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# Characterization and Synthesis Hydroxyapatite from Scallop Mussel Shells Prepared by the Microwave-Assisted Precipitation Methods

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## *ABSTRACT*

*The need for bone implants is constantly increasing every year as the population of older people, accidents, and bone diseases increase. Various solutions, such as autograft, allograft, and artificial endoprosthesis, have been developed by researchers. Ceramic or bio-ceramic materials become attractive as bone implants due to their high biocompatibility. One of the most used bio-ceramics today is hydroxyapatite (HAp). Hydroxyapatite is one of the biomaterials that are widely used as biomedical materials, such as bone fillers, bioactive implant coatings, bone tissue repair systems and drug distribution. Kabupaten Gresik is famous for its fisheries, including the scallop mussel shells. The scallop mussel shells (Ruditapes philippinarum) is a species of shells that is widely cultivated in the Kabupaten Gresik. Using scallop mussel shell wastes as hydroxyapatite raw material can reduce shell waste volumes and production costs. Several methods for producing hydroxyapatites include hydrothermal, sol-gel, mechanochemical, wet chemical and microwave methods. Hydroxyapatite synthesis with the microwave method has the advantages of efficient heating, environmentally friendly and economical. This study aims to characterize and synthesize hydroxyapatite using scallop mussel shell waste prepared using microwave-assisted precipitation methods in the Gresik Regency. The research was carried out to convert the scallop mussel shells resulting from calcination with the crystal phase* $Ca(OH)_2$ *into hydroxyapatite using the microwave method. The synthesis process with the method of microwaves at the power of 450 watts for 2.5 minutes. From the synthesis results, hydroxyapatite was then characterized by XRD and SEM tests. The XRD tests conducted formed 99.1% crystallinity of hydroxyapatite.*

**Keywords:** *Bioceramic, Biomaterial, Hydroxyapatite, Microwave, Scallop Mussel Shells.*

## Introduction

Hydroxyapatite (HAp) is one of the biomaterials used as biomedical materials, such as bone fillers, bioactive implant coatings, bone tissue repair, and drug delivery systems [1, 2]. This can be applied because of the properties of HAp, biocompatibility, bioactive, and osteoconductive. Biocompatibility is the ability of a material to interact with living cells/tissues or metabolic systems that do not cause toxicity, injury or immune reactions when functioning at a specific site. Biocompatibility determines whether the material can be used in the body, in addition to its physical and chemical properties, ease of process, and affordable price.

Hydroxyapatite has the chemical formula $Ca_{10}(PO_4)_6(OH)_2$ that can accelerate the healing of damaged bone tissue because it is easily absorbed by bone tissue (immunogenic) and is nontoxicity, non-inflammation, and chemical content that is almost the same as bone. Cattle bones, mussel shells, fish scales, and fish bones have physicochemical properties similar to human apatite bones so that they can increase potency as HAp synthesis materials [3]. Data from the Badan Pusat Statistika (BPS) in 2022 shows that Indonesia has imported 161 billion kg of hydroxyapatite [4]. Research on hydroxyapatite in Indonesia needs to be done to reduce the number of imports so far. Indonesia, which has abundant marine products, can become an independent producer because hydroxyapatite can be produced from natural raw materials such as shellfish waste [5–9].

Biomineral extraction from natural waste is the best method for producing HAp from biological sources. In addition to some excellent extract properties, it is economical and environmentally friendly [10]. Living organs have a high potential for HAp synthesis due to physicochemical properties similar to human apatite bone [11]. Making HAp using natural waste generally involves several hours of the heating process, during which bone organic matter is removed, leaving pure HAp as residue [12]. Some mussel shells contain 95-99% $CaCO_3$ by weight. Scallop mussel shells are capable of being used as calcium precursors in the manufacture of HAp. Ni and Ratner explain that the surface of nacre shell pieces can be transformed into HAp in phosphate buffer solutions at atmospheric temperature by surface reaction processes. The mineral phase of the surface of the nacre shells was found to change from the CaCO3 aragonite phase to the HAp phase. They found lesser amounts of nacre surface rapidly turned into HAp after one day of soaking in a buffer solution. Mussel shells are high in calcium which can be utilized into hydroxyapatite [13–19].

Rapid technological advances can increase productivity, shorten production time, and reduce production costs. There are several synthesis methods to produce hydroxyapatite, such as mechanochemical, microwave, precipitation, and hydrothermal. Each synthesis method will have advantages and disadvantages in hydroxyapatite [20–25]. The hydroxyapatite synthesis method using microwaves is an effective method because of its short synthesis time, simplicity, efficiency, and ease of repetition, and it can be optimized for mass production [12, 26–28].

Microwave radiation provides an efficient, environmentally friendly heating method due to its increased reaction kinetics and rapid preheating. The dipole interaction between water molecules and the solvent in the microwave causes the temperature and pressure of the solvent to rise, resulting in diffusion from the sample to the solvent with a high extraction speed. Extraction conditions for natural materials can vary depending on several parameters, such as the solvent used, shaking, extraction time, solute/solvent ratio and power used. Compared with conventional heating methods, microwave synthesis methods require a shorter time to obtain good hydroxyapatite with high purity, homogeneity, and smaller crystal size [29–31]. The small size of hydroxyapatite crystals is beneficial in the implant process because it has a compression effect on cancer cells and can penetrate the cell membrane into the cytoplasm [32]. Hydroxyapatite formed by microwave has an intertwined fiber surface covered with hydroxyapatite crystals, while on the fiber surface of hydroxyapatite by conventional hydrothermal methods, hydroxyapatite crystals formed are found scattered and more significant. The size of the crystals formed depends on the nucleation balance and the crystal growth rate. Smaller crystals are formed when the nucleation rate is more dominant than the crystal growth rate [26].

The characterization used to determine the hydroxyapatite formed is XRD and SEM testing. X-ray diffraction (XRD) is now a common technique for studying crystal structure and atomic distance. XRD is based on the constructive interference of monochromatic X-rays and crystalline samples. These X-rays are produced by cathode-ray tubes, filtered to produce monochromatic radiation, collected to concentrate, and directed to the sample, as described in [33]. Scanning Electron Microscope (SEM) is a device that utilizes a centralized electron beam to obtain information. High-resolution and three-dimensional images are generated by SEM and present topography, morphology, and composition information. SEM provides detailed surface data of solid samples. A beam of low-energy electrons is radiated onto the specimen and into the material, emitting photons and electrons from or around the sample surface. After this interaction, the electrons scattered from the surface can be analysed with various detectors that provide topographic, morphological and compositional information regarding the sample surface [34, 35]. This study aims to characterize and synthesize hydroxyapatite by utilizing scallop mussel shell waste prepared using microwave-assisted precipitation methods in the Gresik Regency.

## Research Methods

The method used in this study is a laboratory experiment that includes two stages. The first stage was calcined experiments on scallop mussel shells, and the second stage was HAp synthesis from calcined shells. The materials used in this study were scallop mussel shells and diammonium phosphate (Merck). Figure 1 describes the stages of the hydroxyapatite synthesis process that have been conducted. The first stage for the scallop mussel shells is cleaned and then crushed using ball milling and in a mesh size of two hundred. The next step is the calcination process of scallop mussel shells at a temperature of 900°C for 5 hours.

The hydroxyapatite synthesis process begins by mixing calcined scallop mussel shells with diammonium phosphate. The mixing process is conducted by titration method, where diammonium phosphate is dripped with a burette and assisted by a magnetic stirrer for the mixing process to keep the solution homogeneous. Hydroxyapatite synthesis was conducted with a microwave SHARP 728 S IN with a power setting of 450 watts for 2.5 minutes. After the synthesis process, hydroxyapatite is washed using aquadest to neutralize the pH value and then dried in an oven at a temperature of 110°C for 2 hours. Material characterization is conducted by conducting XRD and SEM testing. Tests were performed on scallop mussel shells before calcining, after calcining and after synthesis into hydroxyapatite. XRD testing is performed to determine the characterization of the crystal structure crystal size of the sample.

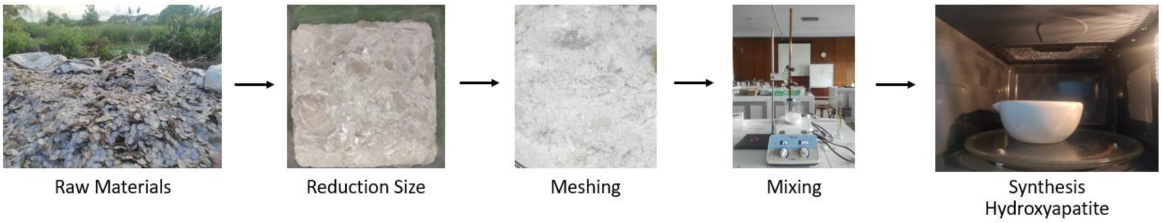


Figure 1. The Process of Synthesis of Hydroxyapatite from Scallop Mussel Shells.

## Results and Discussion

Figure 2 shows the XRD testing of scallop mussel shells, calcined scallop mussel shells, and hydroxyapatite synthesis results. From the XRD test chart was obtained and then analysed with high score plus software. Analysis with high score plus software using the Rietveld method of the dominant peak was then compared with COD (Crystallography Open Database) data. The COD code numbers used are calcite (96-101-0929), vaterite (96-150-8971), aragonite (96-900-0227), porlandite (96-100-1770), and hydroxyapatite (96-900-2214).

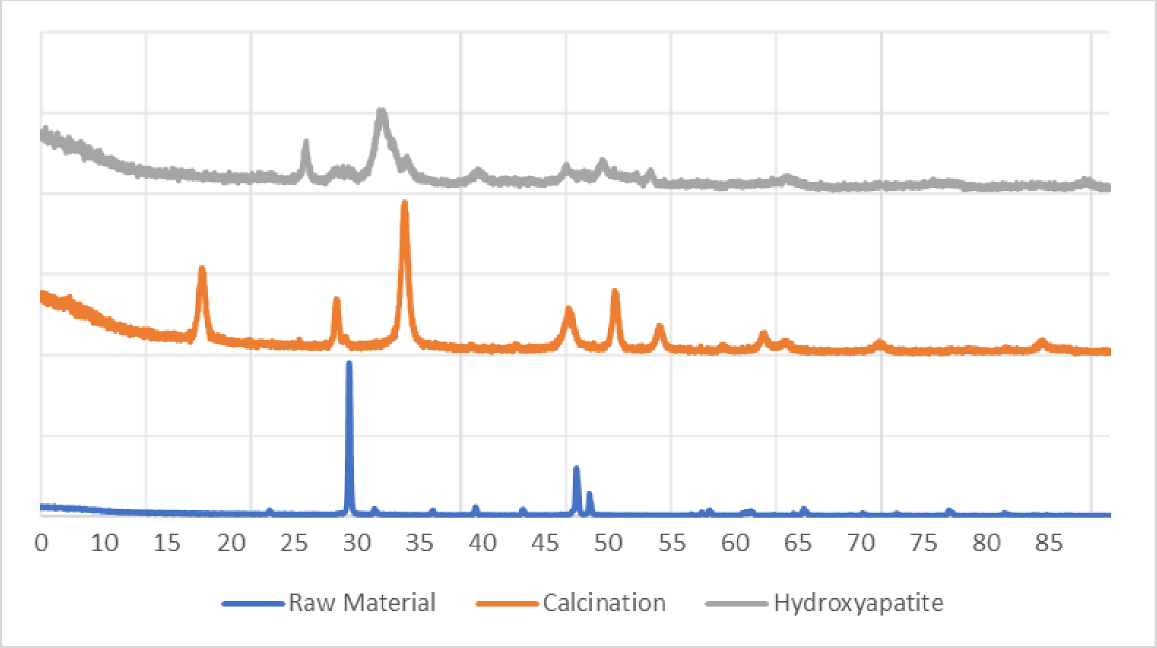


Figure 2. XRD Graph

Figure 3 shows the results of the analysis using high score plus software. The figure shows that the calcite phase dominates the scallop mussel shell with a crystallinity percentage of 66.3%. Meanwhile, the aragonite phase produces a crystallinity percentage of 33.7%, and no vaterite phase is formed. This shows that scallop mussel shells have the potential to be the basic material of hydroxyapatite because the calcite phase contained is quite high [18].

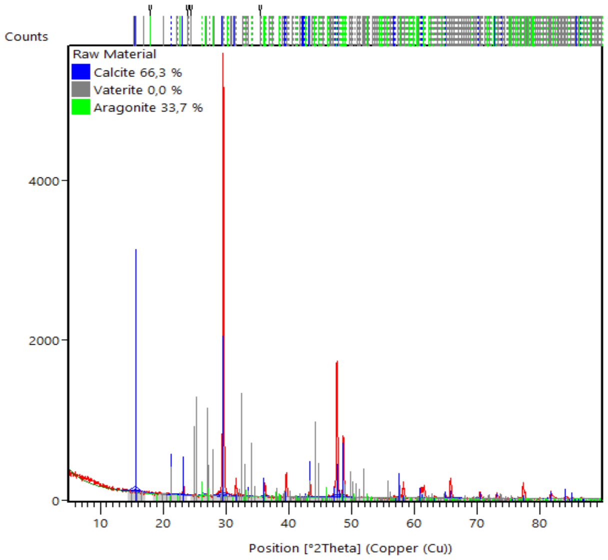


Figure 3. XRD analysis results of scallop mussel shells.

The calcination process aims to remove organic components in a material, increasing the content of inorganic elements, especially Calcium (Ca) [36]. Calcining was conducted using the Furnace Chamber Thermolyne F6010 furnace at the Chemical Engineering Laboratory of the University of Muhammadiyah Gresik. The calcining temperature used was 900°C for 5 hours, this is because the temperature is considered the optimum temperature to remove organic compounds contained in green mussel shell powder [16, 36].

Figure 4 shows the porlandite phase formed after the scallop mussel shells were calcined. It was found that the porlandite phase dominated with a percent crystallinity of 98.1%. While the calcite phase produces a crystallinity percent of 0.6%, the aragonite phase with a crystallinity percent of 1.3% and no vaterite phase is formed. The formation of Ca(OH) 2 is possible from water molecules in the air absorbed on the surface of CaO during the cooling process in the furnace because CaO is hygroscopic [37].

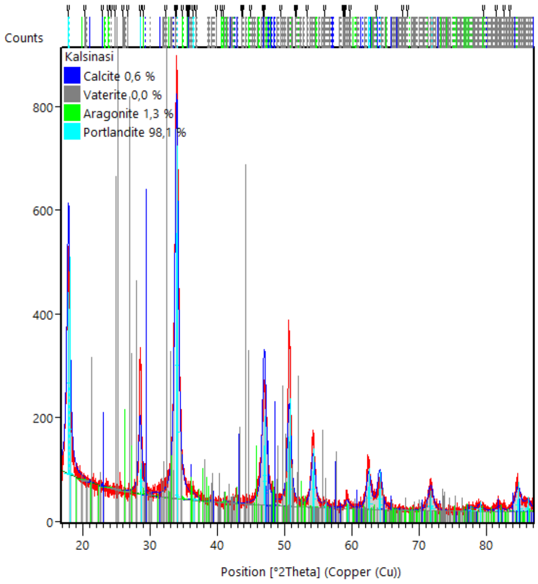


Figure 4. XRD Analysis Results of Calcination Scallop Mussel Shells.

From the results of the synthesis that has been carried out and analysts with high score plus software, the percent crystallinity of hydroxyapatite is formed at 99.1%. The porlandite phase has a crystallinity percentage of 0.9% after synthesis, as shown in Figure 5. The formation of the dominant hydroxyapatite phase proves that the hydroxyapatite synthesis method by microwave method is successfully carried out in a shorter time. The size of the hydroxyapatite crystals produced after the synthesis process is 10.35 nm. Hydroxyapatite produced from microwaves has a smaller crystal size than other methods of hydroxyapatite synthesis. The small crystal size has advantages in the application of hydroxyapatite during the implant process.

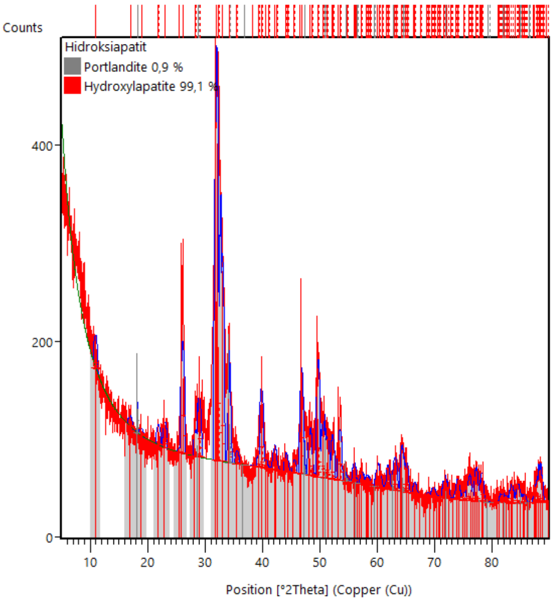
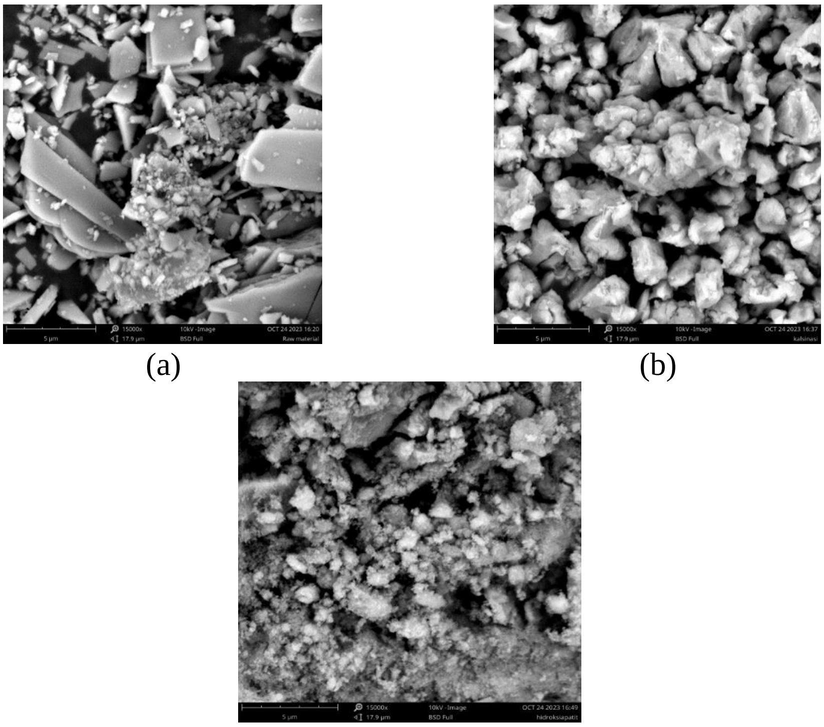


Figure 5. Analysis results of hydroxyapatite synthesis process

Figure 6 shows the SEM test results of scallop mussel shells, calcined scallop mussel shells, and results of hydroxyapatite synthesis by microwave method. Observations were made with magnifications of three thousand times, ten thousand times and twenty thousand times. The SEM images obtained were then compared with Sadat Shojai's journal to determine the characteristics of hydroxyapatite made from green mussel shells [6]. The crystal shape of the scallop mussel shells has not been processed like a rod. On the calcined of scallop mussel shells formed crystal morphology like flowers. Meanwhile, the crystalline form of hydroxyapatite synthesis looks spherical, has various dimensions, and goes through agglomeration. The various shapes of crystals occur because more than one phase is contained in hydroxyapatite.



(a) (b)

(c)

Figure 6. Morphology of crystals formed on (a) scallop mussel shells, (b) scallop mussel shells after calcination, and (c) hydroxyapatite synthesis.

Research has been done on the synthesis of hydroxyapatite and similar materials using microwave-assisted precipitation methods. This method has the benefit of being quick and effective in producing high-purity products with distinct properties. For example, a study that used a microwave-assisted precipitation method to synthesize carbonated hydroxyapatite (CHAp) discovered that the resulting powder had good morphology and crystallinity, making it appropriate for use in bone implant applications [38]. Another study investigated the microwave-assisted sol-gel synthesis of hydroxyapatite nanoparticles and reported that the method exhibited higher efficiency than conventional methods, leading to the preparation of HAp nanoparticles with desirable properties [39]. Furthermore, studies on the co-precipitation synthesis of high-purity β-tricalcium phosphate crystalline powders with microwave assistance have been conducted, indicating the potential of microwave-assisted techniques for the synthesis of diverse calcium phosphate materials [40]. These findings highlight the effectiveness of microwave-assisted precipitation methods for the synthesis of HAp because it allows for the simple, reliable, efficient, and constant regulation of the reaction temperature [38].

The synthesis result of HAp from scallop mussel shells using the microwave method have a good result and can be applied for many things. Recently, HAp has also been studied for medical such as drug delivery, collagen stimulation, skin regeneration, sun protection in the pharmaceutical industry, water purification, wastewater treatment, and in other chemical, optical, and electronics industries, and has the potential to be anti-cancer drug [41,42]. From those studies, HAp can be used for various biomedical applications.

## Conclusion

Synthesis of hydroxyapatite by microwave method from scallop shells raw materials has been successfully carried out. A total of 99.1% crystallinity of hydroxyapatite is produced from the calcite, vaterite, and aragonite phases. The resulting crystal shape resembles a spherical, various shapes and undergoes agglomeration. In this study, it can be concluded that the microwave synthesis method takes a short time and is easy to do. In addition, scallop mussel shells also have the potential to be a source of biomaterials that can be applied in the medical world.

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