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Quality Study of Hydroxyapatite (HAp)-TiO_2 Composites from Pokea (Batissa violacea var. celebensis) Shell Waste by Hydrothermal, Microwave and Precipitation Methods

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ABSTRACT: Research on the quality study of hydroxyapatite (HAp)-TiO_2 composites from Pokea (*batissa violacea var. celebensis*) clamshell waste by hydrothermal, microwave and precipitation methods have been successfully conducted. This study aims to determine the characteristics and quality of HAp-TiO composites$_2$ synthesized from Pokea clam shell using three different methods, namely hydrothermal, microwave and precipitation. FTIR characterization showed the absorption of PO_4^{-3} functional groups in the wave number range 567-1091 cm^{-1}, -OH and -NH groups at wave numbers 3444-3448 cm^{-1}, and Ti-O groups at 567-632 cm^{-1}. XRD analysis revealed a diffraction pattern at $2\theta = 26.67^o$, with crystal sizes of HAp-TiO_2 material of 80.20 nm, 71.47 nm, and 73.28 nm for hydrothermal, microwave and precipitation methods, respectively. The mechanical properties showed that the highest compressive strength of the HAp-TiO_2 composite was obtained in the hydrothermal method (6.07 MPa), followed by the precipitation method (5.19 MPa) and microwave (5.08 MPa). The material density test results for the hydrothermal, microwave and precipitation methods were recorded at 2.27 MPa, 2.12 MPa, and 1.73 MPa. The test results showed the highest value in the hydrothermal method of 24.24 MPa, while the microwave and precipitation methods produced hardness of 18.61 and 18.83 MPa, respectively. Digital optical microscope analysis showed the surface morphology of the material to be uniform, refined grains with pores. The results in this study indicate that the hydrothermal method produces composites with better mechanical quality and crystal structure than other methods.

KEYWORDS: Hydroxyapatite; Hydrothermal; Microwave; Precipitation

I. INTRODUCTION

Along with current technological advances, various efforts are made to improve the quality of life. One of the growing programs is an effort to improve the body's health through synthesizing biomaterials. Biomaterials are materials designed for use in the human body to improve the quality of life [1]. Natural or synthetic biomaterials are specialized functional materials that interact with living systems. Their functions include diagnosis, treatment, replacement, repair or even induction of regeneration in cells, tissues, and organs. Biomaterials are at the intersection and unification of various disciplines, such as materials science, medicine, and pharmacology [2]. One material that is being intensely developed as a synthetic biomaterial is bio ceramic hydroxyapatite (HAp).

Hydroxyapatite (HAp) has recently taken center stage in bone tissue engineering applications [3]. Hydroxyapatite, which has the molecular formula $Ca_{10}(PO_4)_6(OH)_2$, is an essential component in bone and tooth structure, forming most of the bone skeleton and tooth enamel. The thermodynamic context in HAp is considered the most stable phosphate phase in the human body [4]. The scientific link to calcium phosphate (CaP) as a bone substitute material in biomedical applications began with the use of CaP as a filler material to repair keratitis bone defects in rabbits [5]. Due to its physicomechanical properties similar to human bone and its osteogenic potential both in vivo and in vitro. CaP is currently widely used in various fields of medicine, including otolaryngology, skull and maxillary reconstruction, spinal surgery, orthopedics, treatment of fractures and bone disorders, reinforcement implants and dental/periodontal surgery [6]. A study in the United States showed that in 2010, approximately 1.3 billion dollars were invested in CaP-based bone replacement [7]. One of the alternatives that can be used as a substitute for CaP can be made synthetically or extracted from calcium-rich biological sources, such as corals and mollusks.

One of the raw materials that can be processed as raw material for the HAp synthesis process is Pokea shell waste. Pokea clam (*Batissa violacea var. celebensis*) is a freshwater clam from the Corbicullidae family, mainly found in Southeast Sulawesi. This organism is generally widespread in large rivers in the region and is a vital economic resource for local communities. Clam shells have a high calcium carbonate ($CaCO_3$) content; the more complex the shell, the higher the $CaCO_3$ content. Pokea shells have a $CaCO_3$ content of 98%, so they have the potential to be used as a material in HAp synthesis [8]. The problem arising from the low mechanical strength of HAp made from CaP must be overcome by adding reinforcing materials to improve its mechanical properties.

The choice of filler material in this application context becomes very important; it can improve mechanical properties and inhibit bacterial growth. Titanium dioxide (TiO_2) fulfils this requirement due to its inert nature and ability to inhibit microorganisms' growth [9]. Combining HAp with metals or metal alloys results in composite materials with superior mechanical properties, toughness, and biocompatibility. For example, titanium alloys reinforced with HAp particles are often used in orthopedic implants due to their high level of biocompatibility and ability to stimulate bone growth. The metal component provides structural integrity, while the HAp reinforcement improves osseointegration and mimics the mineral composition of bone [10].

Synthesis methods commonly used in HAp production are hydrothermal, sol-gel, ultrasonic and microwave irradiation techniques, and precipitation. HAp is synthesized in an aqueous and high-pressure environment using the hydrothermal method. At the same time, sol-gel involves a chemical process where inorganic precursors react in solution to form a gelled material [11]. Ultrasonic and microwave irradiation techniques use sound waves or microwave energy to accelerate the synthesis process. At the same time, precipitation methods involve the formation of HAp from solution by adding chemical reagents. Due to the variety of synthesis methods available, it is possible to customize the conditions and characteristics of the final product according to the desired applicative needs [12].

This research leads to a comparative study of the quality of HAp-TiO_2 composites produced through three methods, namely hydrothermal, microwave and precipitation, using Pokea shell waste as the source material. The main focus of this research is to find an efficient and potential method to produce HAp-TiO_2 composites from pokea clam shell waste (*Batissa violacea var. celebensis*) with superior quality and contribute to developing environmentally friendly materials.

II. METHOD

The tools used in this study are Furnace (Carbolite), hydrothermal (Welljoin), X-ray diffraction (XRD) (D2 phaser Merck Bruker), Fourier transform infrared (FTIR) (Spectrum two system (L160000A) perkin elmer), Digital optical microscope and Universal testing machine (UTM).

The materials used in this study Are Pokea shells taken from the Lasolo River, Sawa District, North Konawe Regency, diammonium hydrogen phosphate ($(NH_4)_2HPO_4$) (Sap Chemicals), and titanium dioxide (TiO_2) (Star Grace).

Research Procedures

Pokea (*Batissa violacea var. celebensis*) Shell Preparation

The Pokea shells were cleaned of impurities by washing them with water and then drying them under direct sunlight for two days until they were dry. Cleaning the back of the black Pokea shell was done using sandpaper. The clean shells were then pulverized with a mortar and pestle and sieved with a 200-mesh sieve. The shell powder was then calcined using a furnace at 900ºC for 4 hours to produce calcium oxide (CaO).

Synthesis of Hydroxyapatite (HAp)-TiO_2 by Hydrothermal Method

HAp synthesis was made by hydrothermal method in situ by mixing 14 g of CaO, 17.25 g of $(NH_4)_2HPO_4$ and TiO_2 as much as 1.56 g with a mole ratio of 1.67. The mixture was stirred using a magnetic stirrer for 1 hour, then put into a hydrothermal device and heated in an oven at 150ºC for 12 hours. The resulting solid was then washed with distilled water until the pH was neutral and filtered using vacuum equipment. The resulting solid was heated at 110ºC for 12 hours, then continued with the sintering process at 900ºC for 4 hours [13].

Synthesis of Hydroxyapatite (HAp)-TiO_2 by *Microwave* Method

The synthesis method of HAp-TiO_2 was adapted from the method by Hutabarat *et al.* (2019). The synthesis was conducted with microwave assistance by mixing 14 g of CaO, 17.25 $(NH_4)_2HPO_4$ and TiO_2 as much as 1.56 g with a mole ratio 1.67. The mixture was stirred using a magnetic stirrer for 1 hour and then calcined in a microwave oven for 10 minutes operated at 700 W using a microwave frequency of 2.45 GHz. The resulting solid was then washed with distilled water until the pH was neutral and filtered using vacuum equipment. The resulting composite was heated at 110º C for 12 hours, then continued with the sintering process at 900º C for 4 hours [14].

Synthesis of Hydroxyapatite (HAp)-TiO_2 by Precipitation Method

The synthesis method of HAp-TiO_2 was adapted from the method carried out by Suci and Yulius (2020), and the synthesis was carried out using the precipitation method. Starting with mixing 14 g of CaO, 17.25 g of $(NH\ )_{42}$ HPO_4 and TiO_2 as much as 1.56 g with a mole ratio of 1.67. The mixture was stirred using a *magnetic stirrer* for 1 hour and then allowed to stand for 24 hours, then washed with distilled water until the pH was neutral and filtered using a set of vacuum equipment and then dried in an oven at 110º C for 12 hours, then continued with the sintering process at 900º C for 4 hours [15].

III. RESULTS AND DISCUSSION

The HAp synthesis process begins with preparing pokea shells, which are then heated at high temperatures reaching 900º C using a furnace. This step aims to remove carbonates that can inhibit crystal formation and remove all organic matter from Pokea shells. Heating at a high temperature of 900º C to reduce carbonates in Pokea shells so that the crystallization process can take place without interruption and all components are burned out. The reaction occurs in the following equation:

$$CaCO_3 \longrightarrow CaO + CO_2$$

Synthesis of Hydroxyapatite (HAp)-TiO_2

The HAp-TiO_2 synthesis process aims to utilize their respective advantages in biomaterial applications in artificial bone and teeth. HAp is the primary precursor in the synthesis process, and TiO_2 is a reinforcing agent for the mechanical properties of the composite. Diammonium hydrogen phosphate ($(NH_4)_2HPO_4$) serves as a source of phosphate ions (PO_4^{-3}) in the preparation of HAp with the chemical formula Ca_{10} (PO $)_{46}$ $(OH)_2$. The addition of diammonium hydrogen phosphate is essential in the HAp synthesis process because it can provide the phosphate ions necessary for the formation of the HAp structure, which consists of calcium (Ca^{+2}), phosphate (PO_4^{-3}) and hydroxyl (OH^-) [13].

The hydrothermal method uses low temperature with high pressure to produce HAp material with stable crystals and uniform particle size. The microwave method uses microwaves for rapid heating and is efficient in producing HAp materials quickly. Meanwhile, the precipitation method involves mixing calcium and phosphate solutions to form precipitation of HAp material.

Fourier Transform Infra-Red (FTIR) analysis

Fourier Transform Infra-Red (FTIR) was used to analyze the functional groups of HAp-TiO_2 composites using hydrothermal, microwave and precipitation methods. The FTIR test was conducted in the 450-4000 cm^{-1} wave number range. The results of FTIR analysis are shown in Figure 1.

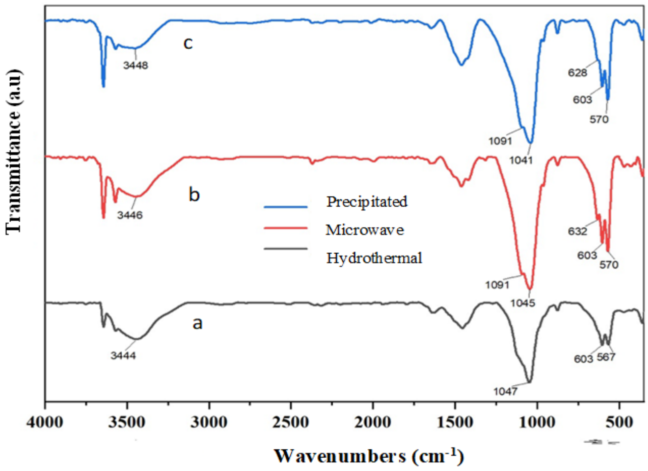

Figure 1. FTIR Spectra of HAp-TiO_2 Based on (a) Hydrothermal, (b) Microwave and (c) Precipitation Methods

Table 1. Results of FTIR Analysis of HAp Based on Hydrothermal, Microwave and Precipitation Methods

Function Group	Vibration	Wavenumbers (cm^{-1})			Reference
		Hydrothermal	*Microwave*	Precipitation	
-OH and -NH	Strain	3444	3446	3448	[16]
PO_4^{-3}	Strain	567; 603	570; 603	570; 603	[17]
PO_4^{-3}	Asymmetry	1047	1045; 1091	1041; 1091	[17]
Ti-O	Strain	567; 603	570; 603; 632	570; 603; 628	[18]

From the results of FTIR analysis, -OH and -NH groups were observed at wave numbers 3444 cm^{-1} to 3448 cm^{-1} and 3446 cm^{-1}. For the stretching vibration of PO group$_4^{-3}$, there are two peaks of wave numbers 567-570 cm^{-1} and 603 cm^{-1} in all methods. The asymmetry vibration of the PO_4^{-3} group is shown by two absorption peaks at wave numbers 1045 cm^{-1} and 1091 cm^{-1} and wave number 1047 cm^{-1}. That shows that the synthesis by microwave, precipitation and hydrothermal methods shows almost the same wave number trend for specific functional groups possessed by hydroxyapatite-TiO_2 composites, so it can be concluded that these three methods are effective to be used as a method of making hydroxyapatite or hydroxyapatite composites in-situ.

Ray Diffraction (XRD) analysis

X-*ray diffraction* (XRD) characterization was performed on HAp-TiO_2 material synthesized using hydrothermal, *microwave* and precipitation methods. The results of the XRD characterization are presented in Figure 2.

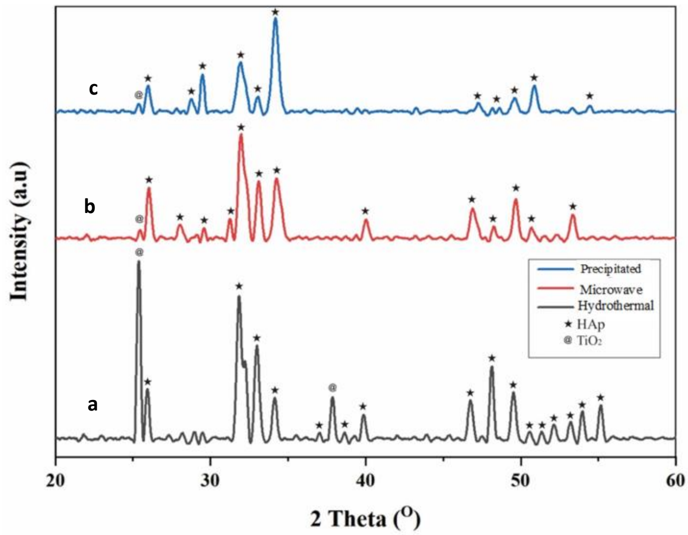

Figure 2. XRD patterns of (a) Hydrothermal, (b) Microwave and (c) Precipitation methods

HAp-TiO_2 composite material has been successfully synthesized. Figure 2 shows that HAp species formed at the peak angle 2θ = 20-57° after calcination at 900°. In the diffractogram of the HAp-TiO_2 composite, there is a peak at an angle of 2θ = 26.76°, which is characteristic of TiO_2; this is to research conducted by Hutabarat (2019)[16] that the specific peak of TiO_2 will appear at an angle of 2θ = 26.68°.

Based on Figure 2, the diffractogram pattern of the synthesized composite shows a relatively high and sharp peak pattern, indicating that the synthesis using the three methods produces quite good quality. The calculation results with the *Scherrer* method show that the three different synthesis methods, hydrothermal, microwave and precipitation, produce composites with varying crystal sizes. The hydrothermal method produced the most significant average crystal size of 80.20 nm, with the highest crystallinity of 99.94%. That shows that the hydrothermal method is more effective in producing materials with a more regular crystal structure than the other methods. The microwave and precipitation methods produced smaller average crystal sizes, 71.47 nm, and 73.28 nm, respectively, with slightly lower crystallinity percentages than the hydrothermal method, 99.51% and 99.28%. That indicates that both methods can also produce materials with a high degree of crystallinity but smaller crystal sizes than hydrothermal methods. The crystal size and crystallinity variation are due to the different reaction conditions, such as temperature, pressure and reaction time used in each method.

Test Properties Using UTM

The compressive strength and density tests were conducted using a Universal Testing Machine (UTM) to measure the resistance and density of the sample when given a certain amount of force or load.

Compressive Strength Test

The results of compressive strength testing for HAp-TiO_2 samples synthesized using hydrothermal, microwave and precipitation methods are presented in Figure 3.

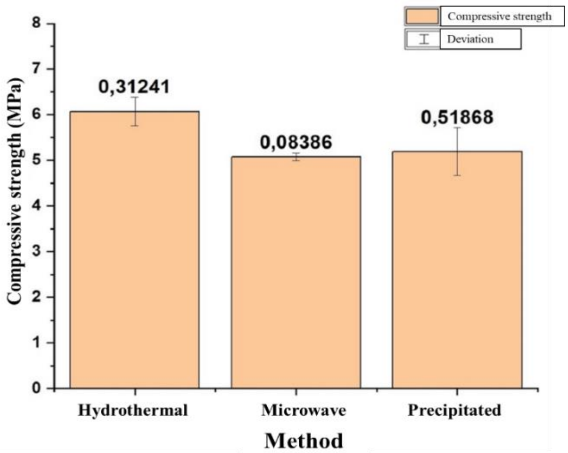

Figure 3. Compressive strength of HAp-TiO_2 material using hydrothermal, microwave and precipitation methods

The results of the compressive strength data presented in Figure 3 show that the hydrothermal method of HAp-TiO_2 synthesis is better at producing materials with the highest compressive strength, which is 6.07 MPa and the most significant standard deviation of 0.312. The precipitation method has a slightly lower compressive strength of 5.19 MPa. Meanwhile, the microwave method produced the lowest compressive strength of 5.08 MPa.

Density Test

The density testing results for HAp-TiO_2 samples synthesized using hydrothermal, microwave and precipitation methods are presented in Figure 4.

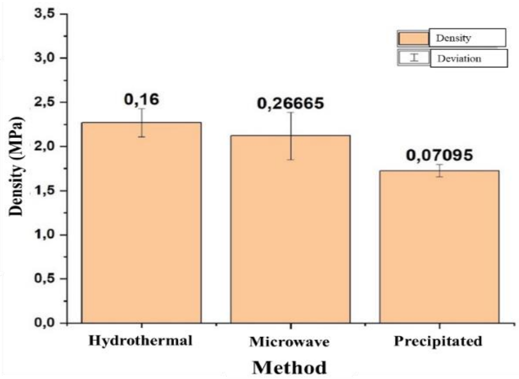

Figure 4. Density of HAp-TiO_2 Material Using Hydrothermal, Microwave and Precipitation Methods

Figure 4 shows the density of HAp-TiO_2 material using hydrothermal, microwave and precipitation methods. The results of the density data presented show that the hydrothermal method has the highest density of 2.27 MPa with a deviation of 0.16. The microwave method has a slightly lower density of 2.12 MPa. Meanwhile, the precipitation method has the lowest density, 1.73 MPa. Overall, the hydrothermal and microwave methods tend to give higher density.

Hardness Test

Hardness test results for HAp-TiO_2 samples synthesized using hydrothermal, microwave and precipitation methods are presented in Figure 5.

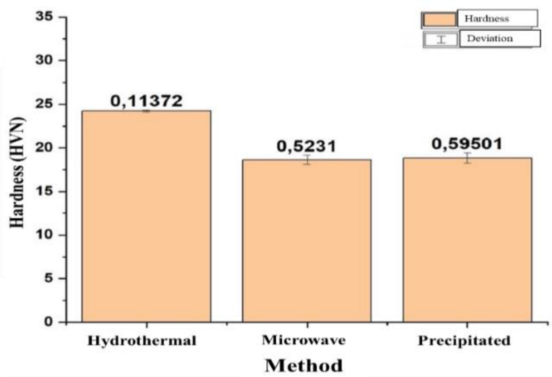

Figure 5. Hardness of HAp-TiO_2 material using hydrothermal, microwave and precipitation methods

Figure 5 shows the hardness of HAp-TiO_2 material using hydrothermal, microwave and precipitation methods. The results of the hardness data show that the highest hardness is 24.24 MPa. That shows that the hydrothermal method provides composite results with the highest hardness value compared to other methods. Furthermore, the microwave and precipitation methods have similar hardness values, 18.61 MPa and 18.83 MPa.

Digital Optical Microscope

Micrographic analysis is used to determine the morphology and particle size of the synthesized composite. The results of micrographic analysis on each sample are shown in Figure 6.

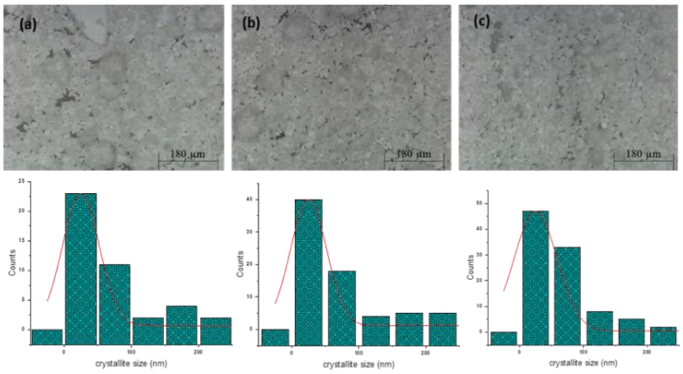

Figure 6. Micrographic analysis results of HAp-TiO_2 composites by (a) Hydrothermal, (b) Microwave and (c) Precipitation methods.

Figure 6 shows that the results of the HAp-TiO_2composite synthesized by hydrothermal, microwave and precipitation methods have a surface morphology in the form of refined grains with uniform size and pores. That is in line with the analysis conducted by Mona sari [19] that the surface morphology of the material that can be used for bone implants is in the form of grains with uniform size and has pores as a place for tissue entry into the implant so that it allows the process of bone regeneration.

CONCLUSIONS

The synthesis of HAp-TiO_2 composites, using hydrothermal, microwave and precipitation methods, has been successfully carried out. The results of FTIR analysis showed PO_4^{-3} groups at wave numbers 567-1091 cm^{-1}, -OH and -NH groups 3444-3448 cm^{-1}, and Ti-O groups at 567-632 cm^{-1}. XRD characterization of HAp-TiO_2 shows diffraction at $2\theta = 26.67^o$ and crystal size in hydrothermal method 80.20 nm, microwave 71.47 nm and precipitation 73.28 nm. The results of the evaluation of mechanical properties obtained by the compressive strength test using UTM on HAp-TiO_2 materials synthesized using hydrothermal, microwave and precipitation methods were 6.07, 5.08 and 5.19 MPa, respectively. The density results were 2.27, 2.12, and 1.73, respectively. The hardness test results were 24.24, 18.61, and 18.83 MPa, respectively. Digital optical microscope analysis shows that the HAp-TiO_2 material has a surface morphology of refined grains with uniform size and pores.

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