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The Role of Hydrothermal Time in Determining the Phase Purity and Compressive Strength of Biogenic Hydroxyapatite from *Meretrix meretrix* Clam Shell

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ABSTRACT

Biogenic hydroxyapatite (HA) can contain calcium hydroxide [$Ca(OH)_2$] as a residual unreacted phase. This study investigates the effect of synthesis duration on the phase purity and compressive strength of HA. HA was synthesized from Meretrix meretrix clam shell (MCS) via a hydrothermal method with reaction times of 15, 18, and 21 hours. Phase composition and purity of HA were characterized using X-ray diffraction (XRD). The results showed that at the 21-hour reaction time, the HA phase purity reached 98.85 %, while the residual $Ca(OH)_2$ content was 1.15 %. Mechanical properties were evaluated using a Universal Testing Machine (UTM), showing that the compressive strength of HA increased with longer synthesis durations. These findings demonstrate that optimizing hydrothermal synthesis time effectively enhances phase purity and compressive strength of HA, while also highlighting the potential of MCS as a sustainable and high-value biogenic source.

ABSTRAK

Hidroksiapatit (HA) biogenik dapat mengandung kalsium hidroksida [$Ca(OH)_2$] sebagai fase sisa yang tidak bereaksi. Penelitian ini mengkaji pengaruh durasi sintesis HA terhadap kemurnian fase dan kekuatan tekannya. HA disintesis dari cangkang kerang *Meretrix meretrix* (MCS) menggunakan metode hidrotermal dengan variasi waktu reaksi 15, 18, dan 21 jam. Karakterisasi fase dan kemurnian HA dilakukan menggunakan X-ray diffraction (XRD). Hasilnya menunjukkan bahwa pada variasi 21 jam kemurnian fase HA didapatkan sebesar 98,85 %, sementara kadar residu $Ca(OH)_2$ sebesar 1,15 %. Sifat mekanik HA dievaluasi menggunakan *Universal Testing Machine* (UTM) dengan hasil menunjukkan bahwa kekuatan tekan HA meningkat seiring dengan bertambahnya waktu sintesis. Temuan ini menegaskan bahwa optimasi waktu sintesis hidrotermal secara efektif meningkatkan kemurnian fase dan kuat tekan HA, sekaligus menunjukkan limbah MCS sebagai sumber biogenik bernilai tinggi dan berkelanjutan.

1. INTRODUCTION

Biogenic hydroxyapatite (HA) is a biomaterial synthesized from unused natural waste. This approach has received considerable attention as an alternative HA synthesis source due to its high availability, relatively low cost, and environmentally sustainable characteristics (Yusuf et al., 2019). Biogenic HA can be derived from a variety of sources, including shells, bones, and marine organisms, which not only support sustainability but also have been demonstrated enhancing biological performance of the material (Chitra et al., 2025). *Meretrix meretrix* clam shells (MCS) can be utilized as a precursor for biogenic HA synthesis due to their substantial calcium carbonate ($CaCO_3$) content, as $CaCO_3$ constitutes the primary and essential compound in biogenic HA

synthesis (Murugiah et al., 2021). The synthesis of biogenic HA using hydrothermal generally comprises the conversion of $CaCO_3$ into calcium oxide (CaO) by high-temperature calcination, succeeded by hydration in an aqueous environment to yield calcium hydroxide ($Ca(OH)_2$). The produced $Ca(OH)_2$ acts as a reactive precursor for HA production through a subsequent reaction with a phosphate source (Pridanti et al., 2020).

The phase purity and compressive strength of biogenic HA are significantly influenced by numerous synthesis factors and circumstances. Prior research indicates that HA produced from eggshells by the hydrothermal process had optimal phase purity at pH 9, achieving 97.8%, with residual $Ca(OH)_2$ at 1.4% and a secondary tricalcium phosphate (TCP) phase at 0.8% (Irwansyah et al., 2022). Hydrothermal synthesis of HA from buffalo bone at different calcination temperatures revealed the influence of temperature on phase purity, with the sample calcined at 1000°C achieving a purity of 88.99 % (Alsharif et al., 2023). Furthermore, alterations in calcination temperature have demonstrated an improvement in the compressive strength of biogenic HA, with pellets derived from mixed animal bones and sintered at 1100°C achieving 0.85 MPa (Obada et al., 2020). Additional essential parameters influencing compressive strength encompass particle size (600 µm) and compaction pressure (6 kN), which were found to produce optimal mechanical performance in HA produced from bovine bone (Abifarin et al., 2023). Although extensive research has addressed the effects of pH, calcination temperature, particle size, and compaction pressure on the phase purity and compressive strength of biogenic HA, the influence of hydrothermal reaction duration on these properties remains underexplored, thereby introducing novelty to this study.

This work systematically evaluates the impact of hydrothermal reaction duration on the phase purity and compressive strength of biogenic HA. Phase purity was evaluated by XRD, while compressive strength was measured using a UTM. This study aims to provide new insights into the correlation between synthesis parameters and the phase purity as well as the compressive strength properties of HA, with a particular focus on the role of reaction time in enhancing material performance.

2. METHOD

The MCS were rinsed with tap water to remove adhering contaminants and subsequently boiled for 1 h. After cooling, the samples were immersed in a 5% (w/v) NaOH solution for 24 h, followed by thorough rinsing with tap water. The treated samples were then dried under solar irradiation and finally in an oven at 100 °C until a constant mass was obtained. The dried MCS were pulverized with a grinder and then sifted through a 200-mesh sieve to get a more homogeneous particle size distribution. The prepared MCS powder was placed in a porcelain crucible and calcined in a furnace at 1000°C for 3 h. The obtained CaO powder was subsequently used as a precursor for HA synthesis.

HA was synthesized using a hydrothermal method following the our procedure reported previously (Arsyad et al., 2025). The CaO powder was mixed with a 2 molar of $(NH_4)\ H_2PO_4$ solution and stirred using a magnetic stirrer while the pH was adjusted to 11–12 by the addition of 3 molar of NH_4OH solution. The resulting suspension was then transferred into a hydrothermal autoclave reactor and subjected to heating at 100°C for synthesis durations of 15, 18, and 21 hours. After completion of the hydrothermal process, the suspension formed was filtered and washed with distilled water until neutral conditions were reached. Finally, the suspension was subsequently dried in an oven at 110 °C to obtain HA powder.

HA samples for compressive strength testing were prepared using epoxy resin as the binding matrix. Each sample contained 10% (w/w) HA, equivalent to 0.3 g, which was mixed with a pre-blended epoxy system composed of 2.7 g epoxy resin and 0.3 g hardener. The mixture was homogenized and molded into cylindrical specimens with a diameter of 8 mm and a height of 16 mm (Said et al., 2021). Then, the sample was dehydrated using a dehydrator at 60 °C for 4 hours. A control sample of pure epoxy resin without HA was also prepared under identical conditions for control sample.

Phase purity of HA was characterized using XRD. The measurements were performed using an X-ray diffractometer with Cu Kα radiation (λ = 1.5406 Å) operated at 40 kV and 30 mA. Data were collected over a diffraction angle range of 2θ = 5–90° with a total scanning time of approximately ±15 min. The obtained data were analyzed using HighScore plus software to identify the phase composition and estimate the percentage of the HA phase.

The mechanical properties of HA were evaluated through a compressive strength test using a UTM. The test was performed by applying a compressive load until specimen failure occurred at a crosshead speed of 5 mm/min. The obtained data were in the form of the maximum load, which was then used to determine the maximum compressive strength of each HA specimen. The compressive strength was calculated using the following equation (1):

$$\sigma = \frac{F}{A} \qquad (1)$$

Where σ denotes the compressive strength (MPa), F is the maximum applied load (N), and A represents the cross-sectional area of the specimen (mm^2).

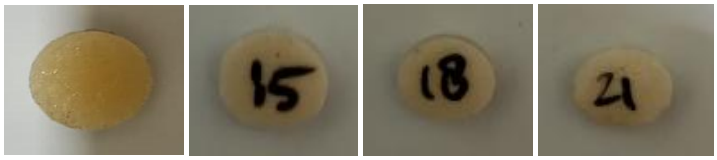
3. RESULT AND DISCUSSION

The raw materials and results of biogenic HA synthesis from MCS using the hydrothermal method with various reaction times are presented in Figure 1. The obtained HA appears as a fine white powder, indicating the presence of phosphate (PO_4) groups and suggesting the formation of HA (Nugroho et al., 2025). The white color indicates that the formed HA does not undergo significant transformation into other compounds and is relatively free from contaminants that may affect the visual appearance of the powder.

(a) (b) (c) (d)

Figure 1. Raw materials (a) and HA samples with different hydrothermal times: (b) 15 h, (c) 18 h, and (d) 21 h

The Figure 2 shows the physical appearance of HA specimens prepared for compressive strength testing, consisting of a control sample (epoxy) and HA samples with different hydrothermal times. Visually, all specimens exhibit a relatively uniform cylindrical shape, indicating that the molding process was well-controlled and consistent. The control sample appears clearer, whereas the addition of HA results in a whitish appearance, indicating the presence of HA particles within the epoxy matrix. In addition, no significant macroscopic defects such as cracks or large pores are observed, suggesting that the specimens possess high density and structural integrity. Therefore, the differences in compressive strength are primarily attributed to the variations in HA characteristics rather than imperfections in specimen geometry.



(a) (b) (c) (d)

Figure 2. Physical appearance of cylindrical specimens used for compressive strength testing: (a) control sample and HA-loaded epoxy samples (b) 15 h, (c) 18 h, and (d) 21 h

Figure 3 shows the XRD patterns of HA (▲) and $Ca(OH)_2$ (■) in all samples, as presented in Table 1, which confirms the formation of the HA phase through the appearance of characteristic peaks at $2\theta \approx 25.9°$, 31.7°, 32.2°, 32.9°, and 39.8°, corresponding to JCPDS No. 09-0432. The obtained peak positions are relatively consistent with slight shifts, which can be attributed to crystal strain or crystallite size effects due to variations in synthesis time (Arsyad et al., 2025). On the other hand, the presence of peaks at $2\theta \approx 18°$, 34°, 47°, 50°, and 54° indicates that the $Ca(OH)_2$ phase has not been fully transformed into HA and remains as a secondary phase, referring to JCPDS No. 44-1481.

Table 1. Observed 2θ values of HA and $Ca(OH)_2$ compared with standard JCPDS references.

Samples	2θ of HA (JCPDS No. 09-0432)					2θ of $Ca(OH)_2$ (JCPDS No. 44-1481)				
	1	2	3	4	5	1	2	3	4	5
JCPDS	25.9°	31.7°	32.2°	32.9°	39.8°	18°	34°	47.1°	50.8°	54.3°
15 h	25.9°	31.8°	32.2°	32.9°	39.8°	18.16°	34.17°	47.20°	50.85°	54.39°
18 h	26°	31.9°	32.3°	33°	39.9°	18.23°	34.27°	47.27°	50.93°	54.5°
21 h	25.8°	31.7°	32.1°	32.9°	39.9°	18.11°	34.13°	47.17°	50.81°	54.37°

The presence of the $Ca(OH)_2$ peak in the XRD pattern indicates that the hydrothermal temperature of 100°C is not sufficient to fully convert all $Ca(OH)_2$ into HA. At relatively low hydrothermal temperatures, the system does not have enough kinetic energy to drive the complete transformation of Ca^{2+} and OH^- ions into the HA crystal structure. As a result, the reaction kinetics are limited, and the crystallinity of the formed HA tends to be lower compared to that obtained at higher temperatures (Ryu et al., 2019). However, this condition can be

advantageous if the goal is to retain some $Ca(OH)_2$ phase within the material, for example, for applications that benefit from its basic nature or its chemical reactivity within the HA system. Previous studies have reported that $Ca(OH)_2$ can provide antibacterial properties, enhance bioactivity, and promote osteogenesis (von Hertzberg-Boelch et al., 2025), suggesting that the presence of $Ca(OH)_2$ in HA may offer additional functional advantages depending on the intended application.

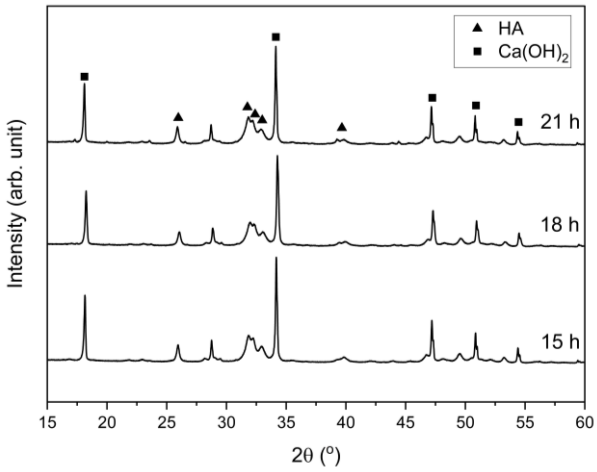


Figure 3. Diffractogram of HA synthesized with different reaction times

Figure 4 shows that the percentage of HA increases and $Ca(OH)_2$ decreases with synthesis time. The graph demonstrates the effect of hydrothermal reaction time on the phase composition changes of HA and $Ca(OH)_2$. As reaction time increases from 15 to 21 hours, HA content gradually rises from approximately 94.03% to 98.85%, while $Ca(OH)_2$ decreases from 5.97% to 1.15%. This trend indicates that $Ca(OH)_2$ is progressively converted to HA through the ongoing hydrothermal reaction process during this period. Longer reaction time provides greater opportunity for Ca^{2+} and OH^- ions to diffuse and react with phosphate sources, thereby promoting better HA crystal growth and reducing residual precursor phases. Thus, it can be concluded that the phase transformation process is time-dependent, with the optimal condition in this system achieved at 21 hours, yielding the highest HA purity with minimal $Ca(OH)_2$ residue. Other studies employing the precipitation method have similarly reported a positive correlation between reaction time and HA yield, with eggshell-derived precursors producing up to 82% HA after 4 hours of reaction time. This result indicates that prolonged reaction time promotes the formation and crystallinity of HA (Wahyuni et al., 2023).

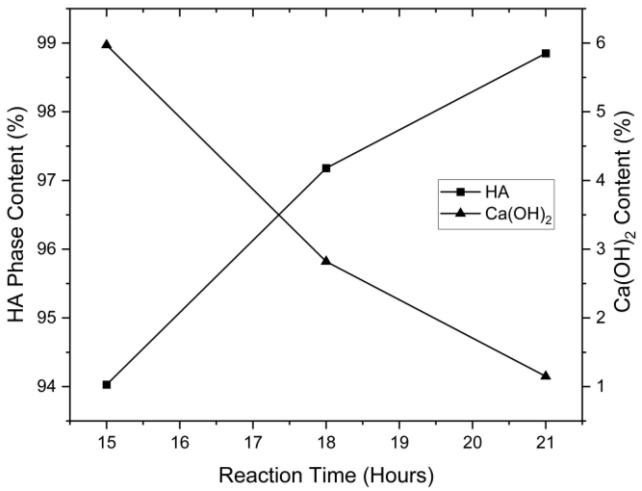


Figure 4. Hydrothermal time-dependent phase composition of HA

The compressive strength results (Figure 5) demonstrate a significant improvement with increasing hydrothermal reaction time, which is attributed to the higher HA content. In addition, the enhanced mechanical performance of HA samples embedded in epoxy is associated with strong interfacial bonding between the polymer matrix and HA particles, promoting more uniform load distribution and improved resistance to

deformation. In comparison, epoxy without HA tends to be stiffer and more brittle, resulting in lower strength values. Such trend suggests that the progressive growth of the HA phase over time contributes to enhanced densification and stronger interparticle bonding, thereby improving the mechanical properties of the material. Conversely, the relatively high presence of $Ca(OH)_2$ in sample 15 h reduces mechanical properties due to the incorporation of OH groups, indicating residual hydroxyl vibrations (Zakaria et al., 2018). Consequently, longer hydrothermal durations allow more complete conversion of $Ca(OH)_2$ into HA, minimizing the weakening effect of OH groups and increasing the compressive strength.

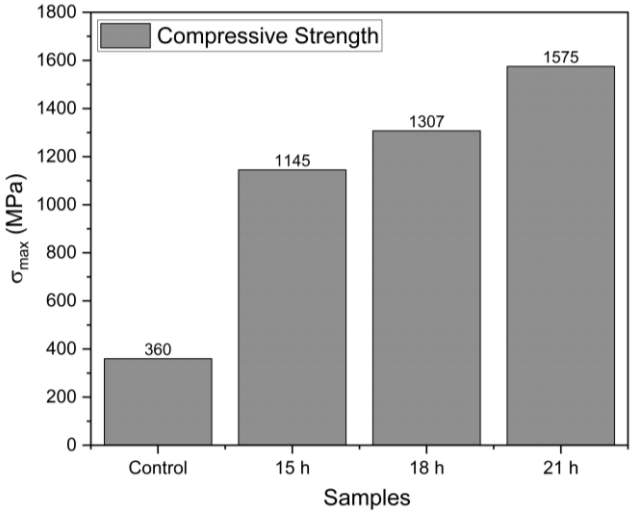


Figure 5. Compressive strength of epoxy resin (control) and HA samples (15, 18, and 21 h).

The control sample, consisting of epoxy resin without HA, exhibits a relatively low strength of approximately 360 MPa. In contrast, the 15-hour sample shows a sharp increase to 1145 MPa, which further rises to 1307 MPa at 18 hours and reaches the maximum value of 1575 MPa at 21 hours. The compressive strength value reaching ~1000 MPa is considered unrealistically high for HA. This is primarily attributed to the use of epoxy as a binding matrix, which significantly enhances load distribution and reduces porosity within the specimen. As a result, the measured strength reflects the combined mechanical contribution of the HA–epoxy system rather than the intrinsic properties of HA itself. In addition, dense, non-porous structures produced during sample preparation can further inflate compressive strength values, whereas pure HA, particularly in scaffold form, typically exhibits much lower mechanical strength due to its brittle nature and inherent porosity. Previous study scaffold based HA and epoxy exhibits a compressive strength of approximately 125 MPa (Kim et al., 2023), which is within the range of natural bone. Therefore, future work should focus on developing HA/epoxy scaffolds to obtain more representative compressive strength values.

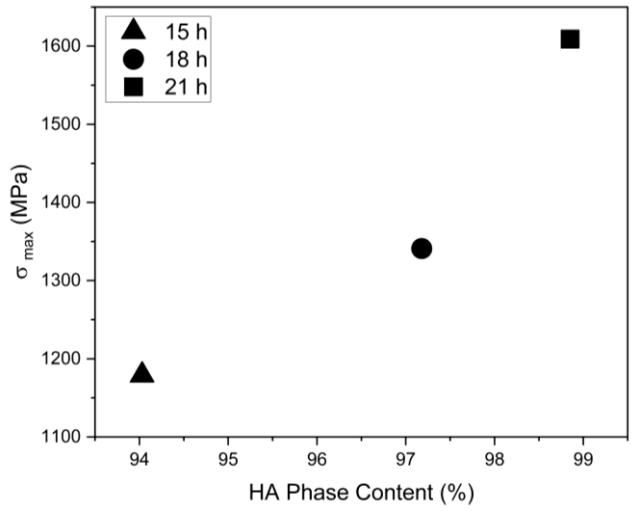


Figure 6. Positive correlation between HA phase and compressive strength.

The graph (Figure 6) demonstrates a positive correlation between HA phase content and compressive strength (σ_{max}), where the increase in HA purity is accompanied by a rise in compressive strength from. This trend indicates that the higher the HA phase fraction, the better the mechanical properties of the biogenic HA, which can be attributed to the reduction of secondary phases like $Ca(OH)_2$ that are mechanically weaker. In the 15-hour sample, the lower compressive strength is associated with reduced HA content and the likely presence of residual $Ca(OH)_2$, whereas in the 21-hour sample, the dominance of the HA phase results in a more compact structure capable of withstanding greater loads. Thus, synthesis duration plays a crucial role in improving HA phase purity, which directly impacts the enhancement of the material's compressive strength.

4. CONCLUSION AND RECOMMENDATION

Hydroxyapatite (HA) was successfully synthesized from *Meretrix meretrix* clam shells (MCS) via the hydrothermal method at varying reaction times (18, 21, and 24 h), with XRD confirming clear phase formation of HA. Longer synthesis durations increased HA phase content and reduced secondary phase of $Ca(OH)_2$. Compressive strength testing revealed a significant enhancement in mechanical performance with extended hydrothermal treatment, underscoring the critical role of synthesis time in optimizing both structural order and mechanical properties. For future studies, HA should be fabricated into porous scaffold structures using epoxy resin as a matrix to ensure that the mechanical evaluation more accurately reflects the intrinsic properties of HA. Moreover, synthesis parameters such as temperature, pH, and Ca/P ratio should be systematically varied to achieve complete phase transformation and provide a more comprehensive understanding of the structure–property relationship of HA for biomedical applications.

5. ACKNOWLEDGE

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