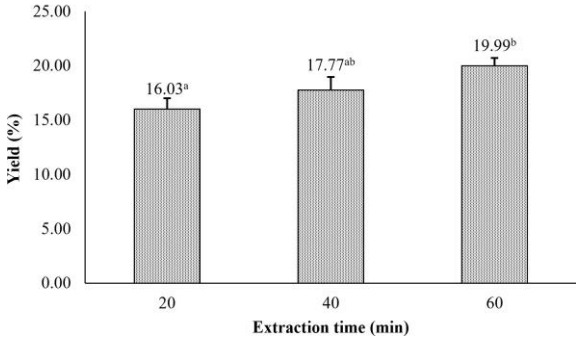

Figure 1. The yield of snakehead fish (*C. striata*) bone hydroxyapatite with different extraction time. Data are shown as mean ± SD (*n*=3).

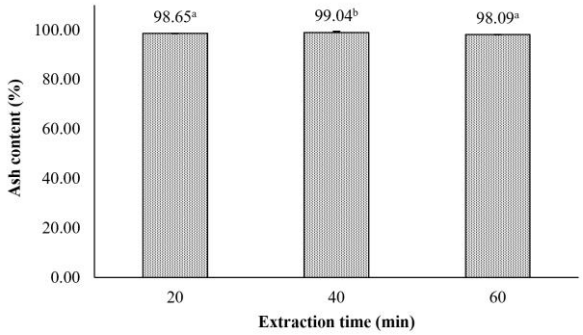

Figure 2. The ash content of the snakehead fish (*C. striata*) bone hydroxyapatite powder. Data are shown as mean ± SD (*n*=3).

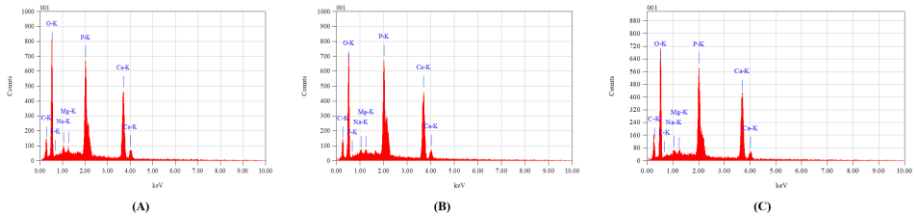

Figure 3. The mineral composition of hydroxyapatite from snakehead fish bone as analyzed by Energy Dispersive X-ray Spectroscopy (EDS).

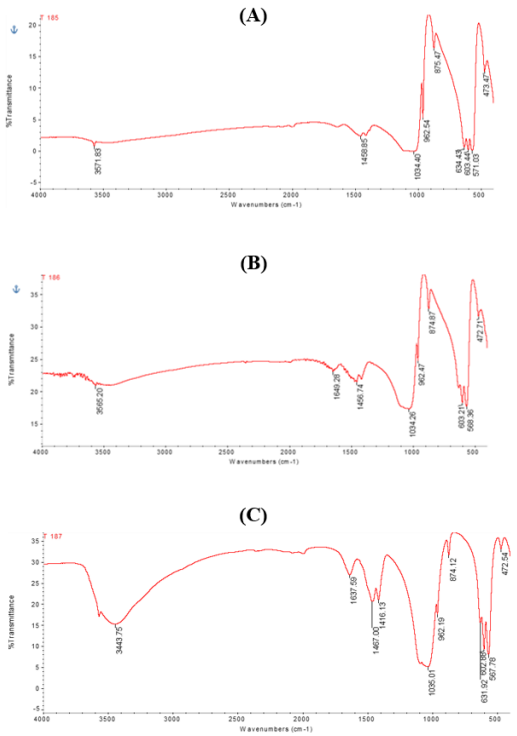

Figure 4. The FT-IR spectra of the snakehead fish bone hydroxyapatite with different extraction times. (A) 20 minutes; (B) 40 minutes; and (C) 60 minutes.

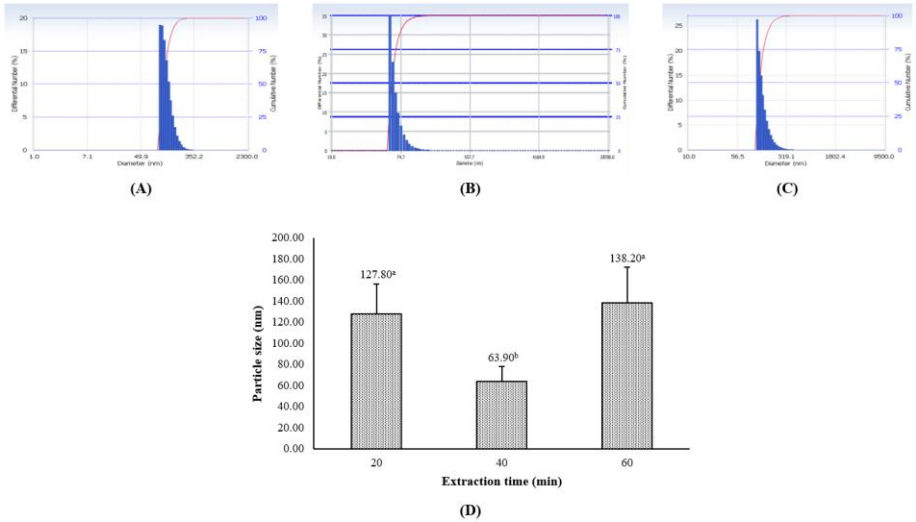

Figure 5. The particle size of the snakehead fish (*C. striata*) bone hydroxyapatite with different extraction times. (A) 20 minutes of extraction time; (B) 40 minutes of extraction time; (C) 60 minutes of extraction time; and (D) the mean ± SD of the hydroxyapatite particle size (n=3).

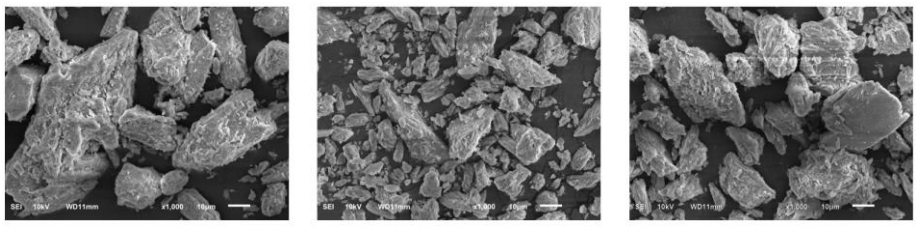

Figure 6. The representative of hydroxyapatite powder morphology by scanning electron microscopy (SEM) with different extraction times. (A) 20 minutes; (B) 40 minutes; and (C) 60 minutes.

Table 1. Mineral compositions of the hydroxyapatite snakehead fish bone to the energy dispersive X-ray spectroscopy.

Minerals	Extraction times (min)					
	20		40		60	
	Energy (keV)	Atom (%)	Energy (keV)	Atom (%)	Energy (keV)	Atom (%)
Ca	3.69	17.95	3.69	18.12	3.69	17.86
P	2.01	10.23	2.01	10.74	2.01	10.36
Mg	1.25	0.52	1.25	0.49	1.25	0.40
Na	1.04	0.78	1.04	0.43	1.04	0.44
O	0.53	55.76	0.53	53.23	0.53	53.90

Table 2. The functional groups of the hydroxyapatite from snakehead fish bone.

Functional groups*	Extraction times (min)					
	20		40		60	
	cm^{-1}	%T	cm^{-1}	%T	cm^{-1}	%T
v_1 PO_4^{3-}	962.54	55.25	962.47	24.80	962.19	17.60
v_2 PO_4^{3-}	1034.40	0.01	1034.26	17.37	1035.01	5.19
v_3 PO_4^{3-}	571.03	0.12	569.36	17.79	567.78	8.12
v_1 CO_3^{2-}	875.47	16.33	874.87	32.82	874.12	31.74
v_2 CO_3^{2-}	1458.85	2.38	1456.74	21.53	1467.00	20.72
OH^-	3571.83	0.84	3565.20	22.09	3443.75	15.99

***** $v_1PO_4^{3-}$, the symmetric stretching of phosphate; $v_2PO_4^{3-}$, asymmetric stretching of phosphate; $v_3PO_4^{3-}$, asymmetric bending vibrations of phosphate; $v_1CO_3^{2-}$, the out-of-plane bending modes of carbonate; $v_2CO_3^{2-}$, the asymmetric stretching of carbonate.

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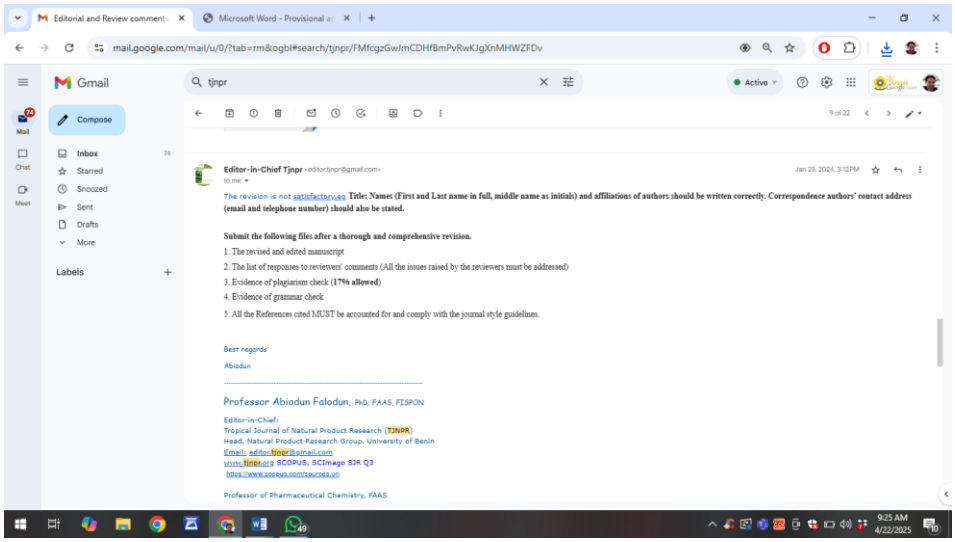

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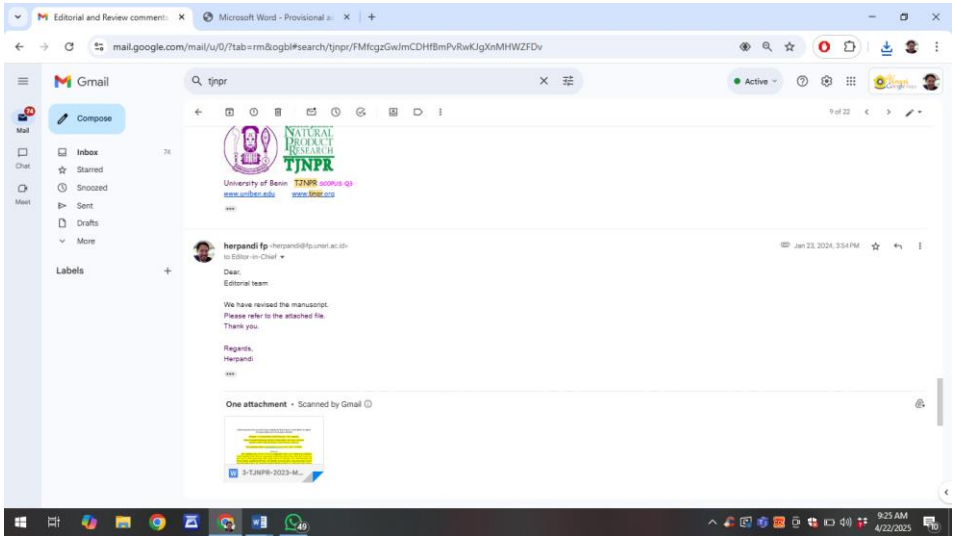

Hydroxyapatite Characteristics from Snakehead Fish (*Channa striata*) Bone via Alkali Treatment followed by Calcination Method

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Abstract

The snakehead fish (*Channa striata*) is commonly used as a raw material in traditional South Sumatran foods. However, some parts of this fish, such as bone, skin, and viscera, are not used in food processing. This study aimed to determine the characterization of hydroxyapatite snakehead fish bone with different extraction times using ultrasound-assisted extraction followed by the calcination method. Hydroxyapatite was extracted using sodium hydroxide with three different extraction times (20, 40, and 60 minutes) before proceeding with calcination. The extraction yield ranges from about 16.03% to 19.99%. The smallest particle size is found at 40 minutes of extraction time (63.90 nm). The ash content of the hydroxyapatite ranges from about 98.09% to 99.04%, calcium from about 17.86% to 18.12%, and phosphorus from about 10.23% to 10.74%. The non-stoichiometric form is present in the hydroxyapatite from snakehead fish bone, with a Ca/P ratio of about 1.69 to 1.72. Analysis of the hydroxyapatite functional groups in snakehead fish bone showed the presence of phosphate groups, carbonate groups, and hydroxyl groups. This data indicates that hydroxyapatite was successfully extracted from snakehead fish bone in nanoparticle form. Therefore, snakehead fish bone can be used as a source of hydroxyapatite.

Keywords: Calcination, *Channa striata*, extraction, hydroxyapatite, phosphorus

Introduction

Consumption of fish products has increased significantly since they were identified as essential to a healthy, balanced diet and way of life. As a result, there has been a sharp rise in the global production of fish waste.[1] Fish waste is a potential source of minerals or trace metals, essential fatty acids, amino acids, peptides, and protein of high biological value as well as vitamins.[2] Therefore, fish waste thus offers enormous untapped potential for value addition. The fish waste includes fish bones, scales, and fins. Whereas, the fish bone waste is around 9-15%.[3, 4]

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The fish bone waste composed of various mineral, such as calcium, phosphorus, zinc, and magnesium that can be used a source of hydroxyapatite.[5, 6]

Hydroxyapatite, with the molecular formula $Ca_{10}(PO_4)_6(OH)_2$, commonly referred to as HA, is one of the most common calcium phosphates that has compositions similar to those of natural bone.[6, 7] Due to its bioactive qualities and osteoconductive properties, hydroxyapatite has garnered interest for use in bone grafting procedures. Additionally, other medical applications of hydroxyapatite have also been studied because hydroxyapatite is non-toxic, bioactive, biocompatible, and non-inflammatory.[2] Natural hydroxyapatite has recently been extracted from various biowastes, including fish waste such as fish bone.[6, 8, 9] Various methods have been used for hydroxyapatite extraction from natural sources, including calcination, alkali treatment, and the combination methods.[10] A previous study reported that calcination method was used for hydroxyapatite extraction.[11, 12] NaOH treatment also was reported for the extraction hydroxyapatite from bovine bone.[13] Additionally, hydroxyapatite successfully extracted from tilapia fish scale using combination method (NaOH treatment and calcination).[14]

Snakehead fish (*Channa striata*) is the primary ingredient used in a traditional fishcake dish from South Sumatra, Indonesia, called "pempek." Generally, this product is made from snakehead fish meat; therefore, some parts of this fish become waste, including the bones. However, the study about the utilization of fishbone waste from "pempek" production has not yet been reported. Therefore, this study aimed to determine the characteristics of hydroxyapatite from snakehead fish bone using a combination of alkali (NaOH) treatment and calcination processes.

Materials and Methods

Sample preparation

The snakehead fish bone preparation was performed according to the previous methods.[6, 15] Briefly, the fish bones were washed and boiled at 80°C for 30 minutes. After that, it was cleaned and mixed with lime water, and then it was boiled for 3 hours. The fish bone was cooled at room temperature for 15 minutes and dried in an oven (Memmert Universal Oven UN55, Germany) at 80°C for 12 hours. The dried fish bone was then ground to powder (80 mesh) then continued to the calcination process.

Hydroxyapatite extraction and calcination process

The hydroxyapatite extraction and calcination processes from fish bones were performed according to the previous methods.[6, 16] Briefly, 10 g of the fish bone powder was mixed with 1 N NaOH and then extracted using an ultrasonic bath at 50°C for different extraction times (20, 40, and 60 minutes). After the extraction times, it was cooled, filtered using filter paper (Whatman No. 42), and neutralized using distilled water. Then, it was oven-dried at 105°C for 3 hours before the calcination process. The calcination process was performed using a muffle furnace (Thermo

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Scientific FB1410M-33, USA) , at 600°C for 5 hours. After that, it was cooled to room temperature and kept for further analysis.

Hydroxyapatite characterizations

The ash content analysis was determined according to the AOAC (2000). Briefly, the hydroxyapatite powder was completely incinerated in a muffle furnace at 550°C (AOAC method 930.05). Whereas the element composition was analyzed using energy dispersive X-ray spectroscopy integrated into scanning electron microscopy (SEM, JEOL JSM-7000F FE-SEM, Japan) and the functional group of the hydroxyapatite powder was analyzed using a Fourier transform infrared spectrometer (FTIR, Brucker Tensor 27) according to the previous method.[2] The particle size of the hydroxyapatite powder was analyzed using a particle size analyzer (Malvern Zetasizer Nano ZS Ver. 7.01) and the hydroxyapatite powder was also viewed by scanning electron microscopy according to the previous methods.[2, 17]

Statistical analysis

The data on yield, ash content, and particle size were expressed as the mean ± standard deviation (SD). It was analyzed using Duncan's multiple range test (p<0.05) using SPSS software (ver. 22.0; IBM Corporation, USA). Whereas, the mineral composition, functional groups and scanning electron microscope image were analyzed using descriptive analysis.

Results and Discussion

Yield of the hydroxyapatite

The yield of the snakehead fish bone hydroxyapatite is shown in **Figure 1.** The yields of the hydroxyapatite are about 16.03% - 19.99%. The highest yield of the hydroxyapatite is found in 60 minutes of extraction time and the lowest in 20 minutes. Whereas, there is no significant (p>0.05) difference between the yield of 40 minutes and other treatments. However, the yield of 20 minutes was significantly (p<0.05) different from 60 minutes of extraction time. A previous study reported that hydroxyapatite yield from bigeye snapper (*Priancanthus tayenus*) bones is about 13.4%.[18] Generally, there are two main methods for obtaining hydroxyapatite: chemical synthesis and biological resource extraction. Compared to synthetic forms, animal bone hydroxyapatite has a few advantages in terms of chemical composition and structure, which are identical to human bone, reduced manufacturing costs, and enhanced biological response.[12] The organic content in the bone is normally removed using an alkaline solution, such as NaOH. The organic component of the bone is hydrolyzed by the NaOH solution, and the remaining calcium phosphate is then washed and filtered. Whereas, calcination was used to completely remove the organic content from the bone.[10]

Ash content

The ash content of the snakehead fish bone hydroxyapatite powder is shown in **Figure 2.** The ash contents of the hydroxyapatite from snakehead fish bone are about 98.09% - 99.04%. The ash content for 40 minutes of extraction shows a significant (p<0.05) difference with the other treatments. Whereas, the ash content of 20 minutes was no significant (p>0.05) different with 60 minutes of extraction time. This condition indicated that the hydroxyapatite powder was composed of high-level minerals. A previous study reported that hydroxyapatite from the sardinella (*Amblygaster sirm*) is composed of 98.24% of ash.[19] Additionally, 99.75% of the ash content is observed in calcined bone from skipjack tuna.[20]

Mineral compositions of the hydroxyapatite

The mineral composition of the hydroxyapatite powder according to energy dispersive X-ray spectroscopy is shown in **Figure 3** and **Table 1**. The main minerals of the hydroxyapatite from snakehead fish bone are calcium (Ca) and phosphorus (P), followed by a small amount of other minerals such as magnesium (Mg) and natrium (Na). According to **Table 1**, the calcium value is about 17.86% - 18.12% and the phosphorus value is about 10.23% - 10.74%. These data indicated that the non-stoichiometric form is found in the hydroxyapatite from snakehead fish bone, with a Ca/P molar ratio of about 1.69 - 1.72. Generally, stoichiometric hydroxyapatite is composed of a Ca/P ratio of about 1.67.[21] Biological sources such as the scale of fish and bones of animals are the sources of non-stoichiometric hydroxyapatite.[22] A previous study reported that the Ca/P ratio of bovine bone hydroxyapatite was about 1.86.[23] The Ca/P ratio of hydroxyapatite from black tilapia fish bone is about 1.56 - 1.65 and it was also reported that calcination temperature affected the Ca/P ratio.[24] Additionally, calcium and phosphorus are the main elements in hydroxyapatite powder.[25]

Functional groups of the hydroxyapatite

The Fourier-transform infrared (FT-IR) spectra and functional groups of the hydroxyapatite are shown in **Figure 4** and **Table 2**. The functional group of the FT-IR spectra was analyzed according to the previous studies.[2, 26] The results showed that asymmetric bending vibrations of phosphate (v_3 PO_4^{3-}) were detected at 571.03 cm^{-1} (20 min), 568.36 cm^{-1} (40 min), and 567.78 cm^{-1} (60 min). The symmetric stretching of PO_4^3 (v_1 PO_4^{3-}) is detected at 962.54 cm^{-1} (20 min), 962.47 cm^{-1} (40 min), and 962.19 cm^{-1} (60 min). Also, the asymmetric stretching of PO_4^3 (v_2 PO_4^{3-}) is detected at 1034.40 cm^{-1} (20 min), 1034.26 cm^{-1} (40 min), and 1035.01 cm^{-1} (60 min). A previous study reported that hydroxyapatite from fish scale indicated the presence of asymmetric bending vibrations of the phosphate group at 563 cm^{-1}, symmetric stretching of PO_4^{3-} at 957 cm^{-1}, and asymmetric stretching of PO_4^{3-} at 1030 cm^{-1}.[2] A previous study also reported that the phosphate groups of natural hydroxyapatites from veal bone were also detected at 560 cm^{-1} and 1000 – 1100 cm^{-1}.[26]

In this present study, the carbonate (CO_3^{2-}) groups were also detected in the hydroxyapatite powder. The out-of-plane bending modes of CO_3^{2-} (v_1 CO_3^{2-}) were detected at 875.47 cm^{-1} (20 min), 874.87 cm^{-1} (40 min), and 874.12 cm^{-1}. Whereas, the asymmetric stretching of CO_3^{2-} (v_2 CO_3^{2-}) was detected at 1458.85 cm^{-1} (20 min), 1456.74 cm^{-1} (40 min), and 1467.00 cm^{-1} (60 min). The hydroxyapatite powder also indicated the presence of hydroxyl (OH^-) groups at 3571.83 cm^{-1} (20 min), 3565.20 cm^{-1} (40 min), and 3443.75 cm^{-1} (60 min). A previous study reported that hydroxyapatite powder from fish scale indicated the presence of out-of-plane bending mode of CO_3^{2-} at 876 cm^{-1} and asymmetric stretching of CO_3^{2-} at 1412-1547 cm^{-1}.[2] Also, CO_3^{2-} natural hydroxyapatite powder from veal bone was detected at 1460 – 1530 cm^{-1}.[26] According to these data, the hydroxyapatite was successfully extracted from snakehead fish bone. These data also support the energy dispersive X-ray spectroscopy (EDS) analysis that confirm the presence of calcium and phosphorus in the hydroxyapatite powder.

Particle size of the hydroxyapatite

The particle size of the snakehead fish (*C. striata*) bone hydroxyapatite powder is shown in **Figure 5**. The particle size of the hydroxyapatite is about 63.9 nm - 138.2 nm. The smaller size of the hydroxyapatite is found in 40 minutes of extraction time. The value was significantly (p<0.05) different from other treatments. Whereas, there is no significant (p>0.05) difference in particle size between 20 minutes and 60 minutes of extraction time. The small particle size in 40 minutes of extraction time was also confirmed by scanning electron microscopy (SEM) as shown in **Figure 6**. The irregular form is observed in the hydroxyapatite powder. A previous study also reported hydroxyapatite extracted from fish bone alkali treatment and calcination, resulting in an irregular form of hydroxyapatite.[27, 28]

Conclusion

In this present study, the hydroxyapatite was successfully extracted from snakehead fish (*Channa striata*) bone. The extraction yield is about 16.03%-19.99%. The smallest particle size is found in 40 minutes of extraction time (63.90 nm). The ash content of the hydroxyapatite is about 98.09% - 99.04%, calcium is about 17.86% - 18.12%, and the phosphorus is about 10.23% - 10.74%. The non-stoichiometric form is found in this hydroxyapatite with a Ca/P ratio of about 1.69 - 1.72. Analysis of the hydroxyapatite functional groups of snakehead fish bone showed the presence of phosphate groups (PO_4^{3-}), carbonate groups (CO_3^{2-}), and hydroxyl groups (OH^-). These data indicated that the hydroxyapatite was successfully extracted from snakehead fish bone in nanoparticle form. Therefore, snakehead fish bone can be used as a source of nanohydroxyapatite.

Acknowledgment

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Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Coppola D, Lauritano D, Esposito FP, Riccio G, Rizzo C, de Pascale D. Fish waste: From problem to valuable resource. Marine Drugs. 2021; 19:116.
2. Huang YC, Hsiao PC, Chai HJ. Hydroxyapatite extracted from fish scale: Effects on MG63 osteoblast-like cells. Ceram. Int. 2011; 37:1825-1831.
3. Maktoof AA, Elherarlla RJ, Ethaib S. Identifying the nutritional composition of fish waste, bones, scales, and fins. IOP Conf. Ser.: Mater. Sci. Eng. 2020; 871:012013.
4. Boronat O, Sintes P, Celis F, Díez M, Ortiz J, Aguayo IA, Gómez HM. Development of added-value culinary ingredients from fish waste: Fish bones and fish scales. Int. J. Gastron. Food Sci. 2023, 31:100657.
5. Lall SP, Kaushik SJ. Nutrition and metabolism of minerals in fish. Animals. 2021; 11: 2711.
6. Marliana A, Fitriani E, Ramadhan F, Suhandono S, Yuliani K, Windarti T. Synthesis and characterization of hydroxyapatite from fish bone waste. AIP Conf. Proc. 2015; 1699: 040006.
7. Jaffri MZ, Ismail ZMM, Dermawan SK, Abdullah HZ. Sample preparation of the natural source hydroxyapatite (HAp) derived from black tilapia fish scales. J. Phys. Conf. Ser. 2022; 2169:012033.
8. Ruiz KLH, Cervantes JL, Machado DIS, Macias MdRM, Murrieta MAC, Silva AS. Hydroxyapatite recovery from fish byproducts for biomedical applications. Sustain. Chem. Pharm. 2022; 28:100726.
9. Granito RN, Renno ACM, Yamamura H, Almeida MCd, Ruiz PLM, Ribeiro DA. Hydroxyapatite from fish for bone tissue engineering: A promising approach. Int. J. Mol. Cell Med. 2018; 7:80-90.
10. Pu'ad MNAS, Koshy P, Abdullah HZ, Idris MI, Lee TC. Syntheses of hydroxyapatite from natural sources. Heliyon. 2019; 5:e01588.
11. Kupiec AS, Wzorek Z. The influence of calcination parameters on free calcium oxide content in natural hydroxyapatite. Ceram. Int. 2012; 38:641-647.
12. Dabiri SMH, Rezaie AA, Moghimi M, Rezaie H. Extraction of hydroxyapatite from fish bones and its application in nickel adsorption. BioNanoScience. 2018; 8:823-834.
13. Miecznik JB, Haberko K, Sitarz M, Bućko MM, Macherzyńska B. Hydroxyapatite from animal bones – Extraction and properties. Ceram. Int. 2015; 41:4841-4846.
14. Zainol I, Adenan NH, Rahim NA, Jaafar CAN. Extraction of natural hydroxyapatite from tilapia fish scales using alkaline treatment. Mater. Today: Proc. 2019; 16:1942-1948.

15. Putranto FH, Andi NA, Indrati K. Properties of belida (*Chitala* sp.) fish bone powder as calcium source based on protein hydrolysis method. Ziraa'ah. 2015; 40:11-20.
16. Mustafa N, Ibrahim MHI, Asmawi R, Amin AM. Hydroxyapatite extracted from waste fish bones and scales via calcination method. Appl. Mech. Mater. 2015; 773-774:287-290.
17. Barre MS, Ali FB, Mirghani MES, Hazri NF, Anuar H, Nasir NAM. Potential food additive of *Boswellia carterii* essential oil encapsulated within gum arabic: A particle size distribution and zeta potential analysis. Food Res. 2020; 4:19-23.
18. Jindapon N, Klinmalai P, Surayot U, Tanadchangsaeng N, Pichaiaukrit W, Phimolsiripol Y, Vichasilp C, Wangtueai S. Preparation, characterization, and biological properties of hydroxyapatite from bigeye snapper (*Priancanthus tayenus*) bone. Int. J. Mol. Sci. 2023; 24:2776.
19. Hasan MR, Yasin NSM, Ghazali MSM, Mohtar NF. Proximate and morphological characteristics of nano hydroxyapatite (Nano HAp) extracted from fish bone. J. Sustain. Sci. Manag. 2020; 15:9-21.
20. Benjakul S, Ali SM, Senphan T, Sookchoo P. Biocalcium powder from precooked skipjack tuna bone: Production and its characteristics. J. Food Biochem. 2017; 41:e12412.
21. Beaufils S, Rouillon T, Millet P, Bideau JL, Weiss P, Chopart JP, Daltin AL. Synthesis of Calcium-deficient hydroxyapatite nanowires and nanotubes performed by template-assisted electrodeposition. Mater. Sci. Eng. 2019; 98:333-346.
22. Dorozhkin VS. Amorphous Calcium orthophosphates: Nature, chemistry and biomedical applications. Int. J. Mater. Chem. 2012; 2:19-46.
23. Barakat NAM, Khil MS, Omran AM, Sheikh FA, Kim HY. Extraction of pure natural hydroxyapatite from the bovine bones bio waste by three different methods. J. Mater. Process. Technol. 2009; 209:3408-3415.
24. Dermawan SK, Ismail ZMM, Jaffri MZ, Abdullah HZ. Effect of the Calcination temperature on the properties of hydroxyapatite from black tilapia fish bone. J. Phys. Conf. Ser. 2022; 2169:012034.
25. Saeed GK, Essa AF, Said SAA. Preparation and characterization of hydroxyapatite powder and study of hydroxyapatite - alumina composite. J. Phys. Conf. Ser. 2020; 1591:012006.
26. Gheisari H, Karamian E, Abdellahi M. A novel hydroxyapatite – Hardystonite nanocomposite ceramic. Ceram. Int. 2015; 41:5967-5975.
27. Pal A, Paul S, Choudhury AR, Balla VK, Das M, Sinha A. Synthesis of hydroxyapatite from *Lates calcarifer* fish bone for biomedical applications. Mater. Lett. 2017; 203:89-92.
28. Venkatesan J, Qian ZJ, Ryu B, Thomas NV, Kim SK. A comparative study of thermal calcination and an alkaline hydrolysis method in the isolation of hydroxyapatite from *Thunnus obesus* bone. Biomed. Mater. 2011; 6:035003.

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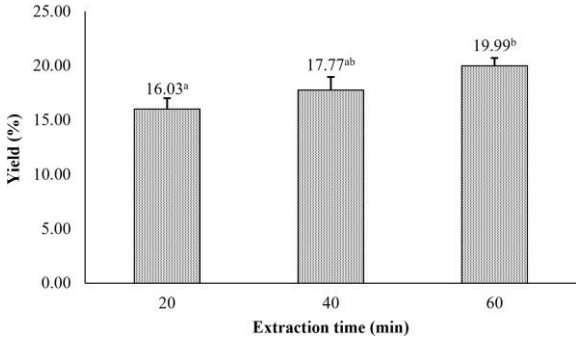

Figure 1. The yield of snakehead fish (*C. striata*) bone hydroxyapatite with different extraction time. Data are shown as mean ± SD (*n*=3).

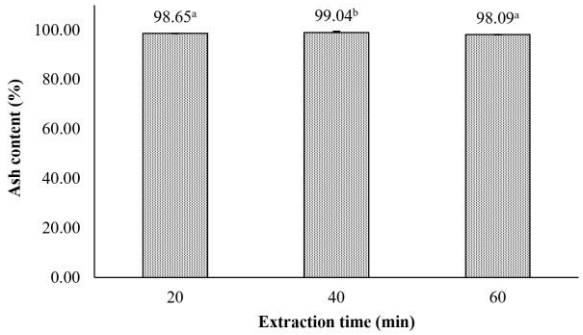

Figure 2. The ash content of the snakehead fish (*C. striata*) bone hydroxyapatite powder. Data are shown as mean ± SD (*n*=3).

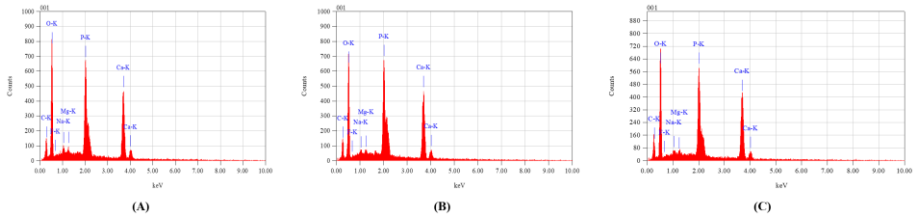

Figure 3. The mineral composition of hydroxyapatite from snakehead fish bone as analyzed by Energy Dispersive X-ray Spectroscopy (EDS).

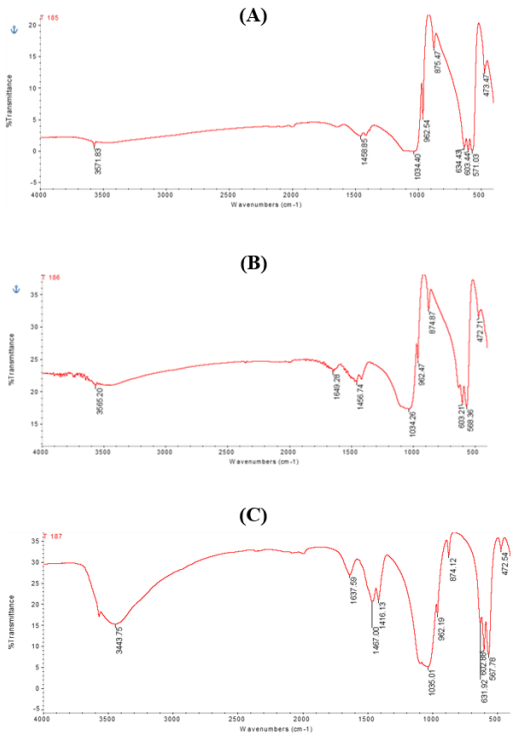

Figure 4. The FT-IR spectra of the snakehead fish bone hydroxyapatite with different extraction times. (A) 20 minutes; (B) 40 minutes; and (C) 60 minutes.

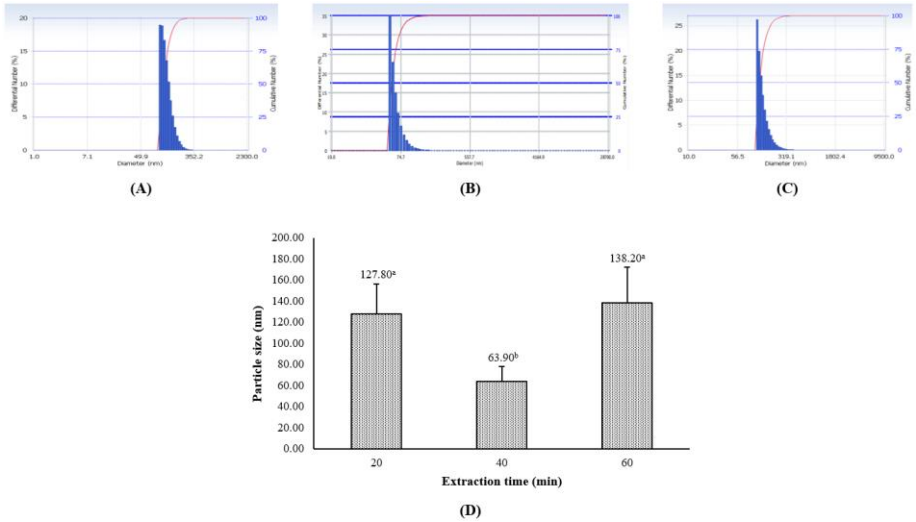

Figure 5. The particle size of the snakehead fish (*C. striata*) bone hydroxyapatite with different extraction times. (A) 20 minutes of extraction time; (B) 40 minutes of extraction time; (C) 60 minutes of extraction time; and (D) the mean ± SD of the hydroxyapatite particle size (n=3).

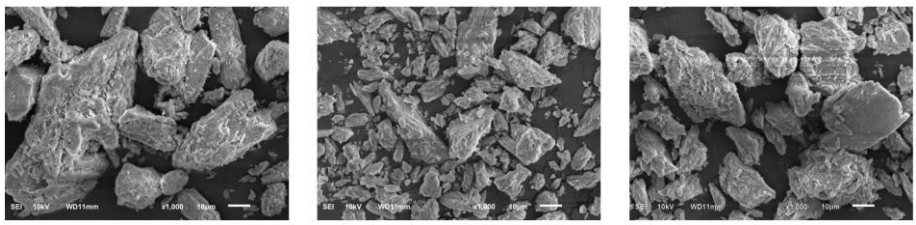

Figure 6. The representative of hydroxyapatite powder morphology by scanning electron microscopy (SEM) with different extraction times. (A) 20 minutes; (B) 40 minutes; and (C) 60 minutes.

Table 1. Mineral compositions of the hydroxyapatite snakehead fish bone to the energy dispersive X-ray spectroscopy.

Minerals	Extraction times (min)					
	20		40		60	
	Energy (keV)	Atom (%)	Energy (keV)	Atom (%)	Energy (keV)	Atom (%)
Ca	3.69	17.95	3.69	18.12	3.69	17.86
P	2.01	10.23	2.01	10.74	2.01	10.36
Mg	1.25	0.52	1.25	0.49	1.25	0.40
Na	1.04	0.78	1.04	0.43	1.04	0.44
O	0.53	55.76	0.53	53.23	0.53	53.90

Table 2. The functional groups of the hydroxyapatite from snakehead fish bone.

Functional groups*	Extraction times (min)					
	20		40		60	
	cm^{-1}	%T	cm^{-1}	%T	cm^{-1}	%T
v_1 PO_4^{3-}	962.54	55.25	962.47	24.80	962.19	17.60
v_2 PO_4^{3-}	1034.40	0.01	1034.26	17.37	1035.01	5.19
v_3 PO_4^{3-}	571.03	0.12	569.36	17.79	567.78	8.12
v_1 CO_3^{2-}	875.47	16.33	874.87	32.82	874.12	31.74
v_2 CO_3^{2-}	1458.85	2.38	1456.74	21.53	1467.00	20.72
OH^-	3571.83	0.84	3565.20	22.09	3443.75	15.99

***** $v_1PO_4^{3-}$, the symmetric stretching of phosphate; $v_2PO_4^{3-}$, asymmetric stretching of phosphate; $v_3PO_4^{3-}$, asymmetric bending vibrations of phosphate; $v_1CO_3^{2-}$, the out-of-plane bending modes of carbonate; $v_2CO_3^{2-}$, the asymmetric stretching of carbonate.

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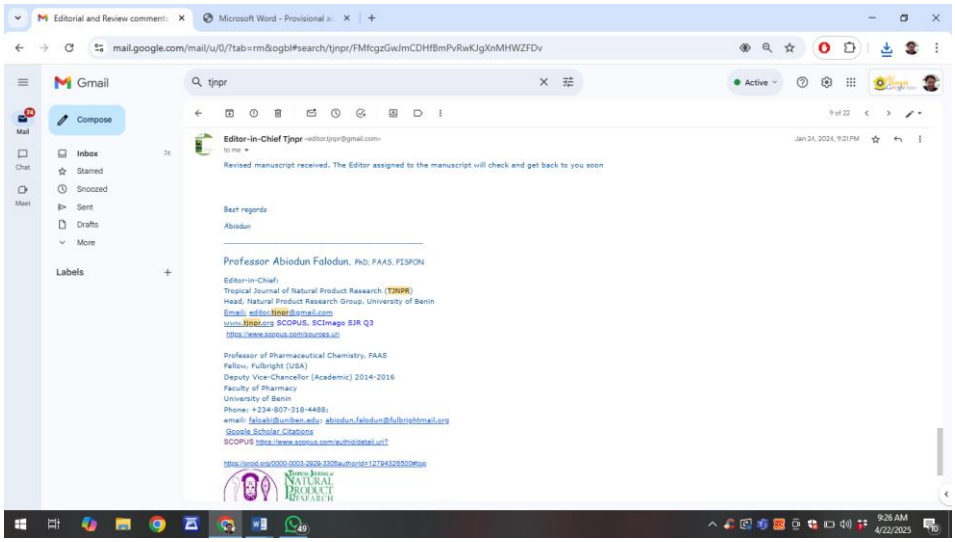

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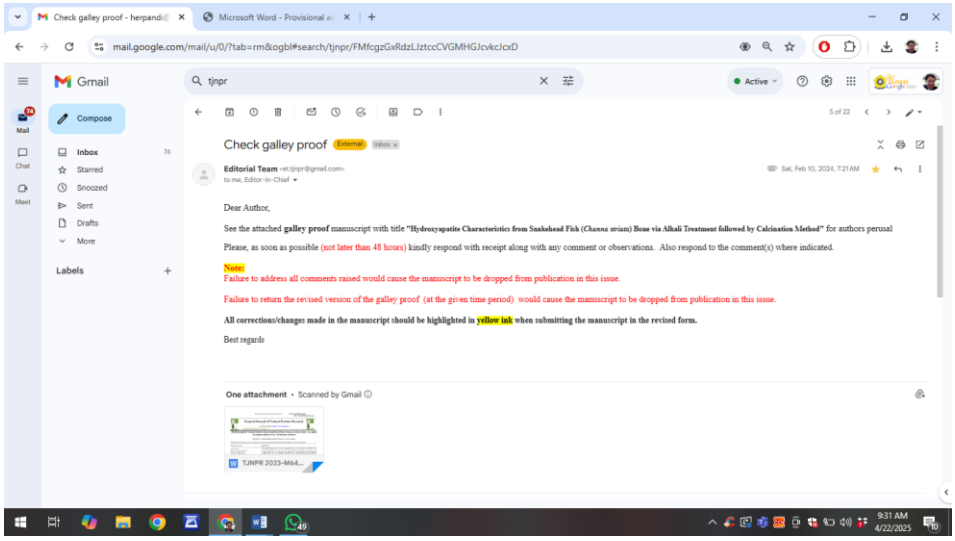

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Original Research Article

Hydroxyapatite Characteristics from Snakehead Fish (*Channa striata*) Bone via Alkali Treatment followed by Calcination Method

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ABSTRACT

The snakehead fish (*Channa striata*) is commonly used as a raw material in traditional South Sumatran foods. However, some parts of this fish, such as bone, skin, and viscera, are not used in food processing. This study aimed to determine the characterization of hydroxyapatite snakehead fish bone with different extraction times using ultrasound-assisted extraction followed by the calcination method. Hydroxyapatite was extracted using sodium hydroxide with three different extraction times (20, 40, and 60 minutes) before proceeding with calcination. The extraction yield ranges from about 16.03% to 19.99%. The smallest particle size is found at 40 minutes of extraction time (63.90 nm). The ash content of the hydroxyapatite ranges from about 98.09% to 99.04%, calcium from about 17.86% to 18.12%, and phosphorus from about 10.23% to 10.74%. The non-stoichiometric form is present in the hydroxyapatite from snakehead fish bone, with a Ca/P ratio of about 1.69 to 1.72. Analysis of the hydroxyapatite functional groups in snakehead fish bone showed the presence of phosphate groups, carbonate groups, and hydroxyl groups. This data indicates that hydroxyapatite was successfully extracted from snakehead fish bone in nanoparticle form. Therefore, snakehead fish bone can be used as a source of hydroxyapatite.

Keywords: Calcination, *Channa striata*, extraction, hydroxyapatite, phosphorus

Introduction

Consumption of fish products has increased significantly since they were identified as essential to a healthy, balanced diet and way of life. As a result, there has been a sharp rise in the global production of fish waste.[1] Fish waste is a potential source of minerals or trace metals, essential fatty acids, amino acids, peptides, and protein of high biological value as well as vitamins.[2] Therefore, fish waste thus offers enormous untapped potential for value addition. The fish waste includes fish bones, scales, and fins. Whereas, the fish bone waste is around 9-15%.[3, 4] The fish bone waste composed of various mineral, such as calcium, phosphorus, zinc, and magnesium that can be used a source of hydroxyapatite.[5, 6]

Hydroxyapatite, with the molecular formula $Ca_{10}(PO_4)_6(OH)_2$, commonly referred to as HA, is one of the most common calcium phosphates that has compositions similar to those of natural bone.[6, 7] Due to its bioactive qualities and osteoconductive properties, hydroxyapatite has garnered interest for use in bone grafting procedures. Additionally, other medical applications of hydroxyapatite have also been studied because hydroxyapatite is non-toxic, bioactive, biocompatible, and non-inflammatory.[2] Natural hydroxyapatite has recently been extracted from various biowastes, including fish waste such as fish bone.[6, 8, 9] Various methods have been used for hydroxyapatite extraction from natural sources, including calcination, alkali treatment, and the combination methods.[10] A previous study reported that calcination method was used for hydroxyapatite extraction.[11, 12] NaOH treatment also was reported for the extraction hydroxyapatite from bovine bone.[13]

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Additionally, hydroxyapatite successfully extracted from tilapia fish scale using combination method (NaOH treatment and calcination).[14] Snakehead fish (*Channa striata*) is the primary ingredient used in a traditional fishcake dish from South Sumatra, Indonesia, called "pempek." Generally, this product is made from snakehead fish meat; therefore, some parts of this fish become waste, including the bones. However, the study about the utilization of fishbone waste from "pempek" production has not yet been reported. Therefore, this study aimed to determine the characteristics of hydroxyapatite from snakehead fish bone using a combination of alkali (NaOH) treatment and calcination processes.

Materials and Methods

Sample preparation

The snakehead fish bone preparation was performed according to the previous methods.[6, 15] Briefly, the fish bones were washed and boiled at 80℃ for 30 minutes. After that, it was cleaned and mixed with lime water, and then it was boiled for 3 hours. The fish bone was cooled at room temperature for 15 minutes and dried in an oven (Memmert Universal Oven UN55, Germany) at 80℃ for 12 hours. The dried fish bone was then ground to powder (80 mesh) then continued to the calcination process.

Hydroxyapatite extraction and calcination process

The hydroxyapatite extraction and calcination processes from fish bones were performed according to the previous methods.[6, 16] Briefly, 10 g of the fish bone powder was mixed with 1 N NaOH and then extracted using an ultrasonic bath at 50℃ for different extraction times (20, 40, and 60 minutes). After the extraction times, it was cooled, filtered using filter paper (Whatman No. 42), and neutralized using distilled water. Then, it was oven-dried at 105℃ for 3 hours before the calcination process. The calcination process was performed using a muffle furnace (Thermo Scientific FB1410M-33, USA) , at 600℃ for 5 hours. After that, it was cooled to room temperature and kept for further analysis.

Hydroxyapatite characterizations

The ash content analysis was determined according to the AOAC (2000). Briefly, the hydroxyapatite powder was completely incinerated in a muffle furnace at 550℃ (AOAC method 930.05). Whereas the element composition was analyzed using energy dispersive X-ray spectroscopy integrated into scanning electron microscopy (SEM, JEOL JSM-7000F FE-SEM, Japan) and the functional group of the hydroxyapatite powder was analyzed using a Fourier transform infrared spectrometer (FTIR, Brucker Tensor 27) according to the previous method.[2] The particle size of the hydroxyapatite powder was analyzed using a particle size analyzer (Malvern Zetasizer Nano ZS Ver. 7.01) and the hydroxyapatite powder was also viewed by scanning electron microscopy according to the previous methods.[2, 17]

Statistical analysis

The data on yield, ash content, and particle size were expressed as the mean ± standard deviation (SD). It was analyzed using Duncan's multiple range test ($p<0.05$) using SPSS software (ver. 22.0; IBM Corporation, USA). Whereas, the mineral composition, functional groups and scanning electron microscope image were analyzed using descriptive analysis.

Results and Discussion

Yield of the hydroxyapatite

The yield of the snakehead fish bone hydroxyapatite is shown in Figure 1. The yields of the hydroxyapatite are about 16.03% - 19.99%. The highest yield of the hydroxyapatite is found in 60 minutes of extraction time and the lowest in 20 minutes. Whereas, there is no significant ($p>0.05$) difference between the yield of 40 minutes and other treatments. However, the yield of 20 minutes was significantly ($p<0.05$) different from 60 minutes of extraction time. A previous study reported that hydroxyapatite yield from bigeye snapper (*Priacanthus tayenus*) bones is about 13.4%.[18] Generally, there are two main methods for obtaining hydroxyapatite: chemical synthesis and biological resource extraction. Compared to synthetic forms, animal bone hydroxyapatite has a few advantages in terms of chemical composition and structure, which are identical to human bone, reduced manufacturing costs, and enhanced biological response.[12] The organic content in the bone is normally removed using an alkaline solution, such as NaOH. The organic component of the bone is hydrolyzed by the NaOH solution, and the remaining calcium phosphate is then washed and filtered. Whereas, calcination was used to completely remove the organic content from the bone.[10]

Ash content

The ash content of the snakehead fish bone hydroxyapatite powder is shown in Figure 2. The ash contents of the hydroxyapatite from snakehead fish bone are about 98.09% - 99.04%. The ash content for 40 minutes of extraction shows a significant ($p<0.05$) difference with the other treatments. Whereas, the ash content of 20 minutes was no significant ($p>0.05$) different with 60 minutes of extraction time. This condition indicated that the hydroxyapatite powder was composed of high-level minerals. A previous study reported that hydroxyapatite from the sardinella (*Amblygaster sirm*) is composed of 98.24% of ash.[19] Additionally, 99.75% of the ash content is observed in calcined bone from skipjack tuna.[20]

Mineral compositions of the hydroxyapatite

The mineral composition of the hydroxyapatite powder according to energy dispersive X-ray spectroscopy is shown in Figure 3 and Table 1.

The main minerals of the hydroxyapatite from snakehead fish bone are calcium (Ca) and phosphorus (P), followed by a small amount of other minerals such as magnesium (Mg) and natrium (Na). According to Table 1, the calcium value is about 17.86% - 18.12% and the phosphorus value is about 10.23% - 10.74%. These data indicated that the non-stoichiometric form is found in the hydroxyapatite from snakehead fish bone, with a Ca/P molar ratio of about 1.69 - 1.72. Generally, stoichiometric hydroxyapatite is composed of a Ca/P ratio of about 1.67.[21] Biological sources such as the scale of fish and bones of animals are the sources of non-stoichiometric hydroxyapatite.[22] A previous study reported that the Ca/P ratio of bovine bone hydroxyapatite was about 1.86.[23] The Ca/P ratio of hydroxyapatite from black tilapia fish bone is about 1.56 - 1.65 and it was also reported that calcination temperature affected the Ca/P ratio.[24] Additionally, calcium and phosphorus are the main elements in hydroxyapatite powder.[25]

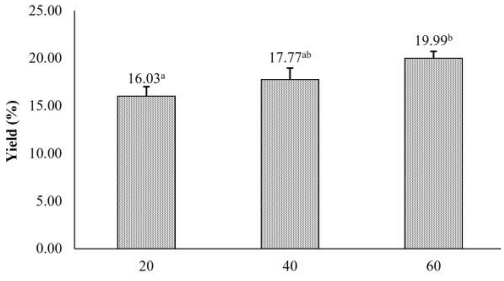

Figure 1: The yield of snakehead fish (*C. striata*) bone hydroxyapatite with different extraction time. Data are shown as mean ± SD (*n*=3).

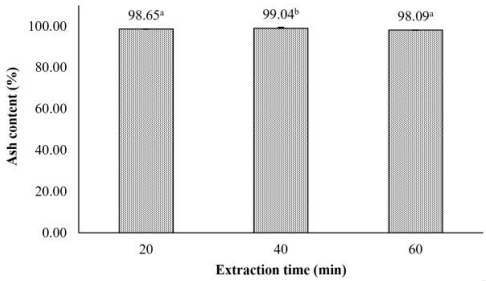

Figure 2: The ash content of the snakehead fish (*C. striata*) bone hydroxyapatite powder. Data are shown as mean ± SD (*n*=3).

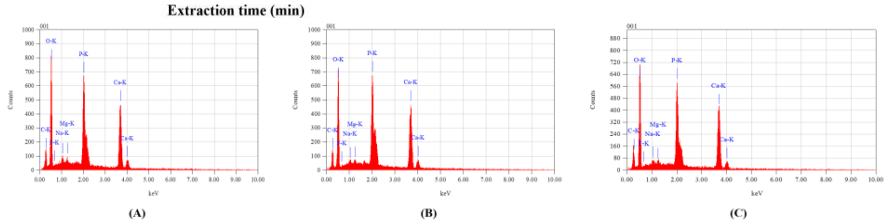

Figure 3: The mineral composition of hydroxyapatite from snakehead fish bone as analyzed by Energy Dispersive X-ray Spectroscopy (EDS).

Table 1: Mineral compositions of the hydroxyapatite snakehead fish bone to the energy dispersive X-ray spectroscopy

Minerals	Extraction times (min)					
	20		40		60	
	Energy (keV)	Atom (%)	Energy (keV)	Atom (%)	Energy (keV)	Atom (%)
Ca	3.69	17.95	3.69	18.12	3.69	17.86
P	2.01	10.23	2.01	10.74	2.01	10.36
Mg	1.25	0.52	1.25	0.49	1.25	0.40
Na	1.04	0.78	1.04	0.43	1.04	0.44
O	0.53	55.76	0.53	53.23	0.53	53.90

Table 2: The functional groups of the hydroxyapatite from snakehead fish bone

Functional groups*	Extraction times (min)					
	20		40		60	
	cm^{-1}	%T	cm^{-1}	%T	cm^{-1}	%T
v_1 PO_4^{3-}	962.54	55.25	962.47	24.80	962.19	17.60
v_2 PO_4^{3-}	1034.40	0.01	1034.26	17.37	1035.01	5.19
v_3 PO_4^{3-}	571.03	0.12	569.36	17.79	567.78	8.12
v_1 CO_3^{2-}	875.47	16.33	874.87	32.82	874.12	31.74

v_2 CO_3^{2-}	1458.85	2.38	1456.74	21.53	1467.00	20.72
OH^-	3571.83	0.84	3565.20	22.09	3443.75	15.99

* $v_1PO_4^{3-}$, the symmetric stretching of phosphate; $v_2PO_4^{3-}$, asymmetric stretching of phosphate; $v_3PO_4^{3-}$, asymmetric bending vibrations of phosphate; $v_1CO_3^{2-}$, the out-of-plane bending modes of carbonate; $v_2CO_3^{2-}$, the asymmetric stretching of carbonate.

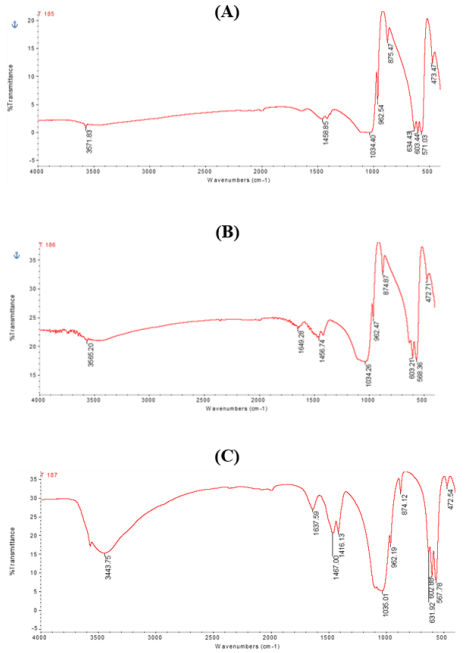

Figure 4: The FT-IR spectra of the snakehead fish bone hydroxyapatite with different extraction times. (A) 20 minutes; (B) 40 minutes; and (C) 60 minutes.

Functional groups of the hydroxyapatite

The Fourier-transform infrared (FT-IR) spectra and functional groups of the hydroxyapatite are shown in Figure 4 and Table 2. The functional group of the FT-IR spectra was analyzed according to the previous studies.[2, 26] The results showed that asymmetric bending vibrations of phosphate (v_3 PO_4^{3-}) were detected at 571.03 cm^{-1} (20 min), 568.36 cm^{-1} (40 min), and 567.78 cm^{-1} (60 min). The symmetric stretching of PO_4^3 (v_1 PO_4^{3-}) is detected at 962.54 cm^{-1} (20 min), 962.47 cm^{-1} (40 min), and 962.19 cm^{-1} (60 min). Also, the asymmetric stretching of PO_4^3 (v_2 PO_4^{3-}) is detected at 1034.40 cm^{-1} (20 min), 1034.26 cm^{-1} (40 min), and 1035.01 cm^{-1} (60 min). A previous study reported that hydroxyapatite from fish scale indicated the presence of asymmetric bending vibrations of the phosphate group at 563 cm^{-1}, symmetric stretching of PO_4^{3-} at 957 cm^{-1}, and asymmetric stretching of PO_4^{3-} at 1030 cm^{-1}.[2] A previous study also reported that the phosphate groups of natural hydroxyapatites from veal bone were also detected at 560 cm^{-1} and 1000 – 1100 cm^{-1}.[26] In this present study, the carbonate (CO_3^{2-}) groups were also detected in the hydroxyapatite powder. The out-of-plane bending modes of CO_3^{2-} (v_1 CO_3^{2-}) were detected at 875.47 cm^{-1} (20 min), 874.87 cm^{-1} (40 min), and 874.12 cm^{-1}. Whereas, the asymmetric stretching of CO_3^{2-} (v_2 CO_3^{2-}) was detected at 1458.85 cm^{-1} (20 min), 1456.74 cm^{-1} (40 min), and 1467.00 cm^{-1} (60 min). The hydroxyapatite powder also indicated the presence of hydroxyl (OH^-) groups at 3571.83 cm^{-1} (20 min), 3565.20 cm^{-1} (40 min), and 3443.75 cm^{-1} (60 min). A previous study reported that hydroxyapatite powder from fish scale indicated the presence of out-of-plane bending mode of CO_3^{2-} at 876 cm^{-1} and asymmetric stretching of CO_3^{2-} at 1412-1547 cm^{-1}.[2] Also, CO_3^{2-} natural hydroxyapatite powder from veal bone was detected at 1460 – 1530 cm^{-1}.[26] According to these data, the hydroxyapatite was successfully extracted from snakehead fish bone. These data also support the energy dispersive X-ray spectroscopy (EDS) analysis that confirm the presence of calcium and phosphorus in the hydroxyapatite powder.

Particle size of the hydroxyapatite

The particle size of the snakehead fish (*C. striata*) bone hydroxyapatite powder is shown in Figure 5. The particle size of the hydroxyapatite is about 63.9 nm - 138.2 nm. The smaller size of the hydroxyapatite is found in 40 minutes of extraction time. The value was significantly (p<0.05) different from other treatments. Whereas, there is no significant (p>0.05) difference in particle size between 20 minutes and 60 minutes of extraction time. The small particle size in 40 minutes of extraction time was also confirmed by scanning electron microscopy (SEM) as shown in Figure 6. The irregular form is observed in the hydroxyapatite powder. A previous study also reported hydroxyapatite extracted from fish bone alkali treatment and calcination, resulting in an irregular form of hydroxyapatite.[27, 28]

Conclusion

In this present study, the hydroxyapatite was successfully extracted from snakehead fish (*Channa striata*) bone. The extraction yield is about 16.03%-19.99%. The smallest particle size is found in 40 minutes of extraction time (63.90 nm). The ash content of the hydroxyapatite is about 98.09% - 99.04%, calcium is about 17.86% - 18.12%, and the phosphorus is about 10.23% - 10.74%. The non-stoichiometric form is found in this hydroxyapatite with a Ca/P ratio of about 1.69 - 1.72. Analysis of the hydroxyapatite functional groups of snakehead fish bone showed the presence of phosphate groups (PO_4^{3-}), carbonate groups (CO_3^{2-}), and hydroxyl groups (OH^-). These data indicated that the hydroxyapatite was successfully extracted from snakehead fish bone in nanoparticle form. Therefore, snakehead fish bone can be used as a source of nanohydroxyapatite.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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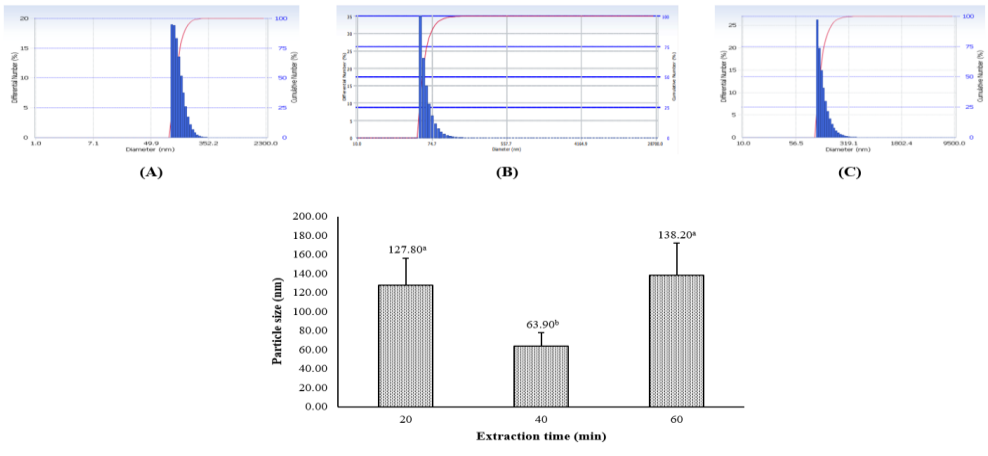


Figure 5: The particle size of the snakehead fish (*C. striata*) bone hydroxyapatite with different extraction times. (A) 20 minutes of extraction time; (B) 40 minutes of extraction time; (C) 60 minutes of extraction time; and (D) the mean ± SD of the hydroxyapatite particle size (*n*=3).

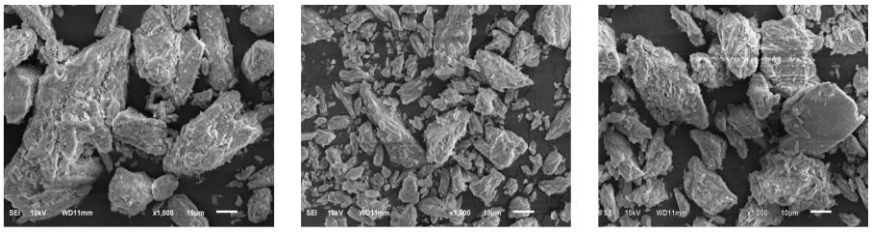
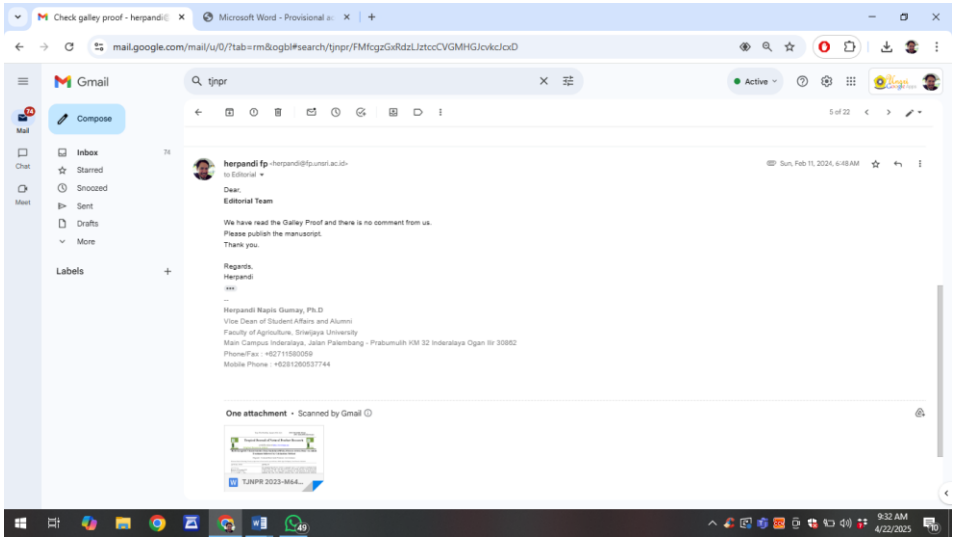


Figure 6: The representative of hydroxyapatite powder morphology by scanning electron microscopy (SEM) with different extraction times. (A) 20 minutes; (B) 40 minutes; and (C) 60 minutes

References

1. Coppola D, Lauritano D, Esposito FP, Riccio G, Rizzo C, de Pascale D. Fish waste: From problem to valuable resource. Marine Drugs. 2021; 19:116.
2. Huang YC, Hsiao PC, Chai HJ. Hydroxyapatite extracted from fish scale: Effects on MG63 osteoblast-like cells. Ceram. Int. 2011; 37:1825-1831.
3. Maktoof AA, Elherarlla RJ, Ethaib S. Identifying the nutritional composition of fish waste, bones, scales, and fins. IOP Conf. Ser.: Mater. Sci. Eng. 2020; 871:012013.
4. Boronat O, Sintes P, Celis F, Díez M, Ortiz J, Aguayo IA, Gómez HM. Development of added-value culinary ingredients from fish waste: Fish bones and fish scales. Int. J. Gastron. Food Sci. 2023, 31:100657.
5. Lall SP, Kaushik SJ. Nutrition and metabolism of minerals in fish. Animals. 2021; 11: 2711.
6. Marliana A, Fitriani E, Ramadhan F, Suhandono S, Yuliani K, Windarti T. Synthesis and characterization of hydroxyapatite from fish bone waste. AIP Conf. Proc. 2015; 1699: 040006.
7. Jaffri MZ, Ismail ZMM, Dermawan SK, Abdullah HZ. Sample preparation of the natural source hydroxyapatite (HAp) derived from black tilapia fish scales. J. Phys. Conf. Ser. 2022; 2169:012033.
8. Ruiz KLH, Cervantes JL, Machado DIS, Macias MdRM, Murrieta MAC, Silva AS. Hydroxyapatite recovery from fish byproducts for biomedical applications. Sustain. Chem. Pharm. 2022; 28:100726.
9. Granito RN, Renno ACM, Yamamura H, Almeida MCd, Ruiz PLM, Ribeiro DA. Hydroxyapatite from fish for bone tissue engineering: A promising approach. Int. J. Mol. Cell Med. 2018; 7:80-90.
10. Pu'ad MNAS, Koshy P, Abdullah HZ, Idris MI, Lee TC. Syntheses of hydroxyapatite from natural sources. Heliyon. 2019; 5:e01588.
11. Kupiec AS, Wzorek Z. The influence of calcination parameters on free calcium oxide content in natural hydroxyapatite. Ceram. Int. 2012; 38:641-647.
12. Dabiri SMH, Rezaie AA, Moghimi M, Rezaie H. Extraction of hydroxyapatite from fish bones and its application in nickel adsorption. BioNanoScience. 2018; 8:823-834.

13. Miecznik JB, Haberko K, Sitarz M, Bućko MM, Macherzyńska B. Hydroxyapatite from animal bones – Extraction and properties. Ceram. Int. 2015; 41:4841-4846.
14. Zainol I, Adenan NH, Rahim NA, Jaafar CAN. Extraction of natural hydroxyapatite from tilapia fish scales using alkaline treatment. Mater. Today: Proc. 2019; 16:1942-1948.
15. Putranto FH, Andi NA, Indrati K. Properties of belida (*Chitala* sp.) fish bone powder as calcium source based on protein hydrolysis method. Ziraa'ah. 2015; 40:11-20.
16. Mustafa N, Ibrahim MHI, Asmawi R, Amin AM. Hydroxyapatite extracted from waste fish bones and scales via calcination method. Appl. Mech. Mater. 2015; 773-774:287-290.
17. Barre MS, Ali FB, Mirghani MES, Hazri NF, Anuar H, Nasir NAM. Potential food additive of *Boswellia carterii* essential oil encapsulated within gum arabic: A particle size distribution and zeta potential analysis. Food Res. 2020; 4:19-23.
18. Jindapon N, Klinmalai P, Surayot U, Tanadchangsaeng N, Pichaiaukrit W, Phimolsiripol Y, Vichasilp C, Wangtueai S. Preparation, characterization, and biological properties of hydroxyapatite from bigeye snapper (*Priancanthus tayenus*) bone. Int. J. Mol. Sci. 2023; 24:2776.
19. Hasan MR, Yasin NSM, Ghazali MSM, Mohtar NF. Proximate and morphological characteristics of nano hydroxyapatite (Nano HAp) extracted from fish bone. J. Sustain. Sci. Manag. 2020; 15:9-21.
20. Benjakul S, Ali SM, Senphan T, Sookchoo P. Biocalcium powder from precooked skipjack tuna bone: Production and its characteristics. J. Food Biochem. 2017; 41:e12412.
21. Beaufils S, Rouillon T, Millet P, Bideau JL, Weiss P, Chopart JP, Daltin AL. Synthesis of Calcium-deficient hydroxyapatite nanowires and nanotubes performed by template-assisted electrodeposition. Mater. Sci. Eng. 2019; 98:333-346.
22. Dorozhkin VS. Amorphous Calcium orthophosphates: Nature, chemistry and biomedical applications. Int. J. Mater. Chem. 2012; 2:19-46.
23. Barakat NAM, Khil MS, Omran AM, Sheikh FA, Kim HY. Extraction of pure natural hydroxyapatite from the bovine bones bio waste by three different methods. J. Mater. Process. Technol. 2009; 209:3408-3415.
24. Dermawan SK, Ismail ZMM, Jaffri MZ, Abdullah HZ. Effect of the Calcination temperature on the properties of hydroxyapatite from black tilapia fish bone. J. Phys. Conf. Ser. 2022; 2169:012034.
25. Saeed GK, Essa AF, Said SAA. Preparation and characterization of hydroxyapatite powder and study of hydroxyapatite - alumina composite. J. Phys. Conf. Ser. 2020; 1591:012006.
26. Gheisari H, Karamian E, Abdellahi M. A novel hydroxyapatite – Hardystonite nanocomposite ceramic. Ceram. Int. 2015; 41:5967-5975.
27. Pal A, Paul S, Choudhury AR, Balla VK, Das M, Sinha A. Synthesis of hydroxyapatite from *Lates calcarifer* fish bone for biomedical applications. Mater. Lett. 2017; 203:89-92.
28. Venkatesan J, Qian ZJ, Ryu B, Thomas NV, Kim SK. A comparative study of thermal calcination and an alkaline hydrolysis method in the isolation of hydroxyapatite from *Thunnus obesus* bone. Biomed. Mater. 2011; 6:035003.



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Indralaya, South Sumatra Indonesia

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Dear Dr. Herpandi,

Provisional Acceptance letter for Article Manuscript Number TJNPR OCT180ARN

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Authors: Herpandi*, I'tishomul Hanif, Indah Widiastuti, Sabri Sudirman

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