

Bioceramics synthesis of hydroxyapatite from red snapper fish scales biowaste using wet chemical precipitation route

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Abstract. Fish scales biowaste contain high collagens and calcium phosphates, therefore have considerable potential as raw material for value-added biomaterial such as hydroxyapatite (HAp). HAp is the main constituent component of hard tissue such as bone and teeth in the human body and is known as bioceramic materials. In this work, wet chemical precipitation method was used to synthesize HAp from Red Snapper Fish (*Lutjanus campechanus*) Scales. Two variations of calcination temperatures of 600°C (FHAp1) and 800°C (FHAp2) were conducted for 5 hours. The results showed calcium content from biowaste of red snapper fish scale was 83.62%. FTIR result shows that PO_4^{3-} , OH^- , and CO_3^{2-} functional groups presence as indicates the formation of HAp. XRD result showed the degree of crystallinity for FHAp1 and FHAp2 were 75.52% and 79.20%, respectively. The degree of crystallinity is in accordance with ISO 13779-2:2000 standard in which the minimum degree of crystallinity of hydroxyapatite used for biomedical materials is 45%. Finally, Particle Size Analyzer (PSA) results show that the particle size distribution is evenly distributed, with the size of micro-scale hydroxyapatite particles, ranging from 5.76 μm to 132.64 μm .

1. Introduction

Ceramics based biomaterial is widely used for instance for dental implant, femur bones and metal implant coating for orthopedic purposes [1-2]. Ceramic biomaterial is also called as bioceramics, especially when it derived from biological materials [3]. Bioceramics made from biological materials including biowaste can be utilized as any ceramic materials, such as membranes [4], however bioceramics has bioactive substances which is very useful for biomedical application, for instance for repairing bones. Bioceramic made from calcium phosphate is one of bioactive ceramics widely used for repairing bones, including betatricalcium phosphat (β -TCP), hydroxyapatite (HAp), and biphasic calcium phosphat (BCP) that consist of HA nuclei and various HA/ β -TCP [5]. Hydroxyapatite (HAp/HA) is promising bioceramics materials, is not only used for biomedical application but also for water efficient fertilizer which is potential for maintaining sustainability [6].



According to International Organization of Standardization (ISO) 13779-2 and American Society for Testing and Materials (ASTM) F1609 on the standardization of HA characteristics for coating as an application of implant for human body, the standard specification requirements for hydroxyapatite are as follows : the calcium-phosphorus ratio must be in the range of 1.67-1.76, the crystallinity content of hydroxyapatite must be at least 45% with the crystallinity content of others maximum 5%, and the adhesive strength must be higher than 15 MPa in both metallic implant and non-metallic implant [7].

HA might be synthesized from natural materials, especially biowastes derived from cow bones waste, egg shell, and the shell of marine organisms. Hydroxyapatite derived from natural materials possesses better physicochemical attributes than synthesized hydroxyapatite [8]. Fish scale is one of the naturally hard tissues that contain organic components such as calcium [9].

Calcium phosphate is the most abundant mineral contained in human body, with 99% contained in hard tissue, such as bones and tooth, especially in the form of $3\text{Ca}(\text{PO}_4)_2 \cdot \text{Ca}(\text{OH})_2$ known as hydroxyapatite. Hydroxyapatite has synthesized from various natural materials that possess high calcium content with several methods of synthesis such as the dry method, hydrothermal method, alkoxide method, flux method, and wet method. However some of the methods above still produce hydroxyapatite with low purity and large crystal structure, therefore additional treatment is needed to minimize the particle size by conducting calcination and sonication on the sample which aim to minimize the produced particle size [10]. In this research, the synthesis of hydroxyapatite is carried out with the wet chemical precipitation route and assisted by sonication treatment and the temperatures used for calcination process are 600°C and 800°C for 5 hours.

2. Materials and Method

Materials

Red snapper fish scales waste were obtained from PT Kelola Mina Laut Gresik. Some chemicals were used for preparation and syntetis, *e.g.*: NaOH 1%, NH_3 25%, H_3PO_4 85% and aquades.

Method

2.1.1. Preparation

Fish scale samples that would be used as calcium precursor are firstly washed in order to release the impurities that could affect the final result, the samples are then boiled with boiling water for 30 minutes. After boiled, samples are washed using aquades, and then drained. Then samples are hydrolyzed using NaOH 1% for 2 hours, in order to remove fat. After that, samples are left at room temperature for 24 hours and washed using aquades and then sterilized using autoclave for 15 minutes at 121°C. After sterilization process is carried out, samples are dried using oven at 110°C for 5 hours. The hydrolyzed fish scales form CaCO_3 compound that needs to be converted to CaO as calcium precursor in calcination at 1000°C for 5 hours.



Figure 1. Calcinated hydroxyapatite.

2.1.2. Synthesis of hydroxyapatite

CaO samples obtained are then dissolved with aquades. Phosphate precursor H_3PO_4 85% was then added. $\text{Ca}(\text{OH})_2$ and H_3PO_4 suspension are titrated with the drop rate of 1.5 ml/minutes. Then, NH_3 was added to reach $\text{pH} > 8$. Samples are then sonicated for 1 hour at 50 Hz. After that, samples are dried in room temperature for 24 hours. Then, the samples are washed and filtered to obtain calcium-

phosphate powder. The powder is then dried using oven at 110°C for 5 hours. Subsequently, the samples are calcinated with the temperature of 600°C and 800°C, for 5 hours. The calcinated samples are then characterized by using FTIR (Fourier Transform Infra Red), XRD (X-Ray Diffraction), XRF (X-Ray Fluorescence), and PSA (Particle Size Analyzer). Calcinated HAp shown in Figure 1.

2.1.3. Characterization of hydroxyapatite

Fourier Transform Infra Red (*Shimazu IR Prestige-21*) was used to analyze chemical properties of the samples, X-Ray Fluorescence (*Philips, PANalytical Minipal 4*) was used to analyze the calcium content in the samples, and X-Ray Diffraction (*Philips, Type: PANalytical Expert Pro*) was used to determine the crystallinity of the samples. The formula used to calculate the crystallinity of the samples is:

$$\% = \frac{\text{Crystalline area fraction}}{\text{Crystalline area} + \text{amorphous area}}$$

Particle Size Analyzer (*Cilas 1090 Liquid*) was to analyze the particle size of the samples.

3. Results and Discussion

3.1 XRF characterization

The purposes of characterization using XRF (X-Ray Fluorescence) were to determine the content of the elements consisted in the ash of red snapper fish scale and the result of hydroxyapatite synthesis. XRF was used to detect the elements consisting in the ash of red snapper fish scale and hydroxyapatite (Table 1). Based on the characterization result using XRF, it can be seen that the Ca content within the ash of red snapper fish scale was 83.62%, and the P content was 12.7%. Then for sample FHAp1 (calcination at 600°C, for 5 hours), the Ca and P content in the sample were 82.30% and 14.9%, respectively. In sample FHAp2 (calcination at 800°C, for 5 hours), the Ca and P content in the sample were 82.09% and 15.1%, respectively. The decrease of Ca content in the sample occurred due to the calcination temperature of 600°C and 800°C.

Table 1 Percentage of elements identified using XRF.

Element	Percentage (%)		
	Red Snapper Scale Ash	HA 600°C, 5 hours (FHAp1)	HA 800°C, 5 hours (FHAp2)
P	12.7	14.9	15.1
Ca	83.62	82.30	82.09
Ti	0.05	0.077	-
Fe	0.21	0.19	0.21
Ni	1.30	0.944	0.931
Cu	0.15	0.12	0.11
Zn	0.17	0.13	0.13
Zr	0.2	-	0.2
Re	0.07	0.15	0.05
Co	-	0.04	-
Sr	-	1.1	1.1

3.2 FTIR spectroscopy

The spectroscopy resulted using FTIR for both samples showed that the main hydroxyapatite component groups are PO_4^{3-} and OH. This can be seen based on the peaks formed in the wave spectrum result shown in Figure 2a and 2b. As shown in Figure 2a, FHAp1 (calcination temperature at 600°C, for 5 hours), in wavenumbers $570.929536 \text{ cm}^{-1}$ and $601.790592 \text{ cm}^{-1}$, PO_4^{3-} groups was observed [11]. Then for sample FHAp2 (calcination temperature at 800°C, for 5 hours), in wavenumbers $555.49908 \text{ cm}^{-1}$ and $603.719408 \text{ cm}^{-1}$, PO_4^{3-} groups were observed. PO_4^{3-} groups existed

at the wavenumber range of 555-602 cm^{-1} . Meanwhile, OH- groups can be seen based on the peaks observed in the wave spectrum result show also in Figure 2b. As shown, OH- groups in sample FHAp1 and FHAp2 observed in wavenumber 3500 cm^{-1} to 3600 cm^{-1} . But, the highest peak is shown in wavenumber 3560-3580 cm^{-1} . Then, for α -TCP and β -TCP also observed in Figure 2c at wavenumber 947.048656 cm^{-1} and 981.767344 cm^{-1} as residual result in small content. This occurred due to imperfect decomposition during calcination that influenced the formation of α -TCP and β -TCP groups [12].

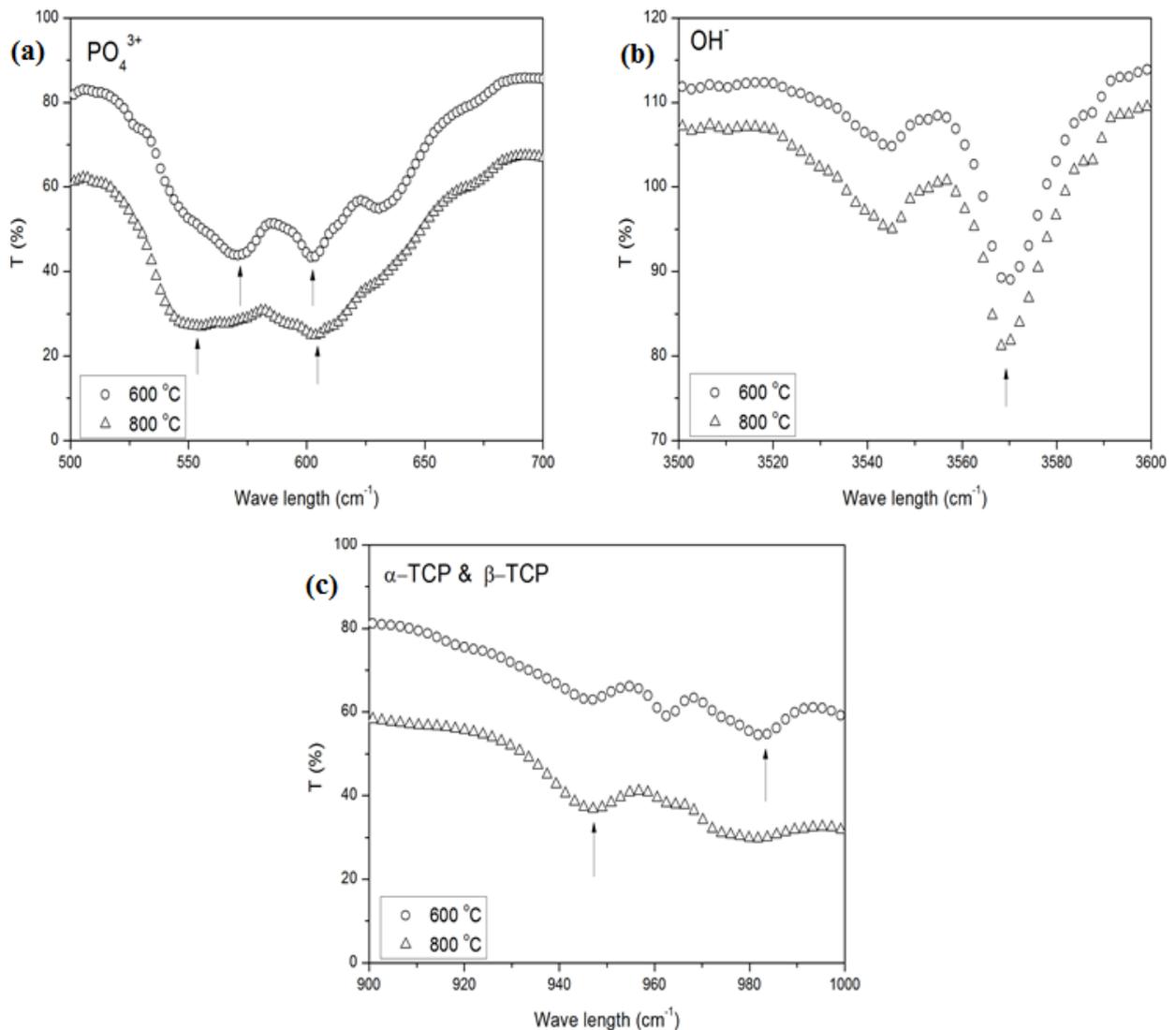


Figure 2. FTIR spectra: Phosphate groups (PO_4^{3-}) (a); Hydroxile groups (OH^-) (b); α -TCP and β -TCP groups (c).

3.3 XRD crystallinity measurement

XRD used to analyze the crystallinity of the samples, by analyzing the resulted diffractogram. The highest intensity shows the increasing HAp crystallinity. The crystallinity level can be found by using the triangle area approach. The crystalline/amorphous area fraction, in which the FWHM level is considered to be half of the base width and *height* as its height.

The diffractogram graphic which resulted from XRD is plotted in form of data as shown in Table 2 and Table 3. Data in both tables are obtained based on the highest existing intensity. Based on the data

of JCPDS 00-001-1008 (*Joint Committee on Powder Diffraction Standards*), for hydroxyapatite phase the main intensity at level 20 is characterized at 25.879; 32.196; 32.902; and 34.04. Based on the data shown, it can be determined that the crystallinity level for sample FHAp1 is 75.52%, and for sample FHAp2 the crystallinity level is 79.20%. The difference of the crystallinity levels from each sample is due to the difference of calcination temperature applied. The crystallinity percentage of a hydroxyapatite sample increases in proportion to the temperature rise during calcination process. Based on ISO standardized specification (ISO 13779-2:2000) to make hydroxyapatite coating which possesses high mechanical strength, it should have at least more than 45% crystallinity.

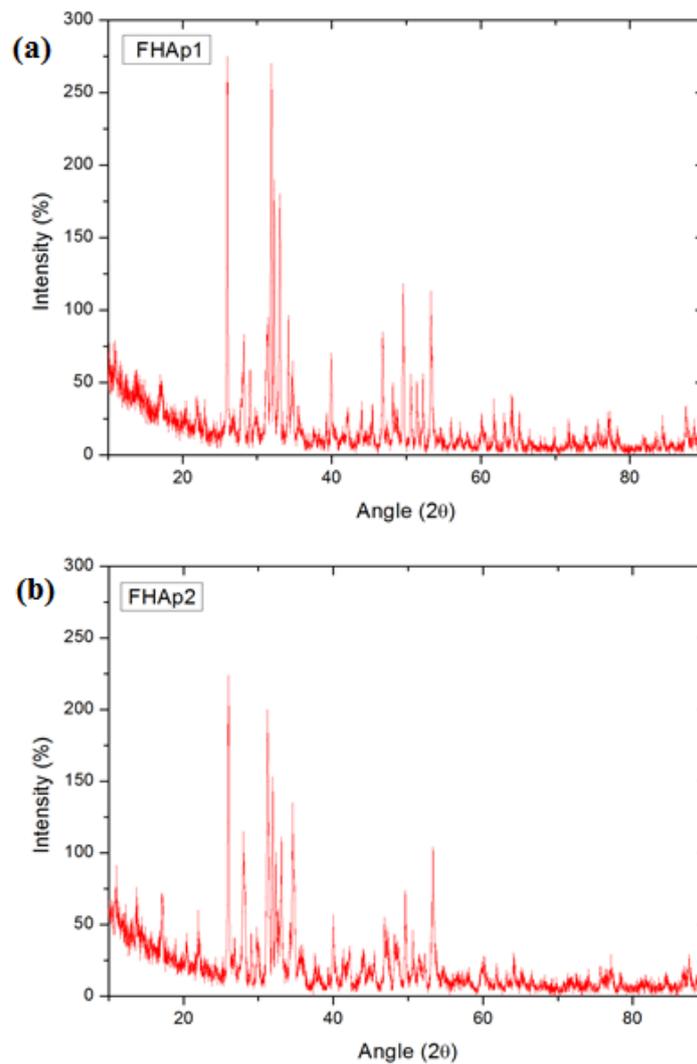


Figure 3. Diffractogram of FHAp1 (a) and FHAp2 (b).

Table 2. Crystallinity of FHAp1.

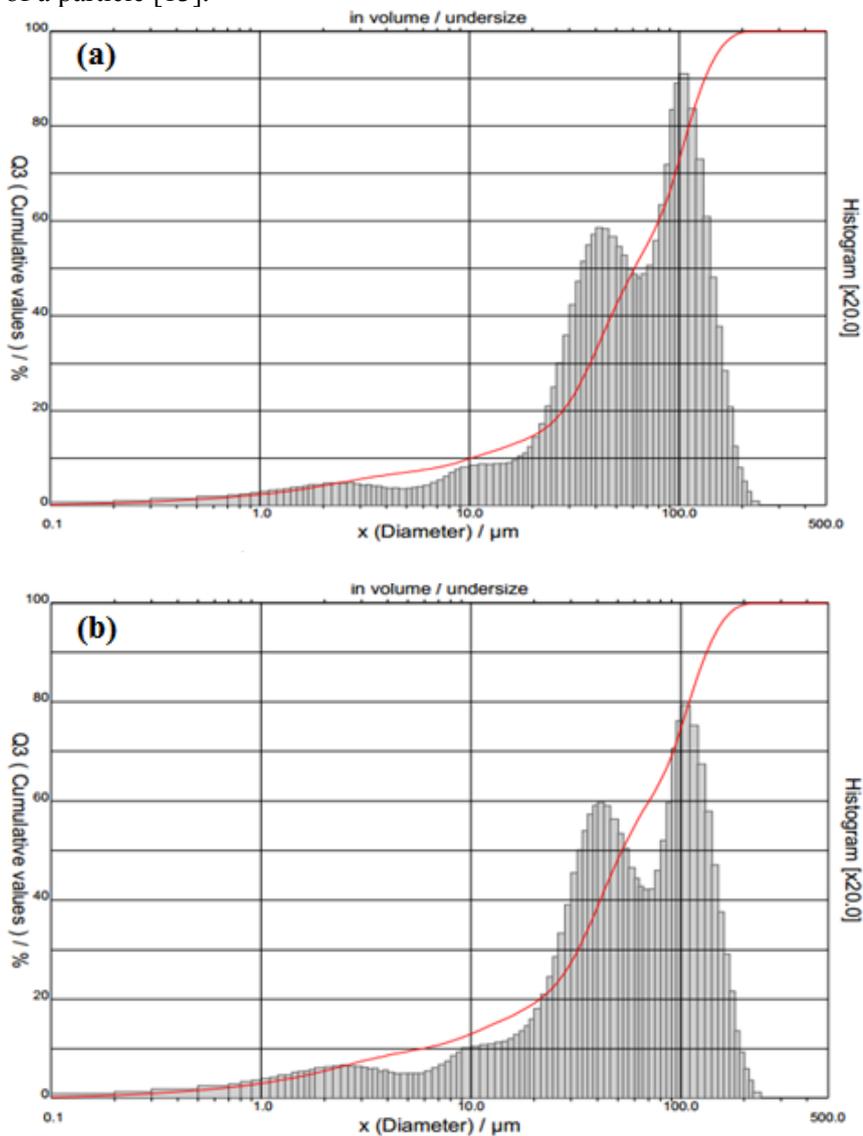
	Pos (2θ)	Height [cts]	FWHM (2θ)	Fraction
Crystal	25.9894	262.71	0.1378	36.2014
	31.9081	260.35	0.1574	40.9790
			∑	77.1804
Amorph	32.3073	181.59	0.1378	25.0231
			∑	25.0231

Table 3. Crystallinity of FHAp2.

	Pos (2 θ)	Height [cts]	FWHM (2 θ)	Fraction
	25.9972	202.49	0.1181	23.9140
	31.2274	178.89	0.2362	42.2538
Crystal			Σ	66.1678
	31.9140	126.09	0.1378	17.3752
Amorph			Σ	17.3752

3.4 Hydroxyapatite particle size

Crystal size of hydroxyapatite was measured using PSA. Particle size of FHAp1 sample ranged from 61.15 μm to 132.69 μm . Similar size was observed for sample FHAp2, with the size ranged from 52.33% to 132.64%. The results shown in Figure 4. Sonication affect the particle size reduction. Ultrasonic (sonicator) is mainly effective to break down the aggregates, reduce the size and reduce the polydispersity of a particle [13].

**Figure 4.** Particle size of FHAp1 (a) and FHAp2 (b).

4. Conclusion

Wet chemical precipitation method was able to synthesize hydroxyapatite from red snapper fish scales. FTIR spectra shows that PO_4^{3-} , OH^- , and CO_3^{2-} functional groups presence as indicates the formation of hydroxyapatite. The degree of crystallinity for FHAp1 and FHAp2 were 75.52% and 79.20%, respectively. Based on the ISO standard (ISO 13779-2:2000), the hydroxyapatite for medical materials must have crystallinity level of at least 45%, and the hydroxyapatite resulted from this research met the those requirements. Finally, the hydroxyapatite particle size distribution is evenly distributed, with the size of micro-scale hydroxyapatite particles, ranging from 5.76 μm to 132.64 μm .

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