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Effect of Calcination Temperature on Characteristics of Hydroxyapatite from Milkfish Bone (*Chanos chanos*)

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Abstract

This study investigates the synthesis of bioceramic hydroxyapatite (HAp) from milkfish bone (Chanos chanos) for medical applications. Due to its high bioactive properties and biocompatibility, HAp is becoming a main selection in biomaterials. Milkfish bone, a biological source abundant in calcium, phosphorus, and carbonate, was utilized in this study due to its high potential in the preparation of biocompatible and economical HAp. Applying various calcination temperatures, this study aims to optimize the HAp synthesis process, analyze the effect of temperature on the crystallinity, phase, and particle size of HAp, and evaluate its chemical composition and crystal structure through XRD and FTIR analysis. Results of this research are aimed to provide new solutions in the development of biomaterials from natural resources that are sustainable and biodegradable for medical applications.

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1. INTRODUCTION

A significant increase in demand for bioactive bioceramic materials for medical applications, particularly in hard tissue substitution and bone regeneration, is due to their advanced biological characteristics. Bioactive characteristics are important in biomaterials [1], [2], [3]. Furthermore, a ceramic/polymer composite was developed, where the bioactive properties of the ceramic particles can be attached to the biodegradable polymer matrix. Such composite is known to have bioactivity properties and can improve the mechanical properties of the membrane when compared to individual polymers [4], [5], [6]. Increasing community needs in the use of bioceramic materials, particularly in the health sector, has stimulated the development of new materials. Hydroxyapatite is one of the bioceramic materials in development. Hydroxyapatite can be used as a ceramic source for the future. Hydroxyapatite for industrial applications is generally obtained by synthesis methods. Several methods are commonly used to obtain HAp, including solid reaction, sintering, co-precipitation reaction, hydrothermal reaction, sol-gel synthesis, and so on. HAp extraction from fish bone is biologically sustainable and economical. HAp has properties that are biocompatible, osteoconductive, non-toxic, non-inflammatory, and not an immunological agent [3], [4], [7], [8], [9].

As one of the biological sources to generate hydroxyapatite is from milkfish bone waste (*Chanos chanos*), because by utilizing this bone waste, it will have a positive impact on the environment, besides that fish bones contain minerals that are relatively high compared to other body parts because the main elements of fish bones are calcium, phosphorus and carbonate [3], [10], [11]. Fish bone products in the form of hydroxyapatite ($Ca_5(PO_4)_3(OH)$) is a natural inorganic element derived from bone that can be used to regenerate bone, repair, replace, expand, and recruit bone tissue. It is due to the hydroxyapatite has good biocompatibility properties. Hydroxyapatite is a compound that has physical and chemical similarity with minerals contained in human bones and Teeth therefore hydroxyapatite can be used as a bone substitute and as a dental filling material. Hydroxyapatite can also be used as an adsorbent because it has porous, inert and wear-resistant to overcome the environmental contamination of heavy metals[2], [6], [11]. The advantages of bone-based adsorbents when compared to other adsorbents are easy to obtain (economically, the price is very cheap), abundant because it is *renewable*, non-toxic, and environmentally friendly.

Hydroxyapatite can be synthesized from milkfish bone waste which is not optimally utilized. Sintering process is very important for hydroxyapatite synthesis because it aims to generate a crystalline structure. However, at certain temperatures, hydroxyapatite can be partially decomposed to form compounds other than hydroxyapatite. Increasing material needs encourage various attempts to find alternative biomaterials that can substitute the missing tissue structure easily without any adverse effects and are affordable by the community.

2. MATERIALS AND METHODS

Implementation of the research is divided into several phases, which begins with a literature study, preparation of milkfish bones, extracting hydroxyapatite from milkfish bone powder, characterization of milkfish bone hydroxyapatite biomaterials such as FTIR analysis and analyzing the type of phase and crystallization of hydroxyapatite with XRD.

Preparation of milkfish bones begins with steaming the fish bones to remove the remaining meat attached. After that, it was cleaned and washed with distilled water then dried in the sun until dry. After that, an acetone solution was immersed for 24 hours and continued by an NaOH solution for 24 hours [3], [10], [12]. Prepared milkfish bones were then dried at 120ºC for 24 hours. The dried milkfish bones were then milled into particles. Extraction was analyzed using various calcination methods (high temperature heating). Calcination temperature was based on the research of Palella *et al*. [13] study that isolated hydroxyapatite from tuna bones (*Thunnus obesus)* at sintering temperatures of 700ºC, 800ºC, and 900ºC for 5 hours. Calcination process begins with the addition of 7 g of milkfish bone powder into a porcelain cup, then heated in the furnace with an increase in heating of 20 ° C per minute, after reaching the heating temperature calcination, the material is allowed to stand until the temperature decreases to room temperature. The hydroxyapatite powder product from milkfish bones obtained was wrapped in plastic and stored at room temperature for analysis. The analysis consist of X-ray diffraction (XRD) and FTIR analysis. Phase type and crystallization of hydroxyapatite were analyzed using XRD. A prepared sample of 2 g was placed in a holder on the diffractometer. FTIR spectroscopy was used to determine the functional groups of the hydroxyapatite sample and possible interactions between the components. 2 mg hydroxyapatite powder was mixed with 100 mg KBr to make a pellet and measured using FTIR at a wavelength of 400-4000 cm^{-1}.

3. RESULTS AND DISCUSSION

Hydroxyapatite Synthesis

Hydroxyapatite (HAp) synthesis was performed using calcium compounds from fish bone powder and phosphate from H3PO4. Before utilized for HAp synthesis, the fish bones were first separated from the meat by steaming for 2 hours, then dried under direct sunlight. After that, it was treated with 1 M NaOH solution for 24 hours followed by acetone treatment for 24 hours and dried in the sun to remove the water concentration. Furthermore, sintering process was carried out on fish bones at a temperature of 800°C, 850°C, and 900°C for 5 hours. Then, the fish bones were milled using milling to obtain fish bone powder.

X-Ray Diffaction (XRD) Analysis

XRD analysis was analyzed to determine the phase and crystallinity. Crystallinity analysis aims to know the effect of calcination temperature on crystallinity. Crystallinity is a quantity that states the amount of crystal content in a material by comparing the crystal area with the total area of amorphous and crystalline crystals.

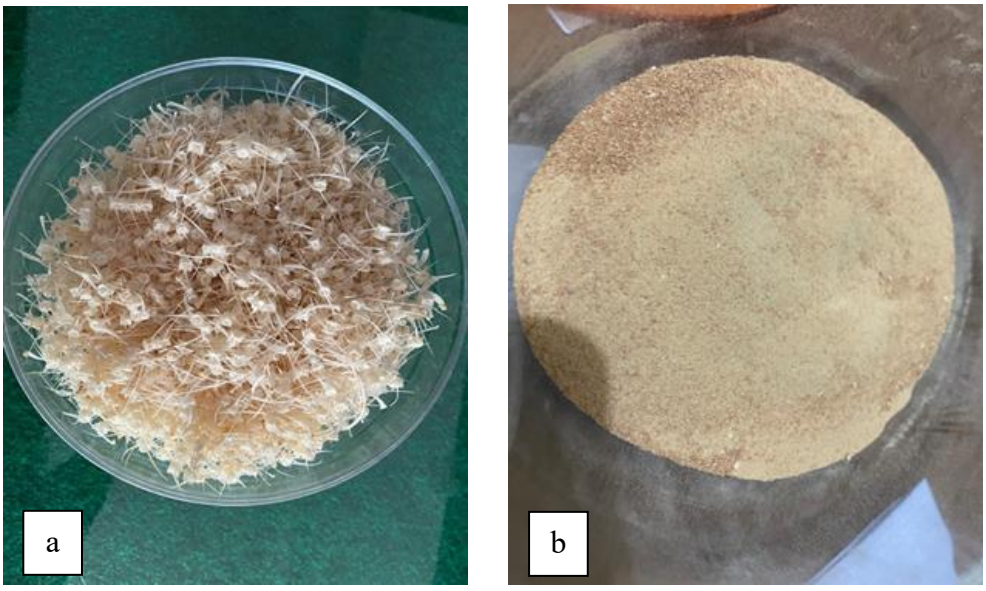

Figure 1 (a) *Chanos chanos* bone (b) *Chanos chanos* bone powder

XRD diffraction pattern shows that the XRD peaks are dominated by hydroxyapatite phase. According to JCPDS no. 09-0432 the HAp phase has 4 distinctive peaks at 31.77°, 32.19°, 32.90° and 34.05°. Figure 2 presents the X-ray Diffraction patterns of fish bone powder at various temperatures, with two separated spectra, the crystal spectrum and the amorphous spectrum. In the crystalline spectrum, this part of the graph shows the XRD pattern for the crystalline structure in the powder at 800°C, 850°C, and 900°C. The graph shows that different peaks indicate the presence of the crystalline phase.

Intensity is measured in units of (a.u), and the x-axis shows the angle 2θ in degrees. Percentage crystallinity at 800°C is 68.01% with a pattern that shows several peaks with varying intensity. The peak at 850°C shows similarities with the peak at 800°C with a small shift, with slightly lower crystallinity at

64.58%. While at 900°C there are some changes in peak intensity and position, resulting in a crystallinity of 64.13%. Amorphous spectra show that the pattern shows broad characteristics typical of amorphous materials, with an amorphous percentage at 800°C of 31.98%, at 850°C of 35.41% with a similar pattern at 800°C with slightly different intensity, while at 900°C there is a small increase in amorphous percentage to 35.86%. As the temperature increases from 800°C to 900°C, there is a small decrease in crystallinity and a corresponding increase in amorphous content. Temperature increase also causes a shift in the position and intensity of the peaks, which is due to changes in the crystalline structure or the formation of new phases. A wide range in the amorphous spectrum also indicates that at high temperatures, there is a significant amorphous phase. Figure 3 is the distribution of particle size and strain at 850°C. The graph shows that the particles have sizes distributed around 20 nm to 60 nm.

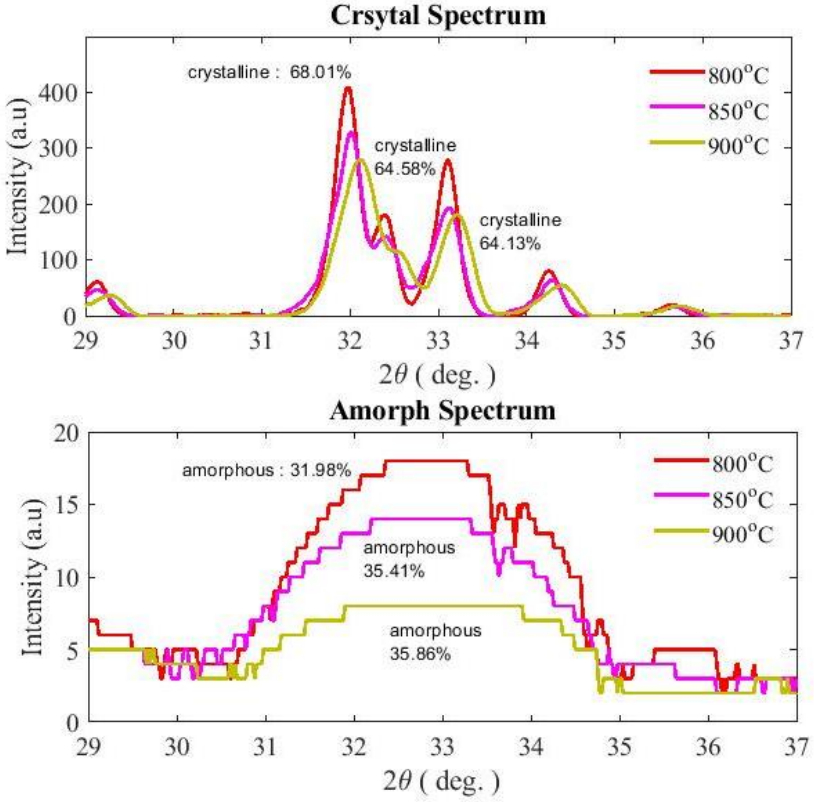

Figure 2 XRD pattern of calcination at 800°C, 850°C, and 900°C for 5 hours

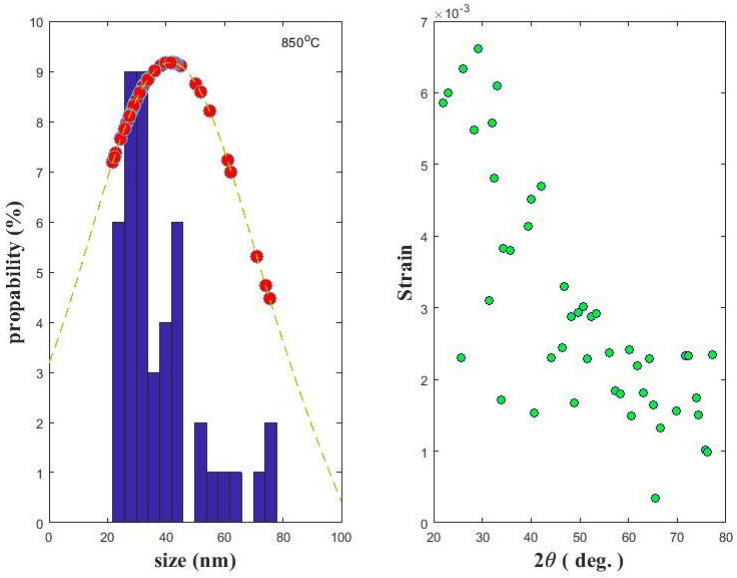

Figure 3 Distribution of particle size and strain at 850°C.

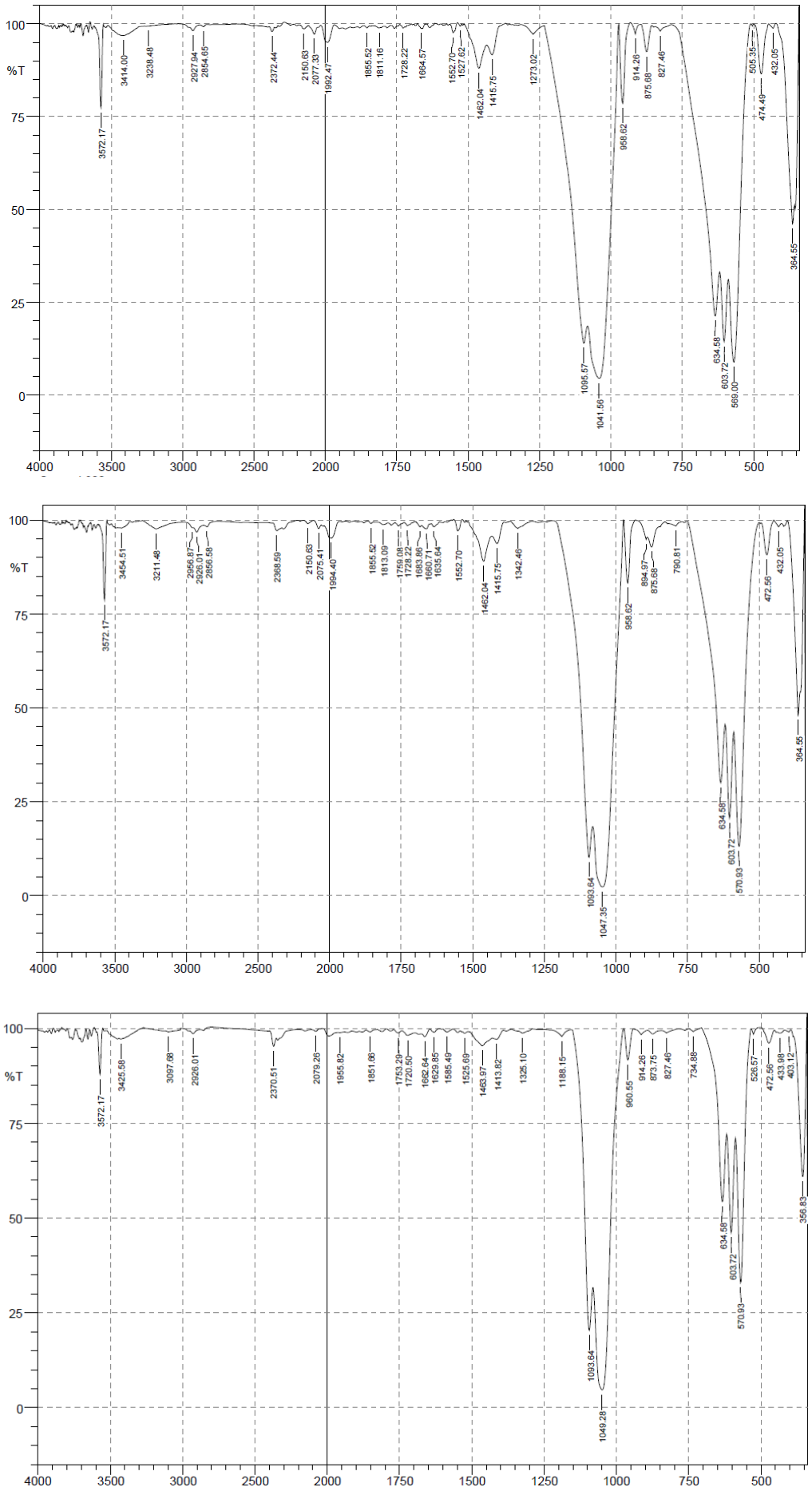

Figure 4. FTIR spectra at 800°C, 850°C, and 900°C

Fourier Transform Infrared (FTIR) Analysis

Functional groups are analyzed qualitatively to identify the constituent elements of milkfish bones through transmission absorption obtained by FTIR. FTIR identification is based on molecular vibrational changes caused by electrostatic valence modification on alkane, alkene, arena, amine, carboxyl and hydroxyl bonds[14], [15]. The analysis of the functional groups of milkfish bones generally consists of two constituents, including organic and inorganic elements [3]. Each molecule has a specific energy to vibrate, this is dependent on the atoms and the bond strength that connects it, as shown in Figure 4. FTIR characterization in this research was carried out to obtain valid information about the vibrations of phosphate compounds, carbonate and amide compounds to confirm the preparation of hydroxyapatite (HAp) compounds without the association of organic groups. Hydroxyapatite has OH^- groups, CO_3^{2-}, and PO_4^{3-} groups [9], [14]. Figure 4 shows the FTIR spectra of fish bone powder calcined at 800°C, 850°C, and 900°C. The O-H^- stretching vibration of the hydroxyl group was detected at around 3572 cm^{-1} both at 800°C, 850°C, and 900°C. This is consistent with research done by Venketesan and Kim [4], [16], [17] which explains that the peak detected at wave numbers 3300-3600 cm^{-1} is an indication of the presence of hydroxyl groups. Hydroxyapatite contains OH^- groups, CO_3^{2-}, and PO_4^{3-} groups. Absorption bands of phosphate groups (PO_4^{3-}) with asymmetric stretching vibrations that are characteristic of hydroxyapatite were detected at wave number 1041.56 cm^{-1} at 800 °C, 1047.35 cm^{-1} at 850°C and at wave number 1049.28 cm^{-1} at 900°C. Increasing the temperature, the phosphate peak also becomes sharper and well-defined, indicating increasing crystallinity. Absorption bands of bending-vibrated phosphate groups were detected at wave numbers 569-634.58 cm^{-1} at 800°C, 570.93-634.58 cm^{-1} at 850°C and detected at wave numbers 570.93-634.58 cm^{-1} at 900°C. Mondal *et al*.[18] mentioned that the first indication of the formation of hydroxyapatite compounds is the formation of molecular complexes at wave numbers 1000-1100 cm^{-1} with asymmetric *stretching* vibrations for phosphate groups and symmetric bending vibrations at wave number 576.30 cm^{-1}. As shown in the graph, when the temperature increases, there is a reduction in the intensity of the hydroxyl group. It is due to water loss, decomposition of organic compounds, and changes in the mineral structure of the bone. Increasing temperature also has an effect on the phosphate peak which changes due to phase transformation of the material, indicating a more substantial change in the hydroxyapatite structure to other phases such as tricalcium phosphate, which has higher thermal stability at high temperatures [7], [19].

4. CONCLUSION

Hydroxyapatite has been synthesized from milkfish bones by utilizing calcination process at different temperatures. XRD and FTIR analysis showed that calcination temperature has a significant effect on the crystallinity, chemical composition, and crystal structure of HAp. Optimal calcination temperature was found to improve the crystallinity of HAp while maintaining a suitable phase and chemical composition for bone regeneration applications. Results confirmed the potential of milkfish bone as a biomaterial source for the biocompatible and economical production of HAp. Given its advantages, HAp from milkfish bone offers a valuable alternative in the potential development of bioactive materials for medical applications, particularly in bone regeneration. The study also highlights the significance of utilizing natural resource waste as an alternative and sustainability measure in the field of biomaterials.

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