Microstructural and Nanostructural Development of Natural Hydroxyapatite Originated from Tamban Bone Waste Through Heat Treatment

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Abstract

Fish bone waste has long been used in research laboratories and it is unfortunate that still many communities fail to understand its importance that makes it necessary for the program to transfer knowledge to the community inorder to improve its usefulness. The morphologic, microstructure and nanostructure development of natural fish bone waste though heat treatment is examined in view of fish bone waste management and as a possible route for hydroxyapatite ceramics. Fish bone heated at temperature < 1300 ^{o}C maintains a porous structure, with a sintered wall and a major crystalline phase of hydroxyapatite. Fish-originated ceramics are potentially useful as bioactive media as well as for environmentally compatible materials.

Key Words : Technology transfer, Fish bone waste, Hydroxyapatite, Development of Microstructure and nanostructure, Porous structure, crystalline phase

1. Introduction

Inorganic phosphate is one of the most important materials for natural animal and biological applications, as well as for use in the ceramics industry. Despite the growing possibilities of phosphorous compounds, the availability of high quality phosphate minerals will be limited in the future[1]. Because natural hydroxyapatite is an excellent biocompatible inorganic substance[2], the animal resourse (from bovin bone) has been applied to the fabrication of practical products for advance biomaterials [3-5]. However, fish bone waste management has recently become serious problem near big cities, specially in Kuala Terengganu Malaysia, because a large anount of seafood and seafood product, including fish, are consumed daily in the coastal areas. Fish waste contains inorganic bone, of which a component is hydroxyapatite. Such natural waste originated hydroxyapatite represents a possible resource for future ceramics.

In addition to developing fisheries and oceanography, the utilization of fish bones is also an alternative to address contamination problem. Hydroxyapatite has currently been used in several areas, such as medical, health, food (gelatin), and overcames contamination.

In this paper, we report on the morphologic, microstructural and nanostructural development of natural fish bone. In regard to the application of fish waste to ceramics, we examine the development of sintered microstructure and crystal phase of natural fish bone waste through heat treatment.

2. Experimental Procedure

From many species of fish, we selected Terengganu Malaysia sea, a high-quality seafood, as the starting ‘fish. The fish waste was washed using a strong water jet to eliminate the fish meat. The washed fish waste was then dried and heat-treated in air at temperature <1300 ^{o}C. Weight change was measured by thermogravimetry (Model SSC580, Seiko Instruments, Inc, PTBIN- BATAN Indonesia. The microstructure was observed by scaning electron microscopy (SEM) JEOL JED-2300. Powder X-ray diffractometry (XRD) Shimadzu XD-610, with a CuKα source (20kV,30mA) was used for characterizing the crystalline phase. Chemical composition of fish bone was performed using a XRF. The fish bone were heated at different temperature 1050 ^{o}C, 1100 ^{o}C, 1150 ^{o}C, 1200 ^{o}C, and 1250 ^{o}C, respectively., FTIR is a promising tool to identify substances and the removal of organic portion from fish bone was confirmed by TGA analysis.

3. Results And Discussion

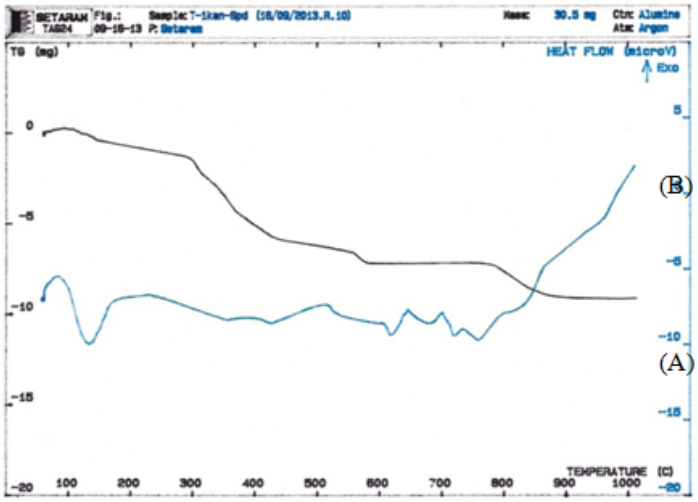


Fig 1. TGA curves of raw fish bone at room temperature in the TGA and DTG (A) TG curve and (B) Exo

The removal of the organic portion from Tamban fish bone and derived HA (900°C) was confirmed by TGA and DTG analysis showed in Figure 1 (A), (B). In the TGA and DTG curves, two infection points were observed in the Tamban fish bone at 100.5 °C and 366°C which corresponds to removal of the water and organic matter. No significant weight loss was observed between 600 °C and 900 °C, indicating the complete removal of organic materials such as collagen, lipids, chondroitin sulfate and keratin sulfate below 600 °C. The average amount of water and organic phase removed during heat treatment was calculated with DTG analysis, and it was found that approximately 4.76 % water and 30.02 % organic matter was removed[1]. Therefore, it can be inferred that about 35% of total weight loss was do to removal of water and organic substances from the Tamban fish bone when it was subjected to heat treatment up 600 °C. Figure 1(B) depicts the derived HA at 900 °C, no inflection points were observed from the starting temperature. This significantly indicated that HA at 900 °C, no inflection point were observed from the starting temperature. This significantly indicated that HA derived at 900 °C lacks organic moieties and water which further confirms that it is the pure form.

Figure 2. shows scanning electron micrograph (SEM) images of fish bone fired at 1050,°C 1150,°C (contains porous) and raw fish at room temperature. The microstructure of the heated bone has characteristic texture, with a weak oreantation of grains of different sizes depending on the heat-treatment temperature The grains measured ~ 200 nm in diameter in the present sample at 1050 °C and ~500 °C in the sample heated at 1150 °C. However, in both sample, the grains were prolonged spherical particles with apparently weak connectivity. The nitrogen asorption isotherms for the bone hydroxyapatite ceramics (heated at 600°, 800° and 1000° C) exhibited Type II isotherms, with no hysteresis loop in the adsorption-to-description cycle. According to Masakuni Ozawa and Suguru [1] the survace area and pure volume of the heatd-treated fish samples are listed in Table 1 [1]. The pore size is distributed in the diameter range > 30 nm for bone fired at 600 °C, >70 nm for the sample heated at 800°C, and >100 nm for the sample heated 1000°C.

The data indicat a change of porous structure (expansion of the open pores), which corresponds to the sintered microstructures (Figs.2(a) and (b) achieved through heat treatment in the fish-bone ceramics.

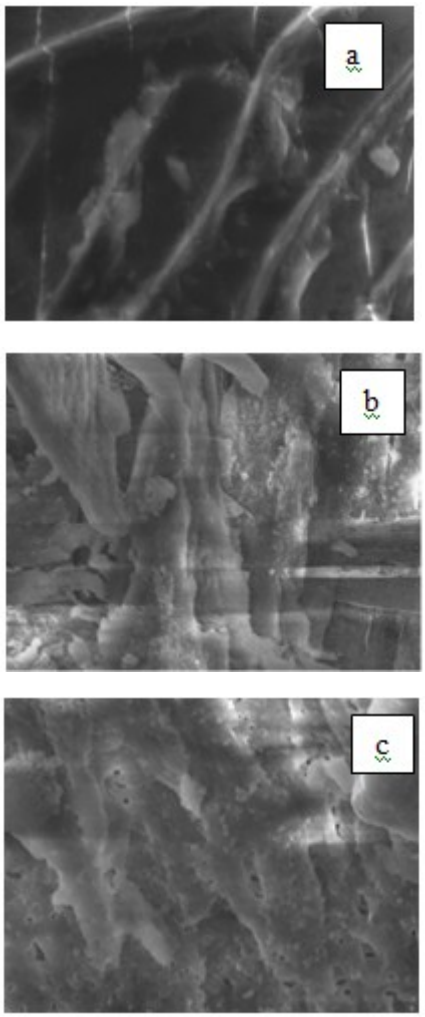


Fig 2. SEM results of (a) raw fish bone at room temperature and treated at (b) 1050 °C 1 hour and (c) 1150°C 1 hour respectively

Table 1. Characterization of Heat-Treated Fish Bone [1]

Heat-treatment Temperature (°C)	Phase	Surfase area (m^2/g	Pore volume (mL/g)
Raw	HAP, am		
600	HAP	10.5	0.28
800	HAP	4.0	0.08
1000	HAP	1.6	0.02
1200	HAP		
1300	HAP, TCP		

HAP, hydroxyapatite, am, amorphous phase (due to organic compout); and TCP, tricalsium phosphate. Relative pressure, $P/P_o = 0.996$.

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FT-Infrared Spectroscopic Analysis (FTIR)

Figure 3. FT-IR is a promising tool to identify unknown substances and to determine the amount of components in a given sample. This test was performed to get authenticated information about th vibrational origin of the phosphate, carbonate and amide groups and to confirm the production of HA with no association of organic moieties. The FT-IR spectrum of raw Tamban fish bone and derived HA at different temperatures are shown in Figure 2. The FT-IR spectrum of Tamban fish bone calcined between temperatures of 1050 °C to 1250 °C revealed the only characteristic peak of HA, which is consistent some previous reports [4,8-7]. A large number of bands in the spectra (601, 631, 873, 962, 1027, 1088, 1413, 1454, 2034, 2157 cm^{-1} and a broad band observed between 3300 – 3600 cm^{-1}) matched the bands in the HA reference spectrum and are in close agreement with the same [4-5]. At lower temperatures (300°C and 400°C), the peak corresponding to the phosphate (PO_4^3group at 1088 cm^{-1} was not observed and it appeared only at temperatures about 500 °C. This may be do to th removal of all organic material from the raw Tamban fish bone and formation of HA crystals.

Thermal stability is an important feature of derived HA. Ooi et al., have reported the formation of calcium phosphate when the bovine bone is subjected to higher temperature, which shows that the derived HA is not thermally stable [5]. On the contrary, no phase transformation due to calcinations was observed in our study even in high temperatures (1200 °C). Subsequently, peaks at 1026-1088 cm^{-1} and 962 cm^{-1} are attributed to PO_4^{3-}. Additionaly; all the FT-IR spectra samples exhibited a peak at 632 cm^{-1} and a broad peak at 3300-3600 cm^{-1} due to the presence of the hydroxyl group. The intense peaks observ'ed at 1413 and 1457 cm^{-1} in the spectrum of calcined Tamban fish bone are attributed to CO_3^{2-} .

Table 2. Chemical composition of raw Fish Bone

COMPOUND	P	S	K	Ca	Mn	Fe	Cu	Zn	Sr	Yb	Re
CONC UNIT	12.20 %	0.59 %	0.20 %	85.41 %	0.087 %	0.19 %	0.082 %	0.17 %	0.58 %	0.45 %	0.07 %

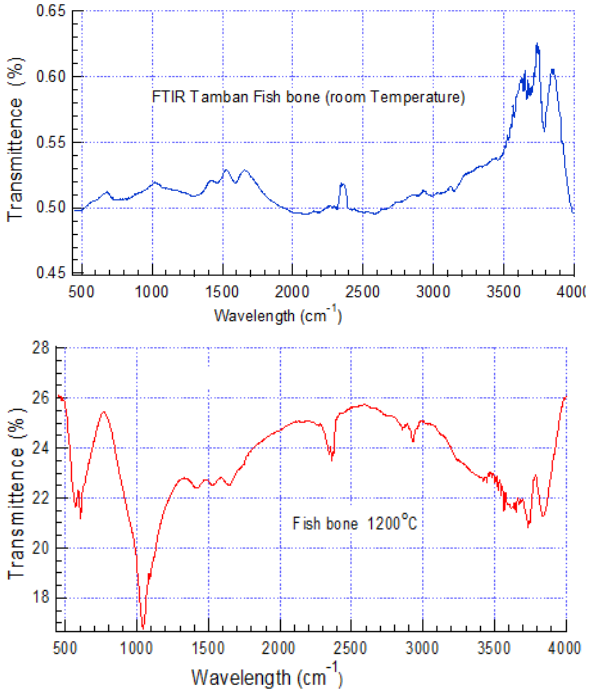


Figure 2. FTIR spctra of Tamban fish bone at room temperature and calcined at 1200°C

The elemental compositions are listed in Table 2. Raw fish bone contains large amount of organics, and even after heat treatment at 600°C, it has partially shown black color with carbon residue (0.5%). Powder become completely white by firing >800°C. The Ca : P ratio of the present fired bone apatite was found to be slightly calcium-deficient is 7.00.

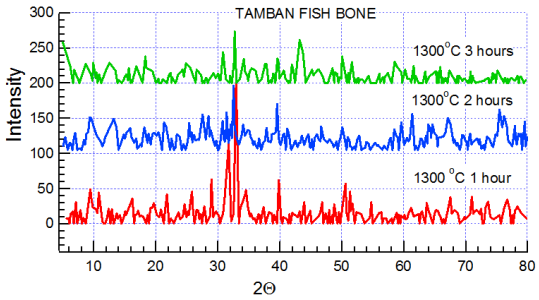


Figure 3. X-Ray Diffraction patterns (for CuKα source) at ranges 2 theta/deg = 05-80 for fish bone and sample heated 1300 °C For tree different time a) 1 hour b) 2 hours and c) 3 hours.

The crystal phase in the fired bodies is assessed in Fig.3, which shows the XRD patterns of original and fired bone. Raw bone is a biologically mineralized hydroxyapatite with very low crystallinity [6,7]. This feature was evident in Japanese sea bream fish bones investigated in present study. Under heat treathment

at 800°-1200°C, the bone initially transformed to a well-crysllized hydroxyapatite crystal (Fig.3). Then at 1300°C, the sintered body became a hydroxyapatite ceramic with a tricalcium phosphate (TCP) phase, as shown in Fig.3. This phenomenon is consistent with a previous report on the phase transformation of synthetic hydroxyapatite to TCP at ~ 1250°C [8]. Thus, we were able to use fish bone to make pure hydroxyapatite ceramics at < 1200°C and a sintered composite of TCP hydroxyapatite with heating to 1300°C 1 hour.

Figure. 3 shows the X-ray diffraction patterns with three different time (hours) of fish bone, sample heated 1300 °C For tree different time a) 1 hour b) 2 hours and c) 3 hours. There are Bragg scattering peaks superimposed on the diffuse fish bone scattering pattern in the hydroxyapatite in 2 and 3 hours. Fish bone heated in 1 hour increasing crystalline precipitate of hydroxyapatite, indicating that the fish bone of hydroxyapatite in fish bone to make pure hydroxyapatite ceramics, while exposure longer (2 and 3 hours) decreases crystalline precipitate of hydroxyapatite, due to increasing heated time. Hence, The fish bone structure is damaged due to exposure to longer heated.

Figure 4 shows first peak in 1 hour (with range 2θ : 32.16°-33.68°), second peaks in 2 hours (with range 2θ : 32.09° -33.51°) and third peaks in 3 hours (with range 2θ : (32.035° - 33.585°). Bragg peaks of the diffraction patterns (dot curves) and the results of Gaussian fitting analysis (solid curves) of fish bone heated 1300°C 1 hour, fish bone heated 1300°C 2 hour and fish bone heated 1300°C 3 hour and it modified. Compared with the diffraction pattern of 1300°C 1 hour it can be seen that the 2θ peaks of fish bone heated 1300°C 2 hour and fish bone heated 1300°C 3 hour were shifted to the lower value of 2θ and intensity, and FWHM become lower value (Table 1)

Tabel 1. Characterization of Heat-Treated Tamban Fish Bone

Material	Peak	2□	FWHM	Crystal size D(nm)	Intensity (count)
Fish bone 1300°C 1 hour	peak 1 hour	32.915	0.1761	90	209.960
Fish bone 1300°C 2 hours	peak 2 hours	32.587	0.0970	160	91.277
Fish bone 1300°C 3 hours	peak 3 hours	32.720	0.0784	190	76.796

The results of fitting analysis to the Bragg peaks in Tamban fish bone at three different time (hour) and three different peaks respectively. With adding different time, in general the peak shifts to the lower angle and intensity respectively. The change is coused by adding longer time of the natural fish bone crystalline in the 1 hour. The data of fitting analysis to the Bragg peak in 1 hour, 2 hours, and 3 hours at three different peaks and at three different hour time are listed in Tabel 1 and Figure 4, respectively.

Figure 4 shows peaks (with range 2θ = 30.0 °-35.0°), Bragg peaks of the diffraction patterns (dot curves) and the results of Gaussian fitting analysis (solid curves) of fish bone and it modified. Compared with the diffraction pattern of fish bone , it can be seen that the 2θ peak of heated 2 hours

3 hours were shifted to the lower value of 2θ and heated 1 hour shifted to the higher value of 2θ, and FWHM become lower (2,3 hours) and higher value (1 hours) data listed in Table 1

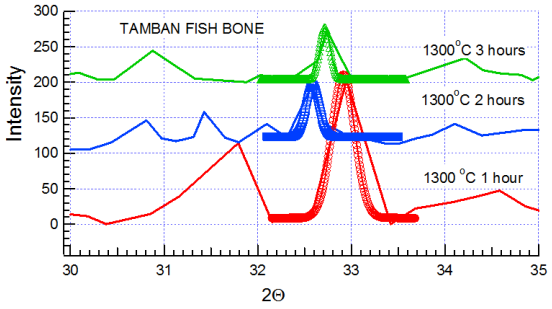


Figure 4. Curve fitting analysis to the Bragg peak in Tamban fish bone for tree different time (hour) at ranges 2 theta/deg = 30-35 and sample heated a) 1300 °C 1 hour b) 1300 °C 2 hours and c) 1300 °C 3 hours.

4. Conclusion

Heat treatment of fish waste lead to nanostructural and microstructural development on both a microscopic and a macroscopic scale. Fish bone ceramics resulting from the heat treatment of fish waste at 600° – 1300°C have a major hydroxyapatite phase and contain macropores with a sintered wall. Such ceramics could be useful as inexpensive ceramic media for biologically and environmentally compatible material. Fish-originated hydroxyapatite is a potential new ceramics resource in the coastal areas of Asia. The present result also suggest a possible materials-resycling technology for future waste management and ecology.

Base on the results of this, we draw a conclusion that a temperature between 600– 1300°C is optimum for isolation of HA from Tamban fish bone with almost no organic portion, high purity, stability, crystallinity, nanostructure and no cytotoxicity, making it appropriate for use in biomedical application, for biologically and environmentally compatible material as well.

5. References

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