

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

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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. 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. 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%. The fish bone waste composed of various mineral, such as calcium, phosphorus, zinc, and magnesium that can be used as source of hydroxyapatite. Hydroxyapatite, with the molecular formula $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, commonly referred to as HA, is one of the most common calcium phosphates that has compositions similar to those of natural bone. Due to its bioactive qualities and osteoconductive properties, hydroxyapatite has gained 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. Natural hydroxyapatite has recently been extracted from various biowastes, including fish waste such as fish bone. Various methods have been used for hydroxyapatite extraction from natural sources, including calcination, alkali treatment, and the combination methods. A previous study reported that calcination method was used for hydroxyapatite extraction. NaOH treatment also was reported for the extraction hydroxyapatite from bovine bone.

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Additionally, hydroxyapatite successfully extracted from tilapia fish scale using combination method (NaOH treatment and calcination). 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. 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 UNS5, 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. 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.

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 functional group of the hydroxyapatite powder was analyzed using a Fourier transform infrared spectrometer (FTIR, Bruker 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 \pm 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 (*Prilancanthus tayerus*) 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

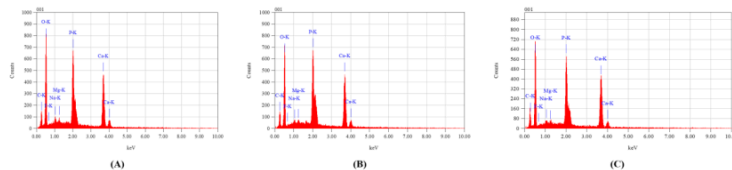


Figure 3: The mineral composition of hydroxyapatite from snakehead fish bone as analyzed by Energy Dispersive X-ray Spectroscopy (EDS).

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 sodium (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

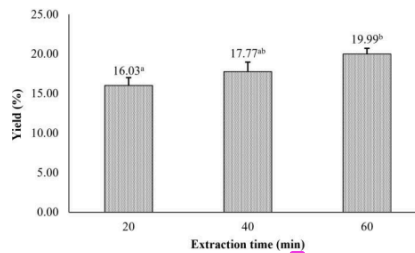


Figure 1: The yield of snakehead fish (*C. striata*) bone hydroxyapatite with different extraction time. Data are shown as mean \pm SD ($n=3$).

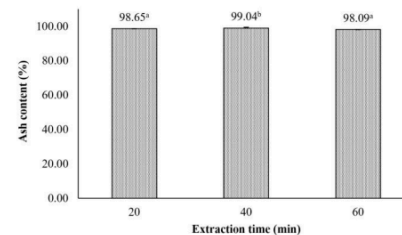


Figure 2: The ash content of the snakehead fish (*C. striata*) bone hydroxyapatite powder. Data are shown as mean \pm SD ($n=3$).

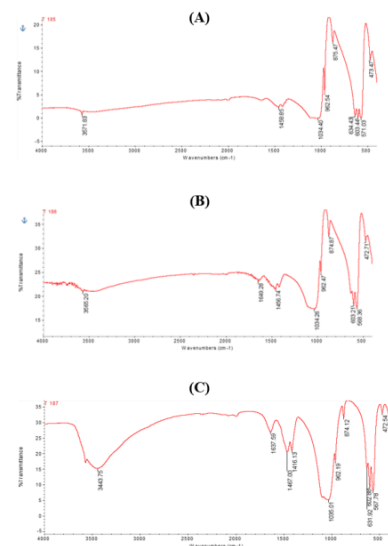
Table 1: Mineral compositions of the hydroxyapatite snakehead fish bone to the energy dispersive X-ray spectroscopy

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
ν_1 PO ₄ ³⁻	962.54	55.25	962.47	24.80	962.19	17.60
ν_2 PO ₄ ³⁻	1034.40	0.01	1034.26	17.37	1035.01	5.19
ν_3 PO ₄ ³⁻	571.03	0.12	569.36	17.79	567.78	8.12
ν_1 CO ₃ ²⁻	875.47	16.33	874.87	32.82	874.12	31.74
ν_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

* ν_1 PO₄³⁻, the symmetric stretching of phosphate; ν_2 PO₄³⁻, asymmetric stretching of phosphate; ν_3 PO₄³⁻, asymmetric bending vibrations of phosphate; ν_1 CO₃²⁻, the out-of-plane bending modes of carbonate; ν_2 CO₃²⁻, 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 (ν_3 PO₄³⁻) 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 PO₄³⁻ (ν_1 PO₄³⁻) 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 PO₄³⁻ (ν_2 PO₄³⁻) 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 PO₄³⁻ at 957 cm⁻¹, and asymmetric stretching of PO₄³⁻ 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 (CO₃²⁻) groups were also detected in the hydroxyapatite powder. The out-of-plane bending modes of CO₃²⁻ (ν_1 CO₃²⁻) 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 CO₃²⁻ (ν_2 CO₃²⁻) 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 CO₃²⁻ at 876 cm⁻¹ and asymmetric stretching of CO₃²⁻ at 1412-1547 cm⁻¹.² Also, CO₃²⁻ 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.

4

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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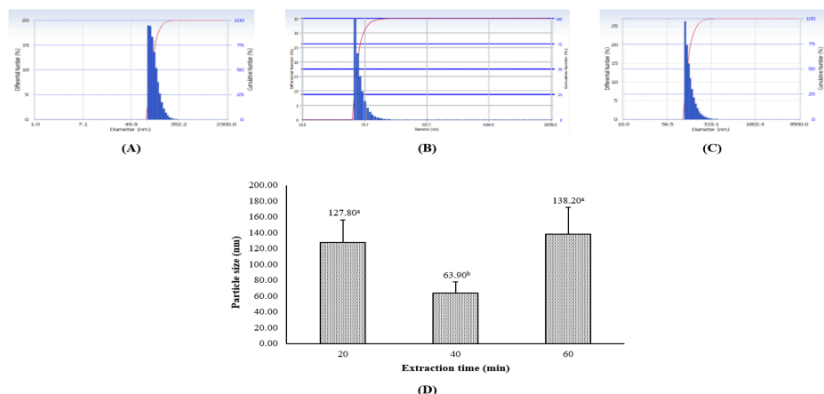


Figure 5: The particle size of the snakehead fish (*C. striata*) bone hydroxyapatite with different extraction times. (A) 20 minutes of extraction time; (B) 40 minutes of extraction time; (C) 60 minutes of extraction time; and (D) the mean \pm SD of the hydroxyapatite particle size ($n=3$).

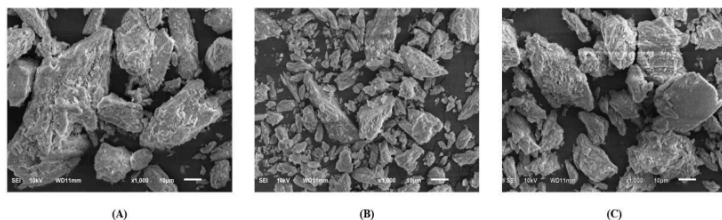


Figure 6: The representative of hydroxyapatite powder morphology by scanning electron microscopy (SEM) with different extraction times. (A) 20 minutes; (B) 40 minutes; and (C) 60 minutes

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