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Synthesis and Characterization of Hydroxyapatite from Crab Shell Waste (*Scylla Serrata*) Using the Precipitation Method

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Abstract
The utilization of crab shell waste as a calcium source represents a value-added material development strategy while simultaneously reducing environmental pollution from fishery waste. This study aimed to synthesize hydroxyapatite (HAp) derived from crab shell waste via the precipitation method and to evaluate the effect of calcination temperature (800, 900, 1000, 1100, and 1200 °C) and Na_2HPO_4 concentration (0.75, 1.3, 1.85, 2.4, and 2.95 M) on the resulting material characteristics. The crab shells were initially calcined to obtain CaO as the calcium source, followed by reaction with Na_2HPO_4 solution as the phosphate precursor to form hydroxyapatite. The synthesized product was subsequently dried and calcined according to the designated temperature variations. Characterization was carried out using XRF, XRD, and SEM analyses. XRF results indicated the dominance of Ca and P elements with a Ca/P ratio approaching the stoichiometric value of hydroxyapatite (1.67). XRD patterns confirmed the formation of crystalline HAp phases, with increasing peak intensity and sharpness at higher calcination temperatures, indicating an enhanced degree of crystallinity. Variations in Na_2HPO_4 concentration influenced phase purity and the Ca/P ratio. SEM analysis revealed sub-spherical particle morphology with a tendency toward agglomeration at higher temperatures due to sintering effects. Overall, the findings demonstrate that controlling calcination temperature and phosphate precursor concentration plays a critical role in determining the composition, crystallinity, and morphology of hydroxyapatite synthesized from crab shell waste.
Keywords: *crab shell waste, hydroxyapatite, precipitation method, calcination temperature, Ca/P ratio.*

Abstrak
Pemanfaatan limbah cangkang kepiting sebagai sumber kalsium merupakan salah satu upaya pengembangan material bernilai tambah sekaligus mengurangi dampak pencemaran lingkungan dari limbah perikanan. Penelitian ini bertujuan untuk mensintesis hidroksiapatit (HAp) berbasis limbah cangkang kepiting melalui metode presipitasi serta mengevaluasi pengaruh suhu kalsinasi (800, 900, 1000, 1100, dan 1200 °C) dan konsentrasi Na_2HPO_4 (0,75; 1,3; 1,85; 2,4; dan 2,95 M) terhadap karakteristik material yang dihasilkan. Cangkang kepiting terlebih dahulu dikalsinasi untuk memperoleh sumber CaO, kemudian direaksikan dengan larutan Na_2HPO_4 sebagai sumber fosfat untuk membentuk hidroksiapatit, dilanjutkan proses pengeringan dan kalsinasi sesuai variasi suhu yang ditentukan. Karakterisasi dilakukan menggunakan XRF, XRD, dan SEM. Hasil XRF menunjukkan dominasi unsur Ca dan P dengan rasio Ca/P mendekati nilai stoikiometri hidroksiapatit (1,67). Pola XRD mengonfirmasi terbentuknya fase kristalin HAp dengan peningkatan intensitas dan ketajaman puncak seiring kenaikan suhu, menandakan peningkatan derajat kristalinitas. Variasi konsentrasi Na_2HPO_4 memengaruhi kemurnian fase dan rasio Ca/P. Analisis SEM menunjukkan morfologi partikel berbentuk sub-spherical dengan kecenderungan aglomerasi pada suhu tinggi akibat proses sintering. Hasil penelitian menunjukkan bahwa pengendalian suhu kalsinasi dan konsentrasi prekursor fosfat berperan penting dalam menentukan komposisi, kristalinitas, dan morfologi HAp berbasis limbah cangkang kepiting.
Kata Kunci: *limbah cangkang kepiting, hidroksiapatit, presipitasi, suhu kalsinasi, rasio Ca/P*

1. Introduction

Hydroxyapatite (HAp), with the chemical formula $Ca_{10}(PO_4)_6(OH)_2$, is a calcium phosphate compound composed of calcium and phosphate elements and is recognized as the primary mineral component of human bones and teeth. Hydroxyapatite can be synthesized from both synthetic and natural calcium sources. Common synthetic calcium sources include CaO, $Ca(NO_3)_2$, $Ca(OH)_2$, $CaCO_3$ and $CaCl_2$, whereas one potential natural source is crab shell waste, which contains a high calcium content [1]. As a bioceramic material, hydroxyapatite has been widely applied in biomedical fields, such as bone tissue

regeneration, dental implant coatings, and as an active ingredient in toothpaste for sensitive teeth due to its biocompatible and bioactive properties [2].

The demand for hydroxyapatite in Indonesia still relies heavily on imported products. According to national statistical data in 2022, the total import volume of hydroxyapatite in Indonesia was significant, reflecting the high domestic market demand. The increasing price of imported hydroxyapatite is attributed to the inability of local producers to meet national demand [3]. This condition highlights the need to develop local raw materials as a more economical and sustainable alternative.

The fisheries and seafood processing industries generate a substantial amount of crab shell waste, reaching approximately 56,200 tons per year [4]. This waste is often underutilized and may cause environmental problems, particularly in coastal areas. Chemically, crab shells contain 53.70–78.40% calcium carbonate ($CaCO_3$), 15.06–23.90% protein, and 18.70–32.20% chitin [5]. The $CaCO_3$ content can be converted into CaO through calcination, which serves as a precursor for hydroxyapatite synthesis [6]. The utilization of crab shells as a calcium source for hydroxyapatite synthesis has been previously reported and demonstrated promising potential [7].

Various synthesis methods for hydroxyapatite derived from natural sources have been developed, including precipitation, sol–gel, and hydrothermal techniques. Hydrothermal synthesis using blue swimming crab shells at a sintering temperature of 700–800°C produced a crystallinity of 86.37% and a Ca/P ratio of 1.67 under optimum conditions at 800°C for 5 hours [8]. The precipitation method applied to chicken eggshells with a sintering temperature of 1000°C for 5 hours also yielded a Ca/P ratio of 1.67 [9]. Meanwhile, synthesis from pleco fish bones at a calcination temperature of 900°C for 7 hours resulted in a CaO content of 81.37% and a crystallite size of 32.39 nm [10]. Other studies have shown that variations in KH_2PO_4 concentration and calcination temperature significantly influence the crystallinity degree of hydroxyapatite in accordance with the JCPDS 09-0432 standard [11].

In this study, the precipitation method was selected due to its simplicity, cost-effectiveness, and ability to control key synthesis parameters such as pH, Ca/P ratio, reaction temperature, and calcination temperature. The calcination temperature range (800°C;900°C;1000°C;1100°C;1200°C) is expected to influence the degree of crystallinity, while variations in Na_2HPO_4 concentration (0,75;1,3;1,85;2,4;2.95 M) affect the Ca/P ratio and phase purity of the resulting hydroxyapatite. The pH was maintained under alkaline conditions pH 11 with a theoretical Ca/P ratio of 1.67 as a reference. This study aims to investigate the effect of calcination temperature and disodium phosphate concentration on the characteristics of hydroxyapatite synthesized from crab shell waste in order to obtain optimal crystallinity and Ca/P ratio. The synthesized hydroxyapatite is expected to serve as a potential biomaterial raw material, particularly for bone implant applications.

2. Materials and Methods

This study employed a laboratory experimental approach to synthesize and evaluate hydroxyapatite material derived from crab shell waste. The research began with washing the crab shell waste, which was then synthesized using the precipitation method. The synthesis process included acidification, precipitation, pH adjustment and aging, filtration, drying, and sintering to obtain hydroxyapatite with the desired characteristics.

The primary raw material used in this study was crab shell waste. The chemicals employed included disodium phosphate (Na_2HPO_4) as the phosphate ion source, hydrochloric acid (HCl) 37% as the acidifying agent to dissolve CaO, sodium hydroxide (NaOH) as the pH regulator, and distilled water as the solvent and washing medium. The equipment used consisted of a beaker glass, hot plate, magnetic stirrer, thermometer, oven, filter paper, glass funnel, stirring rod, mortar and pestle, storage container, measuring pipette, dropper pipette, pH meter, analytical balance, graduated cylinder, spatula, retort stand, clamp, burette, watch glass, and aluminum foil.

Research Procedures

Material Pre-treatment

Crab shells were first boiled for 1 hour to remove any remaining meat and adhering impurities. The shells were then washed under running water and naturally dried under sunlight for approximately ±2 days. The dried shells were crushed using a mortar and pestle and sieved through a 100-mesh sieve.

Calcination of Crab Shells

A total of 250 grams of crab shell powder was subsequently calcined in a furnace at temperatures of 800°C, 900°C, 1000°C, 1100°C, and 1200°C for 5 hours. The resulting CaO powder appeared white and was analyzed using the X-ray Fluorescence (XRF) method to determine the calcium content in the sample.

Synthesis of Hydroxyapatite by the Precipitation Method

The calcined CaO powder weighing 20 g was dissolved in 225 mL of 3 M HCl solution until completely dissolved, forming a calcium chloride ($CaCl_2$) solution. The solution was heated to 90°C, followed by the addition of Na_2HPO_4 solution at varying concentrations of 0.75; 1.3; 1.85; 2.4; and 2.95 M while stirring with a magnetic stirrer at 300 rpm for 1 hour. Subsequently, 1 M NaOH solution was gradually added until the pH reached 11 to create optimal alkaline conditions for hydroxyapatite formation. The mixture was then allowed to stand for 24 hours at room temperature.

The resulting precipitate was filtered and washed with distilled water to remove residual impurity ions such as Na^+ and Cl^-. The precipitate was then dried in an oven at 110°C for 3 hours to remove moisture content. The final step involved sintering at 900°C for 5 hours to enhance the crystallinity and structural stability of the hydroxyapatite. Material characterization was performed using X-ray Diffraction (XRD) to analyze the crystalline structure and Scanning Electron Microscopy (SEM) to examine surface morphology and elemental distribution. The characterization and experimental results were subsequently analyzed comparatively to determine the optimum synthesis conditions. The following illustrates the precipitation apparatus employed in this study.

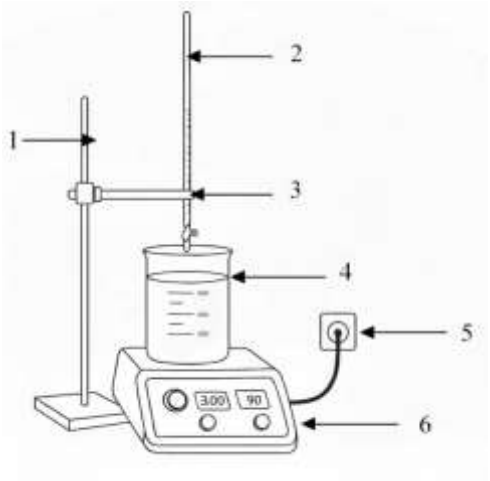

Fig.1: Experimental setup of the precipitation apparatus

Description:
1. Retort stand
2. Burette
3. Clamp
4. Beaker glass
5. Power outlet
6. Hot plate magnetic stirrer equipped with temperature control

3. Results and Discussion

Analysis of CaO Powder Content Derived from Calcined Crab Shells

The calcination stage is required to remove organic compounds and convert $CaCO_3$ into calcium oxide (CaO). During this process, chitin and proteins undergo thermal decomposition, generating volatile gases such as carbon dioxide (CO_2), water vapor (H_2O), and nitrogen-containing compounds such as NH_3 and NO_x, which are released into the atmosphere. Meanwhile, $CaCO_3$ undergoes the following decomposition reaction:

$$CaCO_{3(s)} \rightarrow CaO_{(s)} + CO_{2(g)}$$

The calcination process yielded a CaO-rich solid, which was subsequently analyzed using X-ray Fluorescence (XRF). The XRF data indicate that the dominant components in the post-calcined crab shell powder were CaO at 73.32% and P_2O_5 at 13.93%, as shown in the following table:

Table 1. Original X-Ray Fluorescence (XRF) Results of Crab Shell Waste Raw Material After Calcination at 800°C

Analysis	Component	Content (% w/w)
Element	SiO_2	9,03
	P_2O_5	13,93
	SO_3	0,183
	CaO	73,32
	TiO_2	0,192
	MnO	0,280
	NiO	0,049

Source: Integrated Research and Testing Laboratory, Universitas Gadjah Mada

Characterization of Hydroxyapatite Derived from Crab Shells Based on XRF Analysis

Characterization of the synthesized hydroxyapatite was performed to evaluate its chemical composition with a focus on determining the molar calcium-to-phosphorus (Ca/P) ratio using X-ray fluorescence (XRF) analysis. The theoretical stoichiometric Ca/P ratio of hydroxyapatite, derived from its ideal chemical formula $Ca_{10}(PO_4)_6(OH)_2$, is 1.67 and was used as a reference to assess the conformity of the synthesized samples. A total of 25 sample points were analyzed, revealing variations in Ca/P ratios across different synthesis conditions. The XRF measurements were conducted at the Integrated Research and Testing Laboratory, Universitas Gadjah Mada, based on the excitation of sample atoms by X-ray irradiation and the detection of characteristic fluorescent emissions, enabling the determination of elemental composition. The resulting Ca/P molar ratios obtained from the XRF analysis are presented in **Table 2**.

Table 2. Ratio Ca/P of Hydroxyapatite in Synthesis Using Precipitation Method

Calcination Temperature(°C)	Concentration Na_2HPO_4 (M)	Ratio Ca/P
800	0,75	1,6807
800	1,3	1,6575
800	1,85	1,6427
800	2,4	1,6275
800	2,95	1,6639
900	0,75	1,4794
900	1,3	1,5238
900	1,85	1,5862
900	2,4	1,6128
900	2,95	1,6712
1000	0,75	1,5632
1000	1,3	1,5993
1000	1,85	1,6607
1000	2,4	1,8073
1000	2,95	1,8196
1100	0,75	1,6837
1100	1,3	1,8843
1100	1,85	2,2020
1100	2,4	2,2112
1100	2,95	2,4437
1200	0,75	2,2326
1200	1,3	2,3785
1200	1,85	2,4327
1200	2,4	2,8243
1200	2,95	3,1215

Source: Integrated Research and Testing Laboratory, Universitas Gadjah Mada

Based on the XRF results presented in **Table 2**, the molar calcium-to-phosphorus (Ca/P) ratio of the synthesized hydroxyapatite was strongly influenced by both calcination temperature and Na_2HPO_4 concentration. The obtained Ca/P ratios ranged from 1.47 to 3.12, indicating that only specific synthesis conditions produced Ca/P values close to the theoretical stoichiometric ratio of pure hydroxyapatite (Ca/P = 1.67), derived from its ideal chemical formula $Ca_{10}(PO_4)_6(OH)_2$. At a calcination temperature of 800 °C, samples prepared with Na_2HPO_4 concentrations of 0.75 M and 2.95 M exhibited Ca/P ratios approaching

the stoichiometric value, suggesting that hydroxyapatite retained good structural stability at relatively low calcination temperatures. Similarly, near-stoichiometric Ca/P ratios were also observed at 900 °C with 2.95 M Na_2HPO_4, at 1000 °C with 1.85 M, and at 1100 °C with 0.75 M, indicating the presence of optimal synthesis conditions resulting from the combined effects of calcination temperature and phosphate concentration.

In general, an increasing trend in Ca/P ratio was observed with increasing calcination temperature, particularly at temperatures ≥1100 °C, where most samples exhibited Ca/P values significantly exceeding the stoichiometric ratio. This behavior can be attributed to structural changes in hydroxyapatite at elevated temperatures, where phosphorus species are more susceptible to thermal loss or phase transformation compared to calcium, resulting in a relative enrichment of calcium and an increased Ca/P ratio. Such deviations from stoichiometry are commonly associated with the formation of non-stoichiometric hydroxyapatite or secondary calcium-rich phases, as reported in previous studies [12].

The concentration of Na_2HPO_4 also played a critical role in determining the Ca/P ratio. At low Na_2HPO_4 concentration (0.75 M), the Ca/P ratio tended to be lower or close to the stoichiometric value, indicating that limited phosphate availability restricted complete hydroxyapatite formation. At intermediate Na_2HPO_4 concentrations, such as 1.85 M and 2.95 M, the Ca/P ratios approached the theoretical value of 1.67, suggesting an optimal balance between Ca^{2+} and PO_4^{3-} ions during the precipitation process. However, further increases in Na_2HPO_4 concentration resulted in Ca/P ratios exceeding the stoichiometric value, indicating that excess phosphate does not necessarily promote stoichiometric hydroxyapatite formation, but may instead induce compositional imbalance, reduced structural stability, and the formation of non-stoichiometric hydroxyapatite phases, consistent with observations reported by [13].

Characterization of Hydroxyapatite Derived from Crab Shells Based on XRD Analysis

X-ray diffraction (XRD) was employed to determine the crystallite size, elemental composition, and crystallinity level of the material based on the diffraction peak intensities. The XRD data were analyzed using Origin software and exhibited patterns consistent with the standard XRD data of pure hydroxyapatite (HAp) listed in JCPDS card No. 09-432.

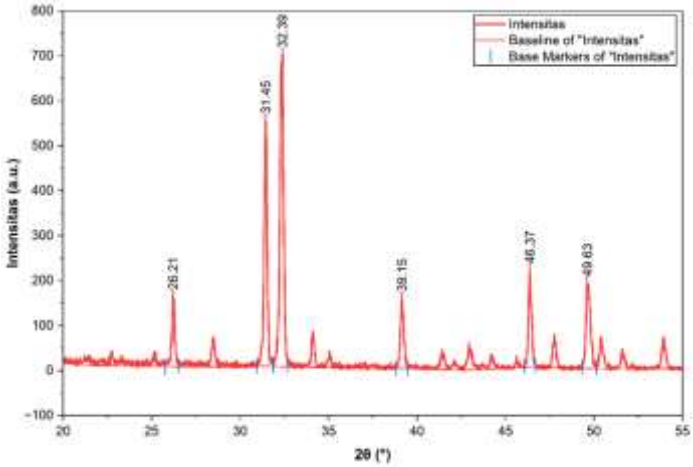

Fig. 2: XRD Results of Hydroxyapatite at 900°C and Na_2HPO_4 2,95M
Source: Advanced Mineral and Materials Laboratory, Universitas Negeri Malang

Based on the X-Ray Diffraction (XRD) pattern in **Figure 2**, it shows the characteristics of hydroxyapatite which is characterized by the emergence of high intensity peaks at 2θ angles of 26.21°; 31.45°; 32.39°; 39.15°; 46.37°; and 49.63°. These peaks are close to the standard data of the Joint Committee on Powder Diffraction Standards (JCPDS), especially at angles of 26°; 31°; 32°; 39°; 46° and 49°. This agreement indicates that hydroxyapatite was successfully formed from crab shells at a calcination temperature variable of 900°C with a Na_2HPO_4 concentration of 2.95 M. The diffraction pattern obtained is similar to the standard hydroxyapatite JCPDS number 09-0432, where each main peak in the graph represents the hydroxyapatite phase. The high peak intensity and relatively sharp peak shape indicate a good level of crystallinity. The higher the intensity and the narrower the diffraction peak, the higher the degree of crystallinity of the hydroxyapatite formed [14].

Table 3. Crystal Size Analysis Results

Peak	2θ	FWHM	L (nm)	Average Size (nm)
1	26,21	0,15937	51,18	
2	31,45	0,18277	45,16	
3	32,29	0,22527	36,71	41,72435
4	39,29	0,20304	41,53	
5	46,37	0,19052	45,36	
6	49,63	0,28784	30,41	

The Scherrer equation was applied to determine the crystallite size and degree of crystallinity, where K represents the shape factor with a value of 0.9. The XRD analysis employed a wavelength (λ) of 0.15406 nm, while the FWHM (Full Width at Half Maximum) was obtained from the diffraction peak at the 2θ position. The crystallite sizes calculated using the Scherrer equation are summarized in Table 2, yielding an average crystallite size of 41.72435 nm. Crystallite sizes below 100 nm are considered suitable for bone implant applications, as nanoscale crystals enhance the interfacial interaction between the implant material and bone tissue, thereby promoting bone regeneration[15].

The XRD analysis results can also be evaluated by examining the degree of crystallinity of hydroxyapatite derived from crab shells. The crystallinity value represents the level of atomic arrangement regularity within the material. The degree of crystallinity was determined using XRD data processed with Origin Graphing Analysis software. Subsequently, the crystallinity degree was calculated by comparing the area of the crystalline phase to the combined area of the crystalline and amorphous phases. Based on the calculation, the crystallinity degree obtained from the XRD data was 81.14%. The development of a well-defined crystal structure contributes to an increase in crystallinity. Furthermore, both crystallinity and crystallite size are affected by the presence of impurities and the accuracy of the stoichiometric composition of the HAp powder. According to the literature [14], synthesized HAp generally exhibits a crystallinity value of approximately 80.93%. High crystallinity enhances the mechanical strength and stability of the material while reducing its reactivity. Therefore, a high degree of crystallinity indicates good-quality hydroxyapatite [15].

Characterization of Hydroxyapatite Derived from Crab Shells Based on SEM Analysis

The SEM characterization of hydroxyapatite derived from crab shells was conducted with the primary objective of determining the surface morphology and particle size, as these results can support and complement the XRD analysis findings. The following presents the SEM results of hydroxyapatite derived from crab shells:

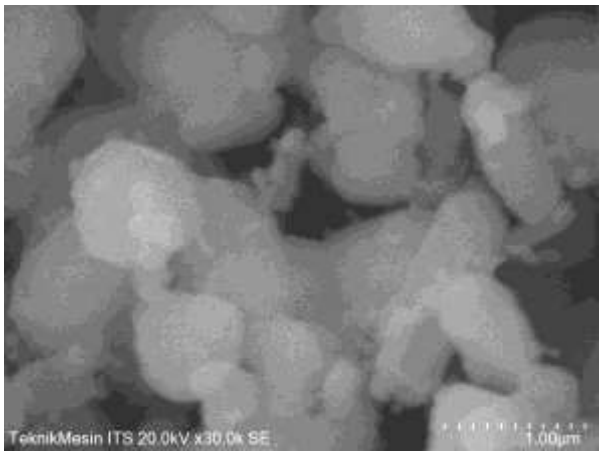

Fig. 3: SEM Results of Hydroxyapatite at 900 °C and Na_2HPO_4 Concentration of 2.95 M at 30,000× Magnification
Source: Mechanical Engineering Laboratory, Institut Teknologi Sepuluh Nopember (ITS)

Based on the Scanning Electron Microscopy (SEM) analysis at a magnification of 30,000× under calcination conditions of 900 °C and a Na_2HPO_4 concentration of 2.95 M, the synthesized hydroxyapatite particles exhibited a predominantly sub-spherical morphology with relatively smooth surfaces. However, agglomeration phenomena were still observed, where primary particles adhered to one another to form larger aggregates. This agglomeration is presumed to result from the high-temperature calcination process,

which increases the kinetic energy of the particles, thereby enhancing surface diffusion and interparticle coalescence [16]. Consequently, smaller particles tend to merge, forming denser and more compact structures. In this study, SEM images were analyzed using ImageJ software to determine particle size. The analysis results showed that the average particle area was 435.548 nm², with an average particle diameter of 33.2745 nm.

4. Conclusion

Hydroxyapatite (HAp) was successfully synthesized from crab shell waste via a precipitation method at a calcination temperature of 900°C and a Na_2HPO_4 concentration of 2.95 M. Compositional analysis using XRF revealed a Ca/P ratio of 1.6712, which is close to the stoichiometric value of hydroxyapatite (1.67) and complies with the requirements of the International Organization for Standardization ISO 13779-3:2008 standard. These results indicate that the applied process parameters were effective in producing a chemical composition suitable for biomaterial applications. Crystal structure characterization by X-ray diffraction (XRD) exhibited the highest diffraction peak at $2\theta = 32.9°$, corresponding to the JCPDS reference 09-0432, confirming that crystalline hydroxyapatite was the dominant phase formed. The calculated crystallinity degree of 81.14% indicates a high level of structural order, attributed to the optimal calcination process.

Morphological analysis using scanning electron microscopy (SEM) showed that the hydroxyapatite particles exhibited irregular shapes with a tendency to form dense and porous aggregates. These characteristics comply with the International Organization for Standardization ISO 13779-6 requirements regarding biomaterial powder characterization, including morphology and physical powder condition. Overall, the synthesis and characterization results demonstrate that the precipitation method is effective in producing hydroxyapatite with high phase purity and crystallinity in accordance with international standards.

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