

Synthesis and Characterization of Hydroxyapatite (HAp) from Eggshell Waste for Biomedical Applications

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Abstract

Approximately 250,000 tons of eggshell waste are generated annually. This study proposes a sustainable and economically viable solution by repurposing this waste into valuable biomaterials like hydroxyapatite (HAp). HAp was successfully synthesized from eggshell waste through a two-step process involving calcination and chemical precipitation. The eggshells were initially calcined at temperatures ranging from 700°C to 1100°C with 100°C intervals to convert calcium carbonate (CaCO₃) into calcium oxide (CaO). The calcined powder was then mixed with distilled water and phosphoric acid before undergoing a 24-hours aging process. The resulting precipitates were centrifuged and re-calcined at 800°C. Characterization of the synthesized HAp was conducted using X-ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), and Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM/EDX). XRD analysis confirmed the formation of HAp through the successful conversion of CaCO₃ to CaO. FTIR spectra indicated the presence of phosphate (PO₄³⁻), carbonate (CO₃²⁻), and hydroxyl (OH⁻) groups, further confirming the synthesis of HAp. SEM analysis revealed an increase in HAp particle size with rising calcination temperatures. Additionally, EDX analysis of the 700°C sample showed a Ca/P ratio of 1.67, which matches the stoichiometric ratio of HAp.

1. Introduction

Hydroxyapatite (HAp), which has the chemical formula of Ca₁₀(PO₄)₆(OH)₂ is a bio-ceramic material that has many applications in biomedical. It belongs to a group of minerals containing calcium cations and can include orthophosphate, metaphosphate, or pyrophosphate, anions. Occasionally, these minerals may also contain hydrogen or hydroxide [1]. Its composition closely resembles human bone, making it the primary inorganic component of bone, enamel, and dentine. Extensive research indicates that HAp exhibits commendable biocompatibility, as it elicits no inflammatory, toxicity, or pyrogenic responses [2]. These distinctive attributes position HAp as a highly desirable and widely employed solution for addressing diverse challenges related to bones.

Inedible by-products from slaughterhouses, catering services, bakeries, and food processing industries, such as bones, seafood shells, and eggshells, are often considered waste, despite being rich in calcium. This leads to the loss of valuable resources and contributes to environmental pollution. To solve these problems, these items can

be utilized to extract HAp. Natural sources offer desirable characteristics in the synthesized HAp, making it highly recommended for biomedical applications. This is because it contains essential elements that aid in the mineralization of calcified tissue, promote bone formation, and lower the sintering temperature for densification [3].

The objectives of this research are to produce calcium oxide, CaO from eggshell waste, to synthesize HAp powder derived from the eggshell waste precursor, CaO, and to characterize the phase composition, functional groups, morphological, elemental composition of hydroxyapatite using XRD, FTIR, and SEM/EDX.

2. Materials and Methods

2.1. Materials

Chicken eggshells, obtained from the UTHM Pagoh student cafe, served as the precursor material for synthesizing and characterizing hydroxyapatite (HAp). Orthophosphoric acid (H_3PO_4) from Bendosen was used as a chemical reagent in the synthesis of hydroxyapatite (HAp) from eggshell waste.

2.2. Preparation of HAp powder

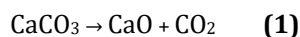
Collected chicken eggshells were washed with soap and water to remove any impurities. Subsequently, the eggshells were boiled (Fig. 1) for one hour and then dried in an oven at 100°C . The dried eggshells were crushed using a crusher and sieved to obtain particles smaller than $100\ \mu\text{m}$, ensuring a homogenous sample. To minimize oxidation during characterization, measurements were taken promptly to avoid prolonged exposure to air, which could potentially alter the observed properties.



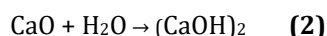
Fig. 1 Eggshells boiled using hotplate stirrer

2.3. Synthesis of HAp powder

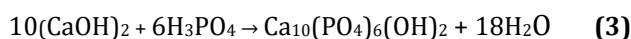
The eggshell waste powder was calcined at various temperatures from 700°C to 1100°C inside a furnace for 2 hours, with an interval of 100°C to produce calcium oxide (CaO). The calcination process converts calcium carbonate to calcium oxide by releasing carbon dioxide as shown in (1).



Then, calcium oxide was dissolved in distilled water producing calcium hydroxide as shown in (2).



Prior to, phosphoric acid (H_3PO_4) was added into the calcium hydroxide produced in (2) in order to produce HAp as shown in (3). The mixture solution was left for 24 hours to allow precipitation to occur. The mixture was then centrifuged at 3000 rpm for 1 hour to separate water and precipitate. The precipitate then was rinsed and filtered with distilled water before drying in the oven at 100°C for 1 hour. Lastly, the dried precipitate was re-calcined at 800°C in the furnace for 2 hours.



2.3.1. Characterization of HAp

XRD, FTIR, and SEM/EDX were employed to characterize HAp after calcination. The phase identification of the crystalline material of the HAp powder was characterized by XRD. The XRD setting was set to a range of 2θ from

10° to 80°. The functional groups of HAp powder were analyzed by FTIR with a range between 400 cm⁻¹ and 4000 cm⁻¹. The morphology and elemental composition of HAp was analyzed by SEM equipped with EDX.

3. Results and Discussion

The results obtained from XRD, FTIR, and SEM/EDX analysis were discussed as in the following sub-topics.

3.1 X-ray Diffraction analysis

Fig. 2 shows the X-ray diffractogram of 5 samples of calcined eggshells at different temperatures (700 °C, 800 °C, 900 °C, 1000 °C, and 1100 °C). As the temperature increased, the intensity of Ca(CO)₃ peak decreased, and at 1000 °C and 1100 °C, no peak of Ca(CO)₃ was observed. This phenomenon is due to Ca(CO)₃ being fully converted to CaO in order to produce HAp. As Ca(CO)₃ decomposes, the CaO peak increases. The appearance of an intense reflection peak at 31.77° of 2θ values represents apatite. This analysis showed that as the calcination temperatures increased, the peak intensity of HAp also increased which indicates the improvement in the crystallinity of the samples [4]. However, there was HAp and biphasic tricalcium phosphate (β-TCP) at 800 °C, 900 °C, 1000 °C and 1100°C [5]. This is due to the decomposition of HAp to β-TCP had occurred at calcination temperatures higher than 700 °C [7]. The figure illustrates the shift in peak intensities in the XRD pattern reflecting the transformation of eggshells from CaCO₃ to CaO and HAp.

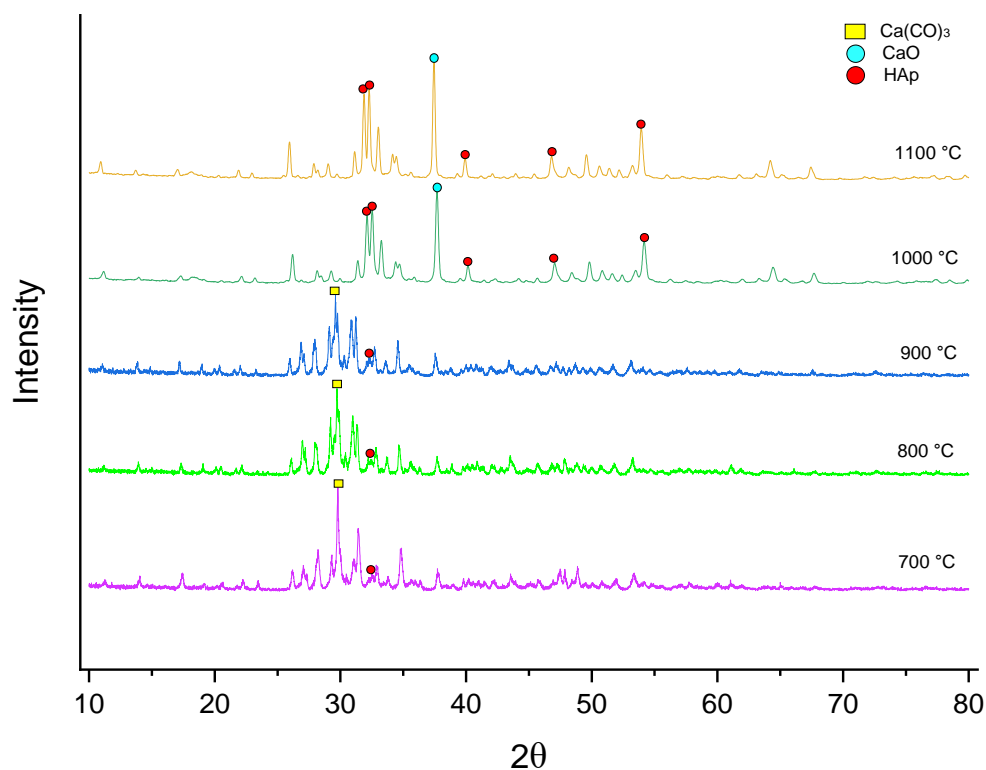


Fig. 2 XRD pattern of calcined eggshell at different temperatures

3.2 Fourier-Transform Infrared Spectroscopy (FTIR) analysis.

Fig. 3 shows the FTIR spectrum of the calcined eggshells in all samples. The analysis showed the presence of phosphate (PO₄³⁻) group at frequencies ranging from 1070 cm⁻¹- 1075 cm⁻¹. Meanwhile, the carbonate (CO₃²⁻) group appears at frequencies of 1400 cm⁻¹ – 1430 cm⁻¹ and gradually disappeared as values of calcination temperatures increased due to the formation of CaO [2]. The hydroxyl (OH⁻) group was also observed at 700 °C, 800 °C, and 900 °C samples which had a peak around frequencies of 3600 cm⁻¹ – 3700 cm⁻¹. However, the bending mode of water (H₂O) slowly disappeared starting at 1000 °C due to increasing calcination temperature. The presence of C-O and O-H bonds in the FTIR spectrum indicates the formation of CaO and (CaOH)₂ in calcined eggshells. Since all the samples exhibited a PO₄³⁻ groups, it confirmed the formation of HAp.

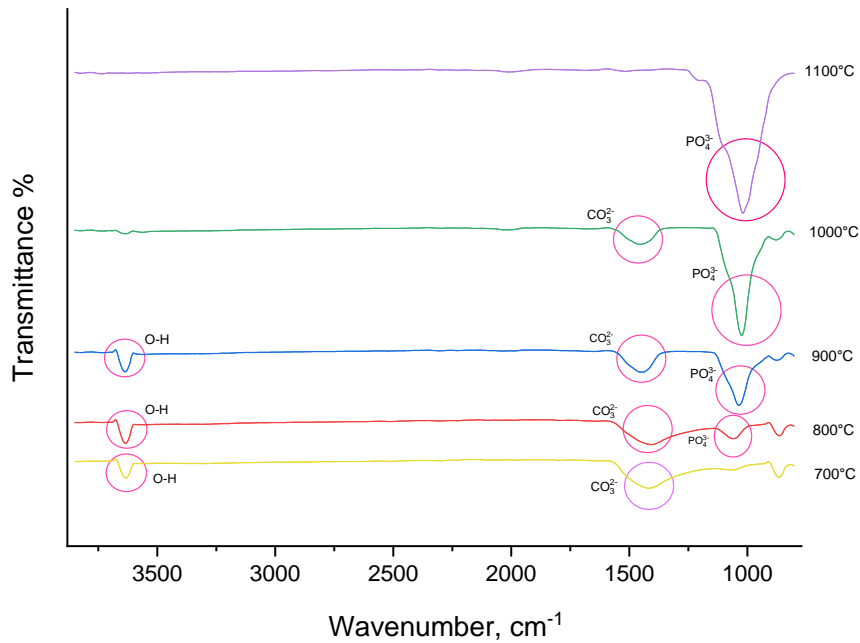


Fig. 3 FTIR spectrum of calcined eggshell at different temperatures.

3.3 Scanning Electron Microscope (SEM) analysis

The surface morphology of Hap was examined by SEM. Fig. 4 shows the morphology of Hap powder calcined at 700 °C, 900 °C, and 1100 °C, respectively. All of the samples were shown to have irregular structures. The formation of larger particles of various sizes was caused by the agglomeration process, which occurred from the chemical precipitation and re-calcination process. The size of the particle increased as the temperature increased. This is due to the higher temperature resulting in the agglomeration of particles [7]. The morphology shown that there were pores between the agglomerates which is advantageous since it allows bodily fluids to circulate throughout the coating in biomedical applications [2].

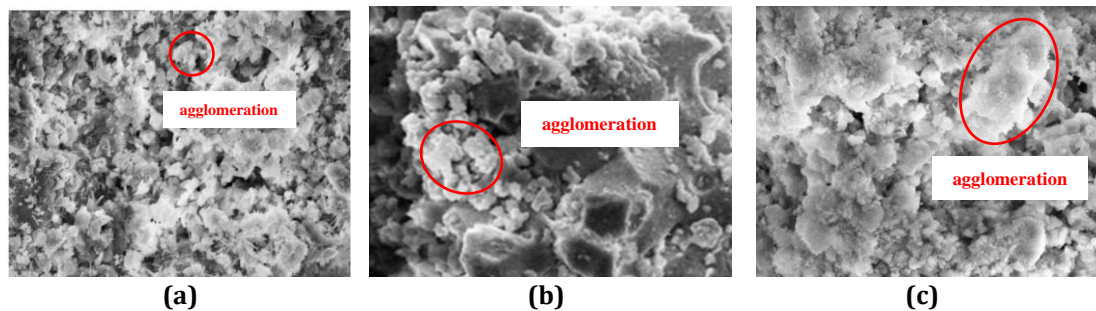


Fig. 4 Morphology of calcined eggshells at different temperatures (a) 700 °C; (b) 900 °C; (c) 1100 °C

3.4 Energy Dispersive X-ray (EDX) analysis

Fig. 5 shows the graph of calcined eggshells at 700°C, 900°C and 1100°C analyzed by EDX. The main elements found in the samples were calcium (Ca) and phosphorus (P). The addition of H_3PO_4 as the source of P in the form of phosphate ions in the sample. The 700°C sample contained 62.6% of Ca and 37.4% of P. This value corresponded to a Ca/P ratio of 1.67 which is equal to the stoichiometric ratio of HAp. However, 900 °C and 1100 °C exhibited 61.4 % and 60.8 % of Ca, respectively. Meanwhile, the percentage of P at 900°C and 1100°C were 38.6 % and 39.2 %, respectively. These values represented a Ca/P ratio ranging between 1.50 to 1.60 closer to the Ca/P ratio of β -TCP which is 1.58. The ranges indicate that HAp and β -TCP were present. The ratio was below stoichiometric HAp due to calcium deficiency in the HAp phase [6,7]. The β -TCP is formed due to the decomposition of HAp as calcination exceeds 700°C [7]. The HAp and β -TCP were present on 900°C and 1100°C samples.

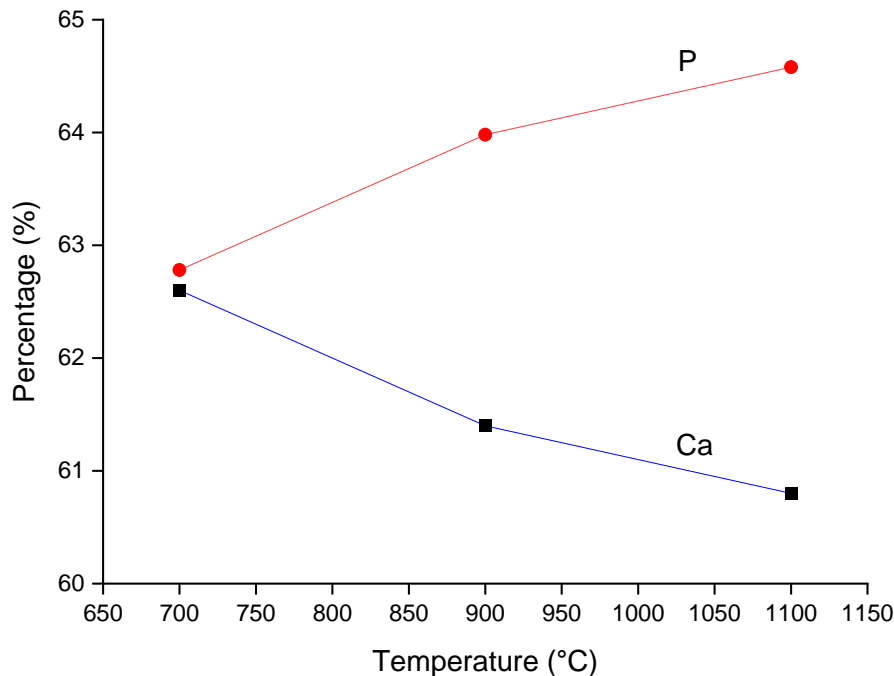


Fig. 5 Percentage of Ca and P at various temperatures

4. Conclusion

The crystalline HAp was able to be synthesized from CaO, a precursor extracted from eggshell waste. The XRD analysis revealed the crystallinity result and showed that the higher the calcination temperatures, the higher the crystallinity of HAp. The FTIR analysis demonstrated the functional group of HAp powder which contains phosphate, carbonate, and hydroxyl groups. The particle size of HAp increases as the temperatures increase shown by SEM analysis. The calcium-to-phosphorus (Ca/P) ratio in HAp was discovered to be 1.67 at 700 °C. In this project, 0.568 mol of H₃PO₄ was utilized to extract the HAp from eggshell waste. Therefore, increasing the phosphorus concentration during the HAp synthesis process may result in higher phosphate content in the finished product. It can be concluded that eggshell waste can be a sustainable alternative for HAp production. In an attempt to offer financial advantages by reducing the cost of HAp production, this also serves as an excellent waste management approach to help reduce environmental issues.

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Conflict of Interest

Authors declare that there is no conflict of interests regarding the publication of the paper.

Author Contribution

The authors confirm contribution to the paper as follows: **study conception and design:** Nur Nadhirah Hani Binti Zahlan Arief, Puteri Nur Hannani Binti Zubairi, Mohamad Naufal Muzammil Bin R Zaiedy, Rozainita Rosley **data collection:** Nur Nadhirah Hani Binti Zahlan Arief, Puteri Nur Hannani Binti Zubairi, Mohamad Naufal Muzammil Bin R Zaiedy **analysis and interpretation of results:** Nur Nadhirah Hani Binti Zahlan Arief, Rozainita Rosley **draft manuscript preparation:** Nur Nadhirah Hani Binti Zahlan Arief. All authors reviewed the results and approved the final version of the manuscript.

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