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

by Herpandi Napis

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Original Research Article

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

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

Fisheries Product Technology, Faculty of Agriculture, Universitas Sriwijaya Indralaya 30862, Ogan Ilir Regency, South Sumatra, Indonesia

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ABSTRACT

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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 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 13 10.74%. The non-stoichiometric form is present in the hydroxyapatite from snakehead fish bone, with a Ca/P rational about 16.9 to 1.72. Analysis of the hydroxyapatite functional groups in snakehead fish bone showed the presence of phosphate groups, carbonate groups, and hydroxyal groups. This data indicates that hydroxyapatite was suc 22 fully 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 we incentified as essential to a healthy, balanced diet and way of life. As a result here 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, epidies, and protein of high biological value as well as vitamise. Therefore, fish waste thus offers enormous untapped potential for value addition. The fish waste two closes the production of the process the fish bone wastes in the process the fish bone wastes in

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%. A The fish bone waste composed of various mineral, such as calcium, phosphorus, zinc, and magnesium that can be used a 15 rec of hydroxyapatite. B Hydroxyapatite of the most common calcium phosphates that has compositions similar to those of natural bone. The first of the state of the most common calcium phosphates that has compositions similar to those of natural bone. The first of the first 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

*Corresponding author. E mail: herpandi@fp.unsri.ac.id Tel: (+62) 711580934

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Additionally, hydroxyapatite successfully extracted from tilapia fish Additionally, Hydroxyapatic successfully extracted from Impair Instacle using combination method (NaOH treatment and calcination). As 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 line 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 [2] formed according to the previous methods. 6 to Briefly, 10 g of the fish bone powder was mixed with 1 N RaoH and then extracted using an ultrasonic bath at 50 °C for different extraction times extracted using an unrasonic but nat 30°C for dirrerent extraction times (20, 40, and 60 minutes). After the extraction times, it was cooled, 22 red 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

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Hydroxyapatite characterizations

The ash content analysis was determined according to the AOAC 2000). Briefly, the hydroxyapatite powder was completely incinera 2 in a muffle furnace at 550°C (AOAC method 93005). Whereas the element composition was an 52°cd using energy dispersive X-ray spectroscopy integrated into scanning electron microscopy (SEM, JEOL JSM-7000F FE-SEM, Japan) and 1 functional group of the hydroxyapatite powder was analyzed using a Fourier transform infrared spectrometer (FTIR, Brue [21 Tensor 27) according to the previous method. The particle size of the hydroxyapatite powder was analyzed using a particle size and 1 certain properties of the hydroxyapatite powder was analyzed using a particle size and 1 certain properties of the hydroxyapatite powder was analyzed using a particle size and 1 certain properties of the hydroxyapatite powder was analyzed using a particle size and 1 certain properties of the hydroxyapatite powder was analyzed using a particle size and 1 certain properties of the hydroxyapatite powder was analyzed using a particle size and 1 certain properties of the hydroxyapatite powder was analyzed using a particle size and 1 certain properties of the hydroxyapatite powder was analyzed using a particle size and 1 certain properties of the hydroxyapatite powder was analyzed using a particle size and 1 certain properties of the hydroxyapatite powder was analyzed using a particle properties of the hydroxyapatite powder was analyzed using a particle properties of the hydroxyapatite powder was analyzed using a functional group of the hydroxyapatite powder was analyzed using a functional group of the hydroxyapatite powder was analyzed using a functional group of the hydroxyapatite powder was analyzed using a functional group of the hydroxyapatite powder was analyzed using a functional group of the hydroxyapatite powder was analyzed using a functional group of the hydroxyapatite powder was analyzed using a functional group of the hydroxyapatite powder was analyzed using a function of

Statistical analysis

The data on yield, ash content, and particle size were express 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.

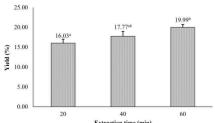
Results and Discussion

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 minute 2f 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 bigory sensor. (Primagathy texpense) that hydroxyapatite yield from bigeye snapper (Prionecanthus tayems) 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 phosometric production of the washed and filtered. Whereas, calcination was used to completely remove the organic content from the bone. ¹⁰

The ash content of the snakehead fish bone hydroxyapatite powder is shown in Figure 2. The ash contents of the hydroxyapatite prom snakehead fish bone are about 98. [31] - 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 continuities with the production of the production in the production of the production o significant (p20,30) different with no minutes of extraction time. I his 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

33 eral compositions of the hydroxyapatite
17 mineral composition of the hydroxyapatite powder according to energy dispersive X-ray spectroscopy is shown in Figure 3 and Table 1. 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-stoichiometri 10 mm is found in the hydroxyapatite from snakehead fish bone, with a CaP molar ratio of about 16.9 - 1.72. Generally, stoichiometric hydroxyapatite is composed of a CaP ratio of about 167. Biological sources such as the scale of fish and bones of animals are the sources of 20no-stoichiometric hydroxyapatite. A previous study reported that the CaP raz2 of bovine bone hydroxyapatite was about 1.86. The CaP 20 of hydroxyapatite from black tilapia fish bone is about 1.56 - 1.65 and it was also reported that calcination temperature affected the CaP ratio. Additionally, calcium and phosphorus are the main elements in hydroxyapatite powder.



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

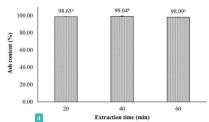


Figure 2: The ash content of the snakehead fish (C. striata) bone hydroxyapatite powder. Data are shown as mean \pm SD (n=3).

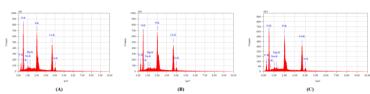


Figure 3: The mineral composition of hydroxyapatite from snakehead fish bone as analyzed by Energy Dispersive X-ray Spectroscopy (EDS).

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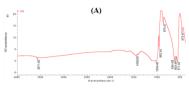
Table 1: Mineral compositions of the hydroxyapatite snakehead fish bone to the energy dispersive X-ray spectroscopy

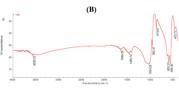
	Extraction times (min)					
Minerals	20		40		60	
	Energy (keV)	Atom (%)	Energy (keV)	Atom (%)	Energy (keV)	Atom (%)
Ca	3.69	17.95	3.69	18.12	3.69	17.86
P	2.01	10.23	2.01	10.74	2.01	10.36
Mg	1.25	0.52	1.25	0.49	1.25	0.40
Na	1.04	0.78	1.04	0.43	1.04	0.44
O	0.53	55.76	0.53	53.23	0.53	53.90

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

F	Extraction times (min)					
Functional	21		40		60	
groups*	cm ⁻¹	% T	cm-1	% T	cm ⁻¹	%T
v₁ PO₄³-	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
v₃ PO₄³-	571.03	0.12	569.36	17.79	567.78	8.12
v₁ 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

* $\nu_i PO_4^2$, the symmetric stretching of phosphate; $\nu_i PO_4^2$, asymmetric stretching of phosphate; $\nu_i PO_4^2$, asymmetric stretching of phosphate; $\nu_i PO_4^2$, the out-of-plane bending modes of carbonate; $\nu_i PO_4^2$, the asymmetric stretching of carbonate.





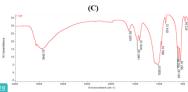


Figure 4: The FT-IR spectra of the 6 hakehead fish bone hydroxyapatite with different extraction times. (A) 20 minutes; (B) 40 minutes; and (C) 60 minutes.

ang of phosphate: v^AVo², asymmetric bending vibrations of phosphate; v²VO², the symmetric stretching of earbonate.

Fune (18) al groups of the hydroxyapatite

The Fourier-transform infrared (F¹-IR) spectra and functional groups of the hydroxyapatite are shown in Figure 4 and Table 2. The functional group of the Ff¹-IR spectra was analyzed according to the previous studies.² The results showed that asymmetric bending vibrations of phosphate (v) PO₂² v) are detected at 571.03 cm³ (20 min), 568.36 cm³ (40 min), and 567.78 cm³ (60 min). The symmetric stretching of PO₄² (v) PO₄²) were detected at 571.03 cm³ (20 min), 568.36 cm³ (40 min), and 567.78 cm³ (60 min), The symmetric stretching of PO₄² (v) PO₄²) is detected at 96.24 cm³ (20 min), 962.47 cm³ (40 min), and 1035.01 cm³ (60 min). A previous study reported that hydroxyapatite from fish scale indicated the presence of asymmetric bending vibrations of the phosphate group at 563 cm³ -symmetric stretching of PO₄² at 1937 cm³, and asymmetric stretching of PO₄³ at 1030 cm³. A previous study also reported that the phosphate groups of natural hydroxyapatites from veal bone were also detected at 560 cm³ and 1000 – 1100 cm³. In this present study, the carbonate (CO₇²) groups were also detected in the hydroxyapatite powder. The out-of-plane bending modes of CO₇² (v) CO₇²) was detected at 1458.85 cm³ (20 min), 1456.74 cm³ (40 min), and 374.12 cm³. Whereas, the asymmetric stretching of CO₇² (v) CO₇²) was detected at 1458.85 cm³ (20 min), 1456.74 cm³ (40 min), and 1467.00 cm³ (60 min), and 544.35 cm³ (60 min), and 544.35 cm³ (60 min), and 546.35 cm³ (60 min), and 546.35 cm³ (60 min), and 546.30 cm³

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

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significant (p>0.05) difference in particle size between 20 minutes and 60 minutes of extraction time. The size 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 mn). 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% - 22 4%. The non-stoichiometric form is found in this hydroxyapatite with a CaP ratio of about 1.69 - 1.72. Analy of the hydroxyapatite functional groups of snakehead fish bone showed the presence of phosphate groups (PO₁*), carbonate groups (CO₂*), 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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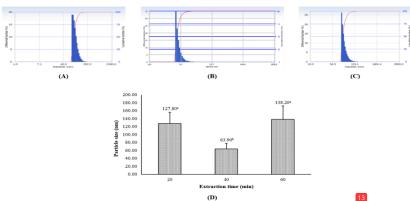


Figure 5: The particle size of the snakehead fish (*C. striata*) bone hydroxyapatite with different extraction times. (A) 20 minutes of extraction time; (B) 40 minutes of extraction time; (C) 60 minutes of extraction time; and (D) the mean ± SD of the hydroxyapatite particle size (n=3).



Figure 6: The representative of hydres apatite 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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