Fabrication of hydroxyapatite from fish bones waste using reflux method

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Abstract. The aim of this present study was to investigate the fabrication of hydroxyapatites, which were synthesized from fish bone wastes using reflux method. The fish bone wastes collected from the restaurant were brushed and boiled at 100°C for 10 minutes to remove debris and fat. After drying, the fish bones were crushed, and ball milled into a fine powder. The fish bone wastes were then processed by refluxing using KOH and H₃PO₄ solutions. The samples were calcined at 900°C and characterized by X-Ray Diffraction (XRD) and Fourier Transform Infrared Spectrometry (FT-IR). The XRD pattern of samples after treatment revealed that the peak of hydroxyapatite was observed and the bands of OH⁻ and PO₄³⁻ were observed by FT-IR. The scanning electron microscope evaluation of sample showed the entangled crystal and porous structure of hydroxyapatite. In conclusion, the hydroxyapatite was successfully synthesized from fish bone wastes using reflux method.

1. Introduction
Hydroxyapatite is one of the most common apatites used in dentistry and medicine. Hydroxyapatite and related calcium phosphate materials have been widely used for bone reconstruction or substitution due to their similarity to the bone. It is believed that natural hydroxyapatite is more biocompatible because it has similar physical-chemical characteristics with bone [1]. The chemical form of hydroxyapatite is Ca₁₀(PO₄)₆(OH)₂ but often written as Ca₁₀(PO₄)₆(OH)₂. Naturally, hydroxyapatite is an inorganic component that can be found in human hard tissue such as tooth and bone. These materials are commonly used as human body implant materials. Natural hydroxyapatite can be synthesized from eggshells, coral, fish bone, chicken bone, etc. [2].

Fish was consumed by humans because of their minerals and vitamins. However, the fish-bone wastes have not been used for anything. The fish-bone wastes have caused the environmental problem such as organic odor. The fish-bone contains calcium phosphate, collagen fiber, calcium carbonate, and hydroxyapatite. Therefore, the possibilities to synthesize hydroxyapatite using natural calcium sources such as fish bones are prominent [3].

Previously, the fabrication of natural hydroxyapatite was conducted using the fish bone of Brazilian river such as pintado (Pseudoplatystomacorruscans), jaú (Paulicealutkeni), and cachara...
(Pseudoplatystomafasciatum) [4]. The fish bone was calcined at 900°C to achieve an optimal natural hydroxyapatite. Most of Indonesia people have consumed both ocean fish and river fish. In West Java Province, especially in Bandung, people usually consume the river fish because it is easier to be obtained than the ocean fish. Minimum production cost, cheap and potential donor for calcium can be achieved by using fish bone [5]. Fabrication of hydroxyapatite from river fish bone required the simple methods. One of these methods is reflux. The reflux is a technique involving the condensation of vapors and the return of this condensate to the system. In the present study, the reflux method was employed using fish bone waste as Ca$^{2+}$ sources. The Ca$^{2+}$ could be bonded with PO$_4^{3-}$ from the solution to form the hydroxyapatite.

2. Method and Materials

2.1 Preparation of fish bones waste
Fish bone wastes were obtained from the restaurant in local area. The collected fish bone wastes were brushed and boiled at 100°C water for 10 minutes to clean these samples from the debris and fat. Bones were dried using a microwave at 90°C for 100 minutes then crushed until they became powder. The powders were dried using dry-heat oven at the temperature of 60°C for 24 hours. Later, the powders were crushed using high-energy ball milling for 24 hours to obtain a fine powder. After milled, bones powder was dried using a microwave at a temperature of 60°C. Powder samples were put into a desiccator to maintain the sample in the dry condition.

2.2 Deproteinization
Powder samples were deproteinized by reflux method using 5% KOH solution. The purpose of 5% KOH addition is to create the strong base solution that can hydrolyse the calcium and protein in the fish bones. The samples and solution were boiled at 130°C for 18 hours.

\[
(Ca^{2+}\text{ Protein}) + KOH \rightarrow Ca^{2+} + \text{ Protein}
\]

\[
Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3
\]

2.3 Reflux using H$_3$PO$_4$ solution
The reflux used a weak acid of H$_3$PO$_4$ 7N $\approx$ 2.33 M as a source of PO$_4^{3-}$. The PO$_4^{3-}$ could act as a donor ion for synthesizing the hydroxyapatite. The solution was boiled at 130°C for 12 hours.

\[
CaCO_3 + H_3PO_4 \rightarrow Ca_3PO_4 + H_2CO_3
\]

\[
/ \quad \backslash
\]

\[
H_2O \quad CO_2↑
\]

2.4 Calcination
Calcination was conducted using high-temperature furnace at 900°C for 8 hours. In this study, the samples were contaminated by excess of H$_3$PO$_4$. By calcination at 900°C for 8 hours, the color of contaminated samples changed from white to grey.

\[
H_3PO_4 \rightarrow H_2O + P_2O_5
\]

2.5 Characterization of the samples
The characterization of the samples was conducted using X-ray Diffraction (XRD), scanning electron microscopy (SEM) JEOL-JSM-6510LV, and Fourier Transform Infrared Spectrometry (FT-IR).
3. Results and Discussion
Table 1 summarized the comparison of hydroxyapatite at three highest peaks (2θ) in XRD between samples that calcined at 900°C and hydroxyapatite (Taihei Chemical Co. Ltd., Osaka, Japan) as a reference.

Table 1. Three highest peaks (2θ) of XRD in hydroxyapatite and samples calcined at 900°C.

| Samples         | Three highest peaks (2θ) |
|-----------------|--------------------------|
|                 | 1           | 2           | 3           |
| Hydroxyapatite  | 32.90       | 32.20       | 31.73       |
| (reference)     |             |             |             |
| 900°C           | 32.52       | 32.05       | 31.84       |

Table 1 and figure 1 showed the result of the sample that calcined at 900°C for three highest peaks (2θ) of XRD. The three highest peaks of hydroxyapatite as reference are 32.90; 32.20; 31.73 [6]. These values are quite similar to the sample calcined at 900°C.

![Figure 1. Three highest peaks of XRD pattern for the sample calcined at 900°C.](image)

The XRD patterns of the sample calcined at 900°C showed that the hydroxyapatite could be observed and no peaks of CaCO₃ were detected. In other words, the reflux method that employed in this study was effective to fabricate the hydroxyapatite.

Figure 2 showed the functional groups of the samples that calcined at 900°C. The functional group of P-O and O-H was clearly observed, indicating that the hydroxyapatite was detected in the sample. For more details, table 2 summarized the comparison of infrared absorption spectroscopy of samples calcined at 900°C and hydroxyapatite as reference (Taihei Chemical Co. Ltd., Osaka, Japan).
Figure 2. The peaks of P-O and O-H functional groups that appeared in the sample.

Table 2. The infrared absorption spectroscopy of sample calcined at 900°C compared to the reference of hydroxyapatite.

| Sample            | Infrared absorption spectroscopy (cm⁻¹) |
|-------------------|-----------------------------------------|
|                   | P-O                                    |
|                   | O-H                                    |
| Hydroxyapatite (reference) | 562-602; 700-725; 1096-1260 | 2700-3000 |
| 900°C             | 563; 725; 1160; 1214                    | 2854; 2368 |

The CO₃²⁻ functional groups were detected in this sample. The carbonate apatite was more useful compared to the hydroxyapatite. The carbonate apatite and hydroxyapatite were used in in vivo study [7]. The transformation of hydroxyapatite to carbonate apatite could also be possible under the low-temperature synthesis [8].

Table 3. The infrared absorption spectroscopy of CO₃²⁻ using FT-IR

| Sample | Infrared absorption spectroscopy of CO₃²⁻ (cm⁻¹) |
|--------|-----------------------------------------------|
| 900°C  | 1029.8; 1079.94; 1650.77                      |

It should be noted that the absorption bands at 1650 cm⁻¹ belonged to the vibration modes of C-O from the CO₃²⁻ group in A-type carbonate apatite, whereby the PO₄³⁻ was partially substituted by CO₃²⁻ [9].

The microstructure of sample observed by SEM can be seen in figure 3. In this image, the morphology of hydroxyapatite is porous. The pore size of sample is less than 1 μm in average and not homogeneous. One of the weaknesses of hydroxyapatite is brittle, but this porosity can be filled with a nano-filler like zirconia [10].
4. Conclusions
The hydroxyapatite was successfully synthesized from the river fish bone wastes using a reflux method. The carbonate functional group (CO$_3^{2-}$) was found in the sample calcined at 900°C.

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