

Effect of CaO Ratio in Relation to $(\text{NH}_4)_2\text{HPO}_4$ and Temperature on Hydroxyapatite from Shells Using The Hydrothermal Method

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ABSTRACT

This study investigates the synthesis of hydroxyapatite (HA) from mussel shells using the hydrothermal method, focusing on the CaO to $(\text{NH}_4)_2\text{HPO}_4$ ratio and reaction temperature as critical parameters. Increasing seafood consumption in the Surabaya region has resulted in greater shell waste, necessitating the exploration of sustainable practices in utilizing this byproduct for bio-materials, particularly in bone implants. The research employs a hydrothermal approach due to its efficacy in controlling HA crystal morphology and enhancing crystallinity. Experimental findings indicate that the optimal yield of 69.64% occurs at a 1.7 Ca/P ratio and 96 °C, validating the importance of both parameters in achieving adequate material characteristics. XRF and XRD analyses confirm the synthesized HA's chemical composition and a high degree of crystallinity at 81.01%, exceeding the minimum requirements for biomedical applications. However, Ca/P ratios slightly above the standard range suggest incomplete reactions during synthesis. This study makes a new contribution by systematically evaluating the combined effects of the Ca/P precursor ratio and moderate hydrothermal temperature on the synthesis of hydroxyapatite derived from local shell waste in general. The results of this study demonstrate the potential of marine shell waste as a sustainable source of calcium for producing hydroxyapatite with high crystallinity suitable for biomaterial applications, thereby supporting the development of environmentally friendly biomaterials for medical use.

INTRODUCTION

Seafood consumption, particularly shellfish, in the Surabaya region reached 0.004 ounces per capita in 2021 and increased to 0.005 ounces per capita in 2022 (BPS, 2023). This increase in consumption has the potential to raise the amount of shellfish processing waste in the form of shells. Mussel shells contain a high amount of calcium, making them potentially useful as biomaterials, one of which is for bone implant materials (Safiera Andyana et al., 2021).

The high CaO content in mussel shells can be used as a raw material for the synthesis of hydroxyapatite. Hydroxyapatite is a biomaterial widely used in the medical field because its

composition is similar to the mineral component of human bones and teeth. This compound has the chemical formula $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ with a theoretical Ca/P molar ratio of 1.67, which is an important parameter in determining the purity of hydroxyapatite (Wijanarko & Irfa'i, 2023). The stable hexagonal unit cell of hydroxyapatite (HA), with $\text{P6}_3/\text{m}$ symmetry, contains two formula units of $\text{Ca}_5(\text{PO}_4)_3\text{OH}$. For simplicity, it is commonly represented as $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. Within each unit cell, there are two distinct crystallographic calcium sites, labeled Ca(I) and Ca(II). These calcium sites form a cationic channel, while phosphate (PO_4)⁻³ and hydroxide (OH)⁻ ions create an anionic channel. This unique crystallographic structure of HA allows for the substitution of various positive and negative ions in bone mineral, making bone a reservoir for essential elements in the human body (Joshi & Shah, 2023).

Mussel shells have a high CaO content of 99.34%, so they can be used as raw material for hydroxyapatite synthesis. Characterization of hydroxyapatite synthesized from clam shells shows good quality. The average particle size of hydroxyapatite is 7.968 nm and the crystallinity value of 87.27% meets the specifications for bone implant manufacturing (Ferdiansyah et al., 2023). The crystallite size was determined from XRD data by measuring the full width at half maximum (FWHM) of the diffraction peaks, converting the values into radians, and applying the Scherrer equation (Pramono et al., 2024).

The crystal structure of hydroxyapatite, which is similar to bone, can be used as a bone implant because it has bioactive, biocompatible, and bioresorbable properties. Bioactive characteristics indicate that hydroxyapatite can trigger certain biological reactions. Biocompatible characteristics indicate that hydroxyapatite has the ability to adapt to bone tissue. The chemical formula for hydroxyapatite is $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ with a hexagonal structure. The standard for hydroxyapatite products according to ISO 13779-2:2018 (E) (International Organization for Standardization) and SNI 3 (Indonesian National Standard) is as follows:

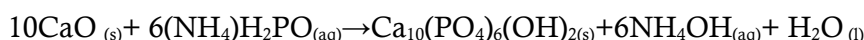
Table 1. Hydroxyapatite Parameters

Parameters	SNI 3	ISO 13779-2:2018 (E)
Calcium to phosphorus ratio (Ca/P)	$1,66 \leq \text{Ca/P} \leq 1,71$	$1,61 \leq \text{Ca/P} \leq 1,76$
Crystallinity ratio	$\geq 95\%$	$\geq 45\%$

Hydroxyapatite can be synthesized using several methods, including hydrothermal methods. Hydrothermal method has advantages because it can produce hydroxyapatite crystals with more controlled particle size and morphology, as well as better crystallinity compared to other synthesis methods (Wijanarko & Irfa'i, 2023). This method is carried out by reacting two materials in a closed container at a certain temperature. The process uses raw materials consisting of materials containing calcium sources and materials containing phosphate. This technique will undergo two main reactions, namely the decomposition of CaCO_3 and the synthesis of hydroxyapatite. The first reaction occurs during the calcination process, as follows (Fahimah et al., 2014):



The synthesis reaction of hydroxyapatite between calcium sources and phosphate sources is as follows (Fahimah et al., 2014):



During the hydroxyapatite synthesis process, several factors influence the process, namely the reaction temperature; if it is too low or too high, the resulting morphological density will be lower (Wijanarko & Irfa'i, 2023). The solution pH during the synthesis process is maintained at around pH 10 because it affects the formation and stability of hydroxyapatite (Torres-Sanchez et al., 2017). The Ca:P ratio; if the calcium-to-phosphorus ratio exceeds the optimal value (1.67:1), this can lead to phosphorus deficiency, thereby reducing yield. If the ratio is lower, it causes

phosphorus excess, potentially reducing both yield and product quality (Fahimah et al., 2014). Stirring speed, where the stirring speed in hydroxyapatite production affects the yield and crystallinity percentage of the hydroxyapatite product (Alhaq & Irfa'i, 2024). Reaction time in hydroxyapatite production affects the Ca/P ratio and the resulting crystallinity (Haruda et al., 2016).

Previous studies have utilized various natural calcium sources for hydroxyapatite synthesis. Khoirudin and Zultiniar (2015) used blood cockle shells through the hydrothermal method and obtained a Ca/P ratio of 1.99, which exceeds the standard value of 1.67. Safiera Andyana et al. (2021) synthesized hydroxyapatite from green mussel shells using the precipitation method and achieved the highest purity of 74%. In addition, Anggresani et al. (2020) reported that a Ca/P ratio of 1.67 produced hydroxyapatite with the best characteristics.

Various studies have shown that the Ca/P ratio and reaction temperature are important parameters that influence the purity and characteristics of the resulting hydroxyapatite. By utilizing mussel shells as a natural calcium-rich raw material, this study focuses on optimizing the parameters of precursor ratio and reaction temperature. Therefore, this research aims to determine the effect of the CaO to $(\text{NH}_4)\text{H}_2\text{PO}_4$ ratio and reaction temperature on the synthesis of hydroxyapatite from mussel shells using the hydrothermal method.

RESEARCH METHODS

Materials and Equipment

The research was conducted at the Chemical Engineering Laboratory of Universitas Pembangunan “Veteran” Jawa Timur, while samples were tested at the Faculty of Mathematics and Natural Sciences of Universitas Negeri Malang (UM). The main equipment used included a furnace, analytical balance, reagent bottles, mortar, pestle, sieve, beaker glass, measuring cup, stirring rod, and oven.

The raw material was red mussel shells obtained from the Ciroyom market in the Bandung area. Meanwhile, distilled water, ammonium dihydrogen phosphate, and sodium hydroxide were obtained from a chemical store.

Set of Tools

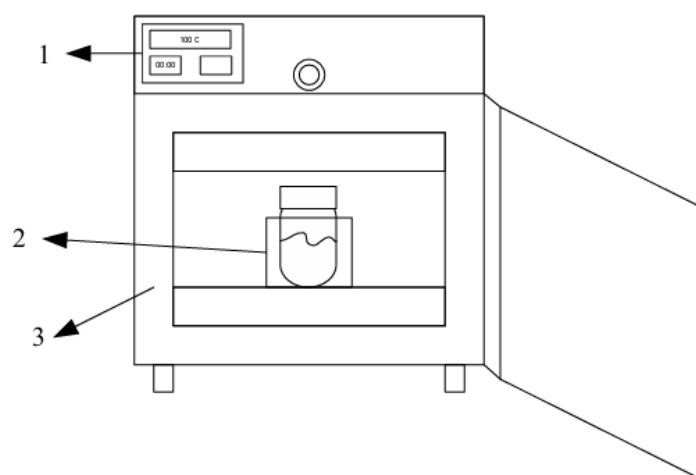


Figure 1. Hydrothermal Reaction Equipment System

The set of tool on the hydrothermal reaction includes a temperature and timer controls, material, and oven.

Variable

The conditions that remain constant in this study were a reaction time of 2 hours, a calcination time of 3 hours, a calcination temperature of 800°C, a drying temperature of 100°C, a mesh size of 100 mesh, and a stirring speed of 200 rpm.

The conditions used in this study were a Ca to P raw material ratio of 1,62; 1,64; 1,66; 1,68; 1,7 (Anggresani et al., 2020), and reaction temperatures of 72°C; 84°C; 96°C; 108°C; 120°C (Wijanarko & Irfa'i, 2023).

Research Process

The process of preparing the materials and combustion involves thoroughly washing the mussel shells with aquadest and drying them, then crushing the mussel shells using a mortar and pestle, followed by filtering the mussel shell powder using a 100-mesh sieve. After that, 250 grams of mussel shells are weighed, and the final process is incineration at a temperature of 8000°C for 3 hours using a furnace.

The hydroxyapatite synthesis process involves weighing $(\text{NH}_4)_2\text{HPO}_4$ and CaO according to the specified variables, dissolve $(\text{NH}_4)_2\text{HPO}_4$ in aquadest according to the variable, mix the calcined CaO powder according to the variable into the $(\text{NH}_4)_2\text{HPO}_4$ solution, then adjust the pH to a range of 11-12 by adding NaOH, place the mixture in an oven in a laboratory bottle to undergo a hydrothermal reaction at a variable temperature for 2 hours, then cool naturally and wash with distilled water until the pH reaches 7-8, and the final process is drying in an oven at a temperature of 100°C until the weight is constant

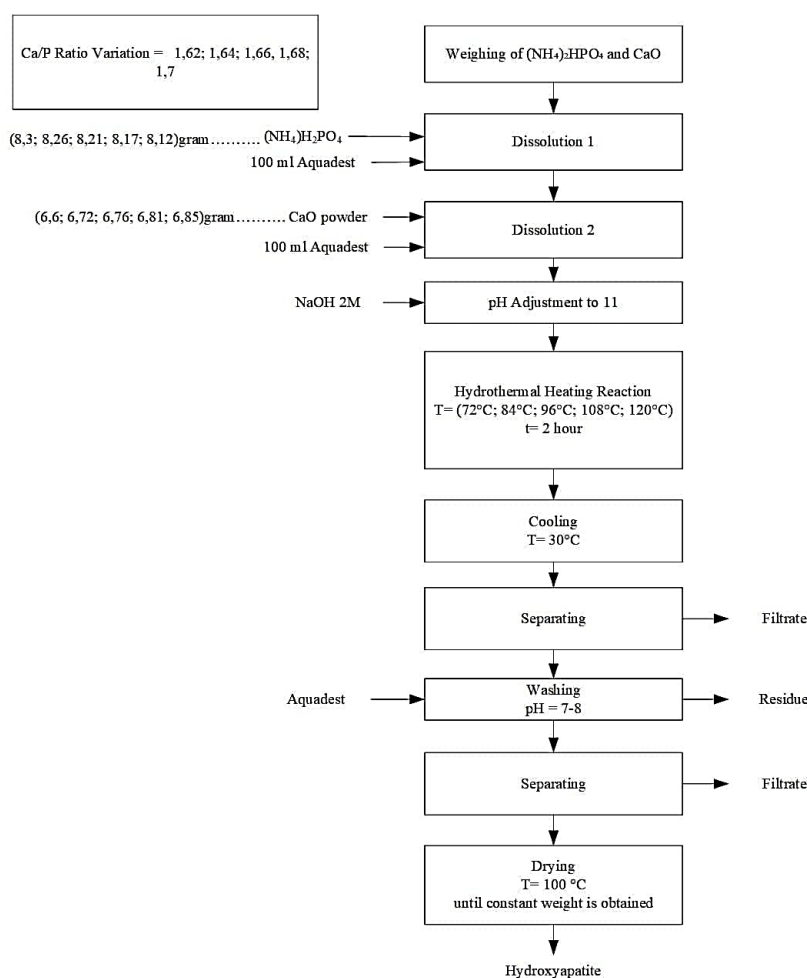


Figure 2. Hydroxyapatite synthesis flow diagram

RESULTS AND DISCUSSION

Effect of Reaction Temperature on the Percentage Yield of Hydroxyapatite

Based on figure 3, indicates that reaction temperature influences the percentage yield of hydroxyapatite. Increasing the reaction temperature up to 96 °C enhances the formation rate of hydroxyapatite, as indicated by the higher yield obtained. This phenomenon is consistent with reaction kinetics theory, where higher temperatures increase molecular collisions and accelerate reaction rates. According to previous research, higher temperatures promote the formation of hydroxyapatite crystals by improving ion mobility in the solution (Ikhsan et al., 2018). However, at temperatures above 96 °C, the yield does not increase significantly, which may be attributed to incomplete reactions or the formation of secondary phases. This indicates that excessively high temperatures do not always favor hydroxyapatite formation.

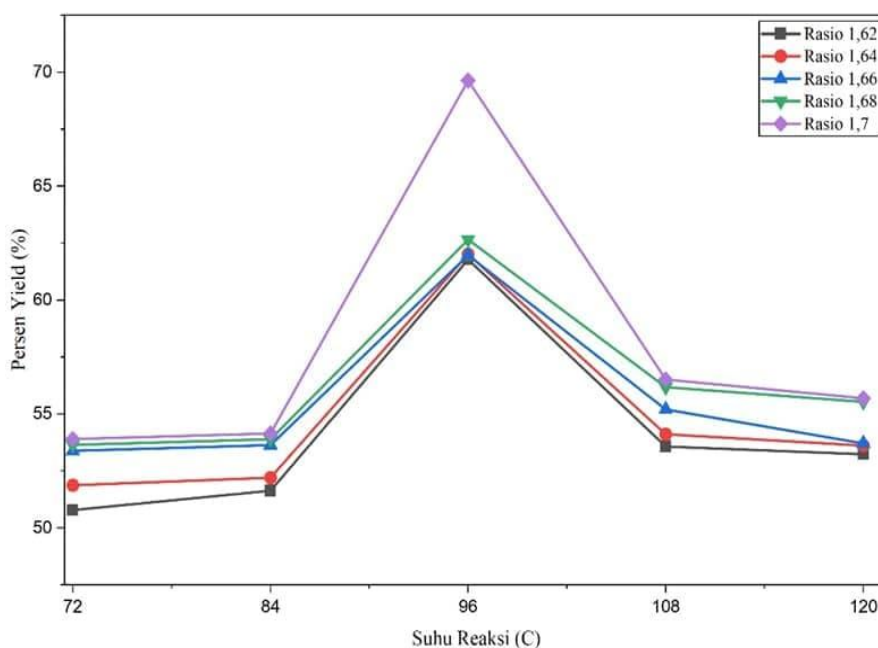


Figure 3. The effect of temperature variable on the percent yield of hydroxyapatite

Effect of $\text{CaO}:(\text{NH}_4)\text{H}_2\text{PO}_4$ Ratio on Hydroxyapatite Yield (%)

Based on figure 4, the increase in the Ca/P ratio leads to a higher hydroxyapatite yield, indicating that calcium availability plays a crucial role in the formation process. This trend is consistent with the stoichiometric principle of hydroxyapatite synthesis, where sufficient calcium ions are required to react with phosphate ions to form $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. At a reaction temperature of 96 °C, the system reaches optimal conditions, allowing the reaction to proceed more effectively due to enhanced ion mobility and reaction kinetics, which results in a more significant increase in yield compared to other temperatures, with the highest yield of 69.64% obtained at a Ca/P ratio of 1.7.

A previous study using blood cockle shells as the raw material for hydroxyapatite synthesis through a low-temperature hydrothermal method which reported that increasing the Ca/P ratio improved hydroxyapatite yield, reaching 77.73% at a ratio of 2.67. However, excessively high Ca/P ratios may lead to the presence of calcium-rich phases or unreacted CaO, which can reduce the purity of the synthesized hydroxyapatite, indicating that although increasing the Ca/P ratio enhances yield, it does not necessarily guarantee the formation of hydroxyapatite with the desired chemical composition and therefore requires careful optimization (Putri et al., 2015).

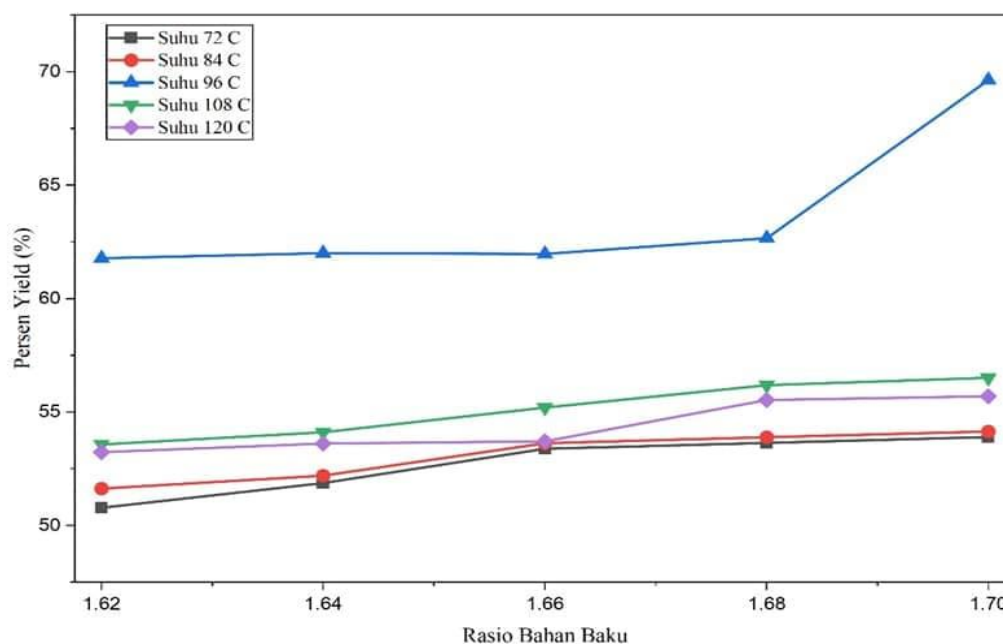


Figure 4. Effect of raw material ratio on hydroxyapatite yield percentage

XRF Analysis

XRF analysis conducted using the XRF (X-Ray Fluorescence) method to determine the elements contained in the raw materials and hydroxyapatite products produced from mussel shell materials. The Ca content of the raw material was examined to determine whether it was suitable for use as a raw material for hydroxyapatite production. In addition, the P_2O_5 and CaO percentage content was evaluated to determine the purity of the Ca/P ratio in the hydroxyapatite product.

Table 2. XRF Result of raw material from Mussel Shells

Component	Percentage (%)
S	0,06%%
Ca	97,62%
Ti	0,07%
Mn	0,086%
Fe	1,13%
Sr	0,56%
Mo	0,0%
Ba	0,2%
Lu	0,22%

Based on the results of the XRF analysis of the raw materials above, the calcium (Ca) content is very high at 97.62%. The minimum Ca content of raw materials for the formation of hydroxyapatite is 39.68% and the phosphorus content is 18% (Tutut Ummul Habibah et al., 2022). This indicates that mussel shells can be used as raw material in the production of hydroxyapatite.

Table 3. XRF Result of Hydroxyapatite from Mussel Shells Ca/P Ratio 1.62

Component	Percentage (%)
P ₂ O ₅	24,3%
CaO	74,49%
TiO ₂	0,05%
MnO	0,041%
Fe ₂ O ₃	0,645%
CuO	0,036%
SrO	0,31%
BaO	0,1%

Table 4. XRF Result of Hydroxyapatite from Mussel Shells Ca/P Ratio 1.7

Component	Percentage (%)
P ₂ O ₅	35,6%
CaO	62,8%
TiO ₂	0,04%
MnO	0%
Fe ₂ O ₃	0,64%
CuO	0%
SrO	0,17%
BaO	0,4%
Cr ₂ O ₃	0,11%
NiO	0,056%
Yb ₂ O ₃	0,2%

XRF analysis showed that the Ca/P ratio purity reached 3.91 at a CaO to (NH₄)H₂PO₄ ratio of 1.62 at a reaction temperature of 96 °C. Meanwhile, at a CaO to (NH₄)H₂PO₄ ratio of 1.7 under the same temperature, the Ca/P ratio purity obtained was 2.24. These results indicate that the synthesized hydroxyapatite has not yet met the standard Ca/P ratio for hydroxyapatite products, where the Indonesian National Standard (SNI) specifies $1.66 \leq \text{Ca/P} \leq 1.71$ and the ISO standard specifies $1.61 \leq \text{Ca/P} \leq 1.76$. The higher Ca/P ratio observed in this study may be attributed to the presence of CaO that did not completely react during the hydroxyapatite formation process, resulting in an excess calcium content in the synthesized product (Khoirudin & Zultiniar, 2015).

Although the initial molar ratio was strictly controlled, the presence of residual CaCO₃ impurities suggests that the hydrothermal process was not entirely effective in extracting and converting all CaCO₃ into hydroxyapatite, indicating incomplete reaction or insufficient process conditions (Fitriyana et al., 2023). In addition, the relatively low content of P₂O₅ suggests that a portion of the phosphorus was not fully incorporated into the hydroxyapatite crystal structure (Fahimah et al., 2014).

XRD Analysis

After conducting the XRF analysis, a further analysis was performed using XRD to determine the diffraction peaks, crystallinity, and crystallite size of the hydroxyapatite product. The test was carried out on the sample with a Ca/P product ratio closest to the standard based on the XRF analysis, namely the sample with a ratio of 1.7.

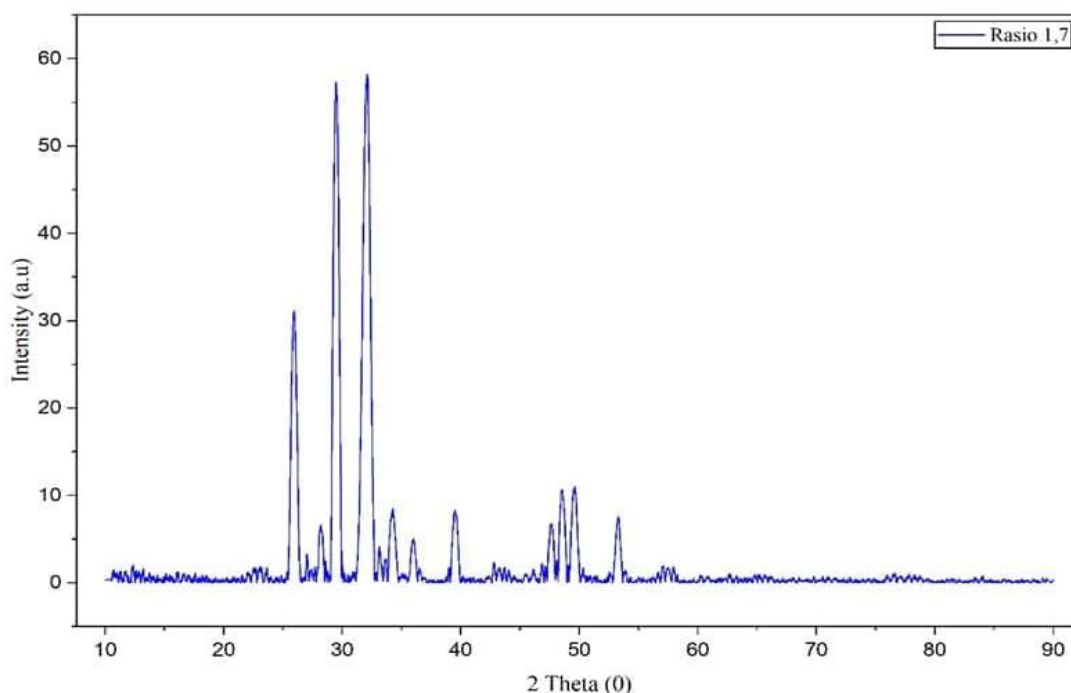


Figure 5. XRD Analysis Results of Hydroxyapatite from Mussel Shells with CaO to $(\text{NH}_4)\text{H}_2\text{PO}_4$ Ratio 1.7 at 96°C

Based on the XRD analysis graph, the results indicate the formation of the hydroxyapatite phase, which is evidenced by the appearance of characteristic diffraction peaks at 2θ around 25.9° corresponding to the (002) crystal plane, as well as a group of strong peaks within the range of $31.7\text{--}32.9^\circ$ associated with the (211), (112), and (300) crystal planes. These diffraction peaks are consistent with the standard data for hydroxyapatite listed in the JCPDS card No. 09-0432.

Based on the XRD data analysis, the crystalline area obtained was 127.42, while the total area was 157.27, resulting in a crystallinity value of 81.01% for the sample. A previous study using calcite rock as the raw material reported a crystallinity value of 60.33% (Rahmawati et al., 2021). This result indicates that the synthesized hydroxyapatite possesses a highly ordered crystal structure compared to the previous study. Furthermore, according to ISO specifications (ISO 13779-2, 2000), hydroxyapatite used for biomedical applications should have a crystallinity degree greater than 45% to ensure adequate mechanical strength within bone tissue.

In the Scherrer equation, K represents the crystal shape factor with a value of 0.9, while λ is the wavelength of the X-ray radiation ($1.5406 \text{ \AA}$). The parameter β corresponds to the full width at half maximum (FWHM) expressed in radians, and θ denotes the diffraction angle. Using these parameters, the crystallite size of hydroxyapatite derived from mussel shells was calculated to be 120.39 nm. An increase in crystallite size generally leads to a more ordered and compact crystal structure, resulting in improved structural stability of the material (Syam et al., 2025).

CONCLUSION

Based on the results of this study, it can be concluded that increasing the reaction temperature accelerates the formation process of hydroxyapatite, which tends to increase the yield. In addition, a higher raw material ratio also results in a higher percentage yield of hydroxyapatite. The hydroxyapatite product obtained from mussel shell raw material using the hydrothermal method at Ca/P ratio variables of 1.62 and 1.7 and at a temperature of 96°C produced Ca/P ratios of 3.91 and 2.24, respectively. These values do not meet the standard Ca/P ratio for hydroxyapatite products, where the Indonesian National Standard (SNI) requires a Ca/P ratio of 1.66–1.71 and the ISO standard (1.61–1.76). The results of this study indicate that although hydroxyapatite can be synthesized from mussel shell waste using a hydrothermal method, further optimization of the

synthesis parameters is still required to achieve the ideal Ca/P ratio needed for biomedical applications. The results of this study contribute to the development of sustainable biomaterial production by demonstrating the potential for utilizing oyster shell waste as an alternative calcium source for hydroxyapatite synthesis. This study also provides a foundation for future studies to improve synthesis conditions to produce hydroxyapatite with properties that meet international standards for medical and biomaterial applications.

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