

Influence of Milling Speed on the Structural and Hardness Properties of Eggshell-Derived Hydroxyapatite

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DOI: <https://doi.org/10.47772/IJRISS.2026.100900430>

Received: 19 September 2026; Accepted: 24 September 2026; Published: 13 October 2026

ABSTRACT

Hydroxyapatite (HA) has incredible compatibility for use in the field of biomedical applications. Nevertheless, conventional HA synthesis frequently uses energy-intensive processing and high-purity chemical precursors, which raise manufacturing costs and have an adverse effect on the environment. This study offers a sustainable method for producing value-added biomaterials from biowaste by assessing the impact of ball milling speed on the structural and hardness characteristics of HA synthesised from eggshell-derived calcium oxide (CaO). Eggshells were calcined to produce CaO, which was then combined with diammonium hydrogen phosphate and ball-milled for four hours at 200, 300, and 400 rpm before being heated to 800 °C. Field-emission scanning electron microscopy combined with energy-dispersive X-ray spectroscopy (FESEM–EDX), X-ray diffraction (XRD) was used to characterize the produced powders. The findings demonstrated that increasing milling speed promoted a more homogenous microstructure while improving phase formation, crystallinity, and particle refinement. EDX analysis revealed that after heat treatment, Ca/P ratios converged toward the stoichiometric value of HA (1.67) across all milling speeds, with the 400 rpm condition achieving 1.67 post-calcination. Additionally, hardness increased dramatically from 551.43 MPa at 200 rpm to 730.83 MPa at 400 rpm, suggesting better densification and less porosity. Overall, the results show that a crucial processing factor affecting the mechanical and structural characteristics of HA produced from eggshells is milling speed. The superior crystallinity and hardness of the optimized HA produced at 400 rpm demonstrated the potential of eggshell waste as a sustainable and eco-friendly precursor for biomedical-HA.

Keywords: Ball milling, Biowaste, Eggshell-derived hydroxyapatite, Hardness, Structural characterization

INTRODUCTION

Hydroxyapatite (HA), with the chemical formula $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, is a biomaterial component that draws context from the structure of the inorganic components of human bone and teeth. This characteristic allows HA vast applicability for a broad range of clinical biomaterial uses. HA has the chemical equivalence to the inorganic constituents of bone and has a large array of desirable properties that include bioactivity and relatively easy integration with living systems. Because of these desirable attributes and for various other reasons, HA has been utilized for tissue engineering scaffolds, drug delivery systems, and other applications of regenerative medicine and positive health practices. HA is also utilized in the orthopedic and dental fields, for bone defect repair and for the modification of the surface of implants [1-3]. HA's properties of bioactivity and non-toxicity have made restorative and regenerative applications for bone and the integration of long-lasting implants a reality. These properties have made HA a subject of intense research to improve its functions. A field of this research is the study of HA of a more advanced state, including doped HA, nanostructured HA, and HA-based composites,

which recently have garnered interest for their potential to improve mechanical properties, bioactivity, antimicrobial activity, and overall biological behavior [4, 5].

Despite these benefits, there are also several intrinsic disadvantages that restrict the practical use of pure HA. Conventional methods for HA production give it inadequate fracture toughness and poor mechanical properties resulting in brittle behavior that makes HA unsuitable for load-bearing biomedical applications [2, 5]. Furthermore, conventional synthetic methods require time-consuming and energy-demanding high-temperature processes and high-purity chemical precursors, increasing the cost and undermining the environmentally friendly aspect of the HA manufacturing process. With the increasing demand for biomaterials, the need for cost-effective and environmentally friendly synthetic processes has become pivotal. To this end, a lot of focus has been given to using other resources, especially waste and renewable resources, as alternative starting materials for HA synthesis.

Calcium-rich biowaste materials provide the opportunity for an approach to biowaste that is inexpensive and has low environmental consequences. Fish bones, corals, bovine bones, oyster shells, and eggshells are all examples of biogenic resources [6–8]. They have adequate amounts of calcium for calcium source planning for HA synthesis. Utilizing waste for raw materials promotes cost efficiency in production. It can also aid in the management of waste by environment protection in the formation of value-added biomedical products from previously discarded materials. In comparison to the plethora of biowaste, eggshells are a great candidate due to how they are a byproduct of food processing and are used in households. They have also been noted to have a high almost 90% weight in Calcium carbonate (CaCO_3). This makes them cheap and accessible options for HA synthesis. In addition to the many biowaste sources, the disposal of eggshell waste can be of high value and promotes the principles of a circular economy along with added value biomaterials and sustainable manufacturing waste from agriculture to landfill [6, 8].

To produce HA from conventional and waste-derived calcium sources, multiple synthesis techniques have been used, such as sol-gel, wet precipitation, hydrothermal, and mechanochemical techniques [9–11]. Of these methods, wet precipitation is widely used since it is a low-cost and simple method to manufacture HA with stoichiometric balance. However, this method leads to powder that is agglomerated and has a heterogeneous microstructure, which may negatively affect the powder's performance in biomedical applications. Because of this negative effect, mechanochemical processing has been incorporated in more HA production routes. Ball milling has various benefits, such as efficient mixing, smaller particle sizes, larger surface areas, and a greater chemical reactivity in powder, which positively affect the formation of phases and the overall crystallinity and mechanical properties of HA synthesized [10, 11]. Furthermore, mechanochemical processing is a scalable method that lacks much of the environmental impact of other methods and leads to a positive effect on holistic design of biomaterials.

Although numerous studies have confirmed that H eggshell waste can successfully produce HA, the specific impact of milling parameters on the material's final properties is not yet fully understood. Milling speed is essential because it defines the amount of energy that is transferred throughout the milling process, impacting on the refinement of the particles, the kinetics of reactions, the crystals, and the microstructures. Because of this, HA's phase composition, morphology, crystallinity and mechanical properties will be changed. There is a noticeable lack of research on the effects of milling speed on HA made from eggshells. For this reason, this research focuses on the sustainable production of HA from eggshell waste and the effects that multiple milling speeds have on the microstructure, crystallinity, and hardness. The end goal of this research is to improve biomaterials that are safe for the environment and can be used in next generation biomedical products that are commercially profitable.

MATERIALS AND METHODS

Preparation of eggshell-derived HA

Waste chicken eggshells were sourced from a restaurant in Bukit Beruang, Melaka. The eggshells were rinsed

with water and then subjected to a water bath at 60 °C for 30 minutes. The purpose of this was to eliminate any surface contamination or organic remnants. The eggshells were then dried in an oven at 60 °C for an hour. The dried eggshells were then ground into a fine powder and subjected to calcination screening at temperatures ranging from 800 °C to 1000 °C for 2 hours each, to identify the optimal conversion temperature. Based on XRD analysis (Fig. 1), 900 °C was selected as the calcination temperature for producing the CaO precursor used in subsequent milling experiments, as it yielded near-complete CaCO₃ decomposition while minimising energy consumption.

Stoichiometric amounts of the synthesized CaO powder and diammonium hydrogen phosphate [(NH₄)₂HPO₄] were combined at a Ca:P molar ratio of 10:6 and processed in a planetary ball mill (Pulverisette 7, Fritsch, Germany) using an alumina milling jar and fifteen alumina balls (diameter: 10 mm), with a total powder charge of approximately 5 g per batch and a ball-to-powder mass ratio of 10:1. Milling was performed continuously at 200, 300, and 400 rpm for 4 h under ambient atmospheric conditions. The milled powders were subsequently sieved and thermally treated at 800 °C for 2 h to promote crystallisation and facilitate the formation of the hydroxyapatite phase.

Characterisation of eggshell-derived HA

The phase composition of the calcined eggshells and the synthesized HA powders at different milling speeds was analyzed using X-ray diffraction (XRD). Data collection was performed on a Rigaku SmartLab diffractometer operating at 45 kV with Cu K α radiation ($\lambda = 0.15406$ nm). Diffractograms were recorded over a 2θ range of 10° to 70° at a scanning rate of 14° min⁻¹. The average crystallite size (D) of each sample was estimated using Scherrer's equation:

$$D = \frac{0.9\lambda}{\beta \cos \theta} \quad \dots (1)$$

Where λ is the X-ray wavelength (0.15406 nm), β is the full width at half maximum (FWHM) of the most intense HA diffraction peak at $2\theta \approx 31.8^\circ$ (corresponding to the (211) plane), and θ is the Bragg diffraction angle. FWHM values were determined by peak profile fitting using Gaussian functions in the HighScore Plus software. No correction for instrumental broadening was applied, as the instrument contribution was determined to be negligible relative to sample broadening under the applied scan conditions.

FESEM–EDX (Hitachi SU5000, 20 kV) was used to analyze the morphology, elemental composition and Ca/P ratio. A Vickers Falcon 400G2 micro-hardness tester was used to evaluate the mechanical parameters. To measure the hardness, the heat-treated powders were uniaxially compressed into 10 mm pellets and pressure sintered at 1000 °C for two hours at a rate of 10 °C per minute. Pellet hardness was evaluated at a load of 100 g and a storage time of 20 s; ten curves of each sample were averaged with a standard deviation.

RESULT AND DISCUSSION

The XRD patterns in Figure 1 demonstrate the temperature-dependent transformation of eggshell-derived calcium carbonate (CaCO₃) into calcium oxide (CaO) during calcination. The raw eggshell powder exhibits sharp diffraction peaks characteristic of crystalline calcite CaCO₃, consistent with the natural composition of eggshells [12]. At 800 °C, the intensity of the CaCO₃ peaks decreases and CaO reflections begin to appear, indicating the onset of thermal decomposition. Raising the calcination temperature to 900 °C leads to significant enhancement of CaO peak intensity with only trace CaCO₃ remaining, suggesting near-complete conversion of the carbonate phase [13, 14]. Subsequently, the presence of the CaO peak at 1000 °C proves complete decomposition and improved crystallinity. However, the choice of 900 °C as the calcination temperature minimizes energy consumption and avoids unnecessary heat exposure, making the process more economical and energy-efficient. In summary, calcination effectively converts CaCO₃ in eggshell into highly crystalline CaO precursors suitable for HA production.

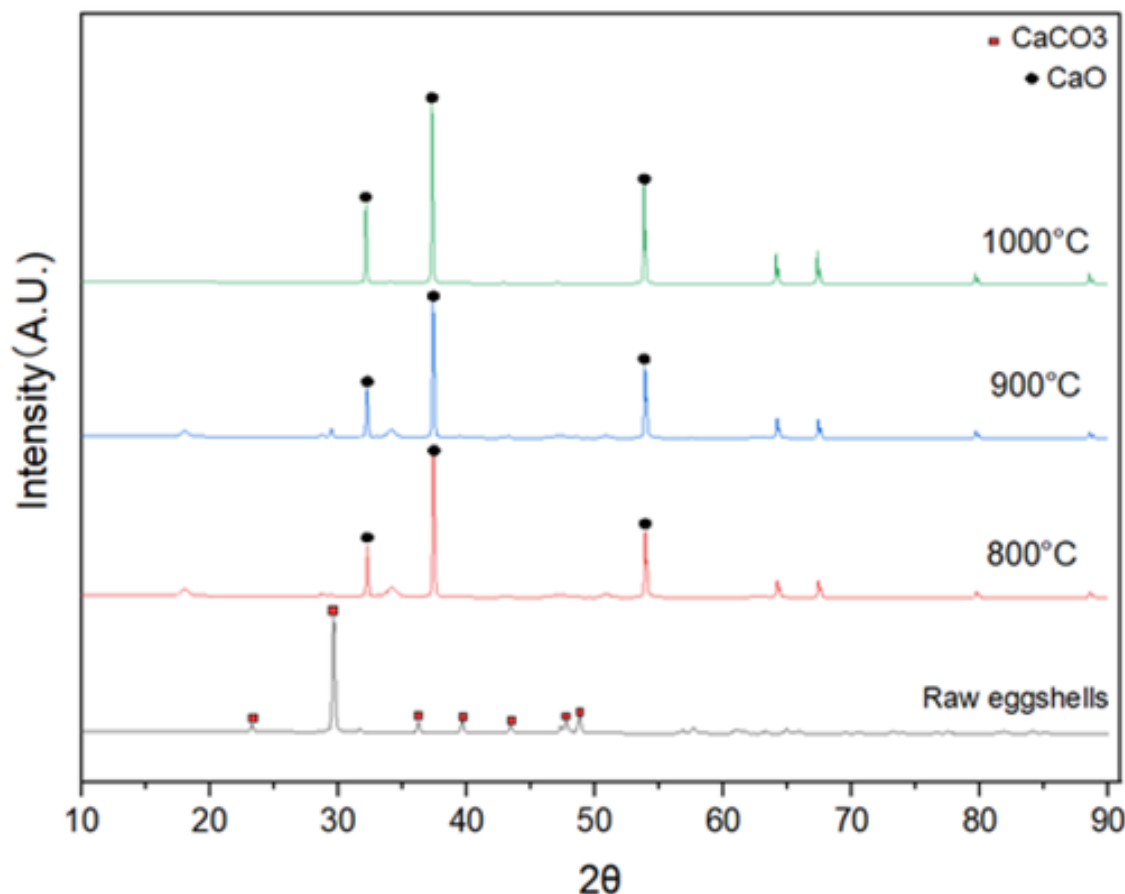


Fig. 1. XRD patterns of raw eggshell powder and calcined samples starting at 800°C up to 1000 °C for 3 hours, showing the phase transformation from CaCO_3 to CaO with increasing temperature.

Figure 2(a) shows the XRD pattern of the ball milled powder before heat treatment. The XRD pattern shows a multi-phase mixture consisting of unreacted CaCO_3 , residual $(\text{NH}_4)_2\text{HPO}_4$, and intermediate beta tricalcium phosphate (β -TCP), indicating that the solid-state reaction is incomplete across all milling speeds. These findings can be attributed to the inherent stability of CaCO_3 obtained from eggshells during mechanochemical processing. This indicates that ball milling induces physical refinement where it can reduce particle size and increase surface area but cannot produce complete chemical transformation. Consequently, thermal activation is necessary to decompose the stable calcite phase into reactive calcium species that can subsequently participate in the formation of calcium phosphate and HA phases [8, 11, 15]. This observation is further supported by Tangboriboon et al. [16], who reported that CaCO_3 retained its crystalline structure even under high-energy milling conditions and only underwent significant decomposition at elevated temperatures.

Following calcination at 800 °C (Fig. 2(b)), the powders undergo a marked phase transition, characterized by the emergence of strong HA reflections and the suppression of precursor-related peaks, confirming successful HA formation through solidstate reaction between thermally derived CaO and phosphate species. This behaviour is consistent with previous studies reporting that eggshell-derived calcium precursors become highly reactive toward phosphate sources following calcination, enabling the formation of crystalline hydroxyapatite during subsequent heat treatment [7, 8]. Minor β -TCP reflections observed in the 200 and 300 rpm samples suggest localized non-stoichiometric Ca/P ratios or insufficient precursor homogenization; phenomena commonly associated with incomplete reaction and phase coexistence in biogenic calcium phosphate systems [11]. In contrast, the 400 rpm sample exhibits the highest HA peak intensity and the lowest β -TCP content, indicating that increased milling energy promotes more uniform precursor mixing and enhanced reaction kinetics, leading to improved HA phase predominance. Although trace residual β -TCP and CaO reflections remain detectable, the 400 rpm condition yields the most HA-dominant phase assemblage among the three conditions studied. Quantitative phase analysis via Rietveld refinement would be required to confirm precise phase fractions.

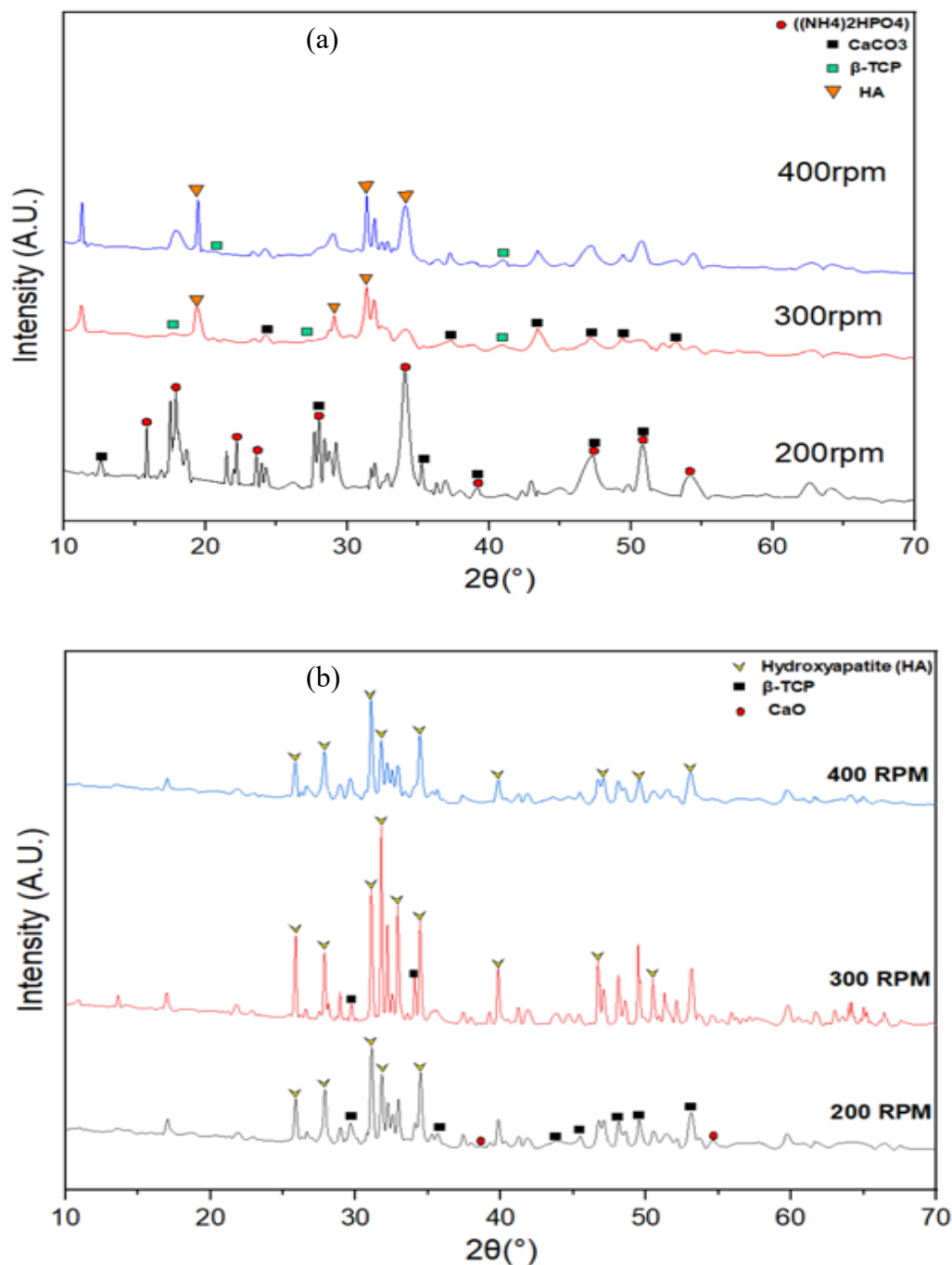


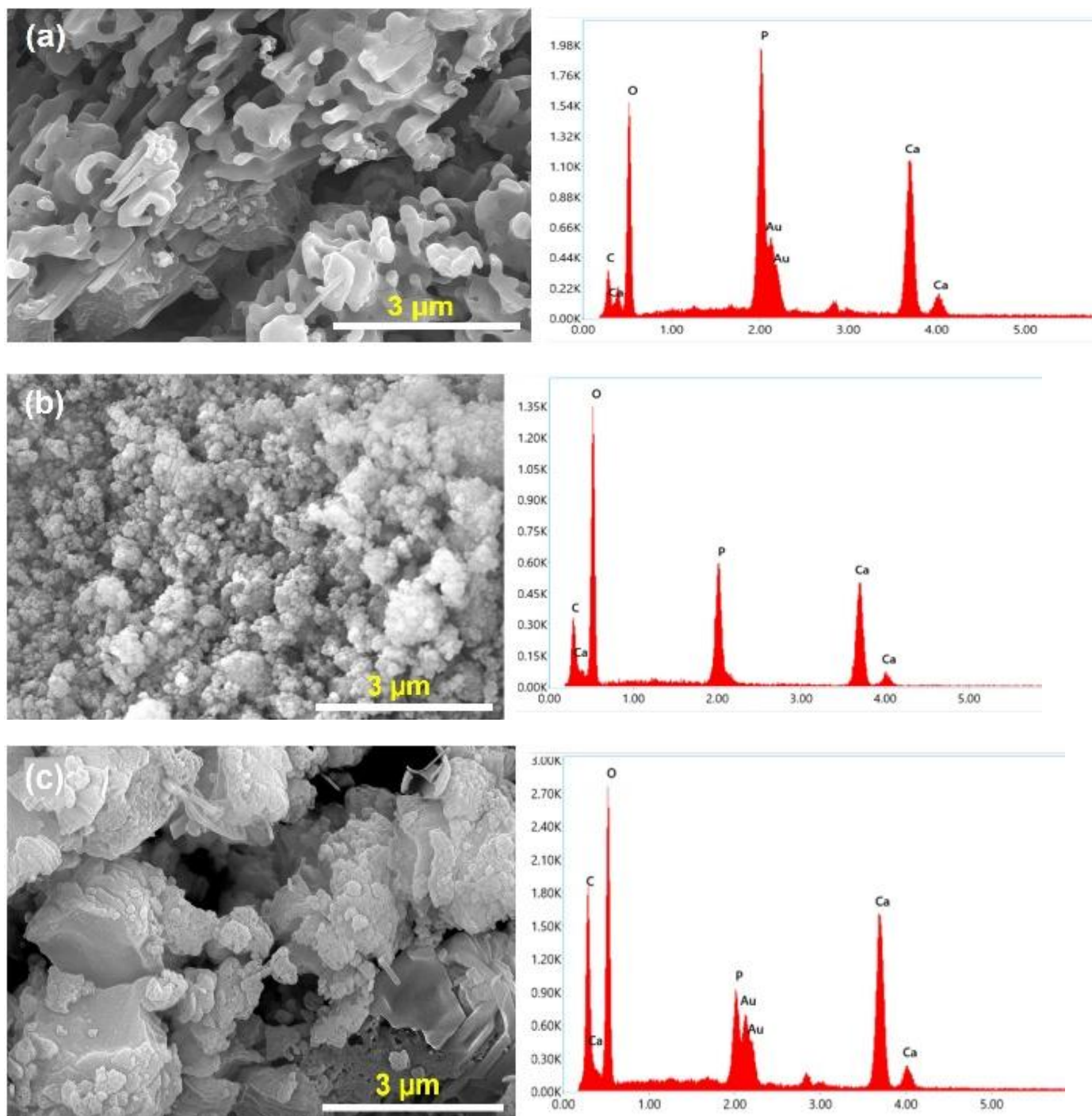
Fig. 2. XRD patterns of ball-milled powders at 200–400 rpm: (a) before heat treatment, showing CaCO_3 , $\beta\text{-TCP}$, HA, and residual $(\text{NH}_4)_2\text{HPO}_4$; (b) after heat treatment at 800 °C for 2 hours, showing dominant HA with residual $\beta\text{-TCP}$ and CaO.

Crystallite size (as seen in Table 1) decreases with an increase in milling speed from 200 to 300 rpm, suggesting the greater milling energy leads to greater lattice distortion and fragmentation. This agrees with Munir et al. [5] and Kareem and Eyler [8], where such grain refinement is associated with an increase in plastic deformation and defect concentration. The slight increase in crystallite size at 400 rpm is attributed to particle agglomeration, cold welding, and/or localized heating because of excessive milling energy. These phenomena have been reported in high-energy milling and milling on calcium phosphate-based material [5, 11]. After calcination, the samples exhibit an increase in crystallite size. This is attributed to grain growth and recrystallization, which are also thermally induced. The increase in crystallinity is agreed with findings in the literature that post calcination, hydroxyapatite bioceramics exhibit improved lattice order, with a reduction of structural defects and improvements in crystal growth and in the size of sequentials [7, 11].

Table 1, Variation of crystallite size as a function of milling speed, illustrating the combined effects of mechanochemical processing and thermal treatment.

Sample	Before heat treatment (nm)	After heat treatment (nm)
200	38.12	41.92
300	26.26	29.78
400	27.59	38.10

Figure 3(a-c) presents FESEM and EDX analyses, which indicate that eggshell-derived HA morphology and its EDX profiles before calcination are impacted by the milling speed. At 200 rpm, powders are coarse and are rich in large plate-like agglomerates. From 300 rpm, milling speed leads to a steady reduction in average particle size, attaining the finest and most uniform powders at 400 rpm. Beyond 400 rpm, powders are the finest; however, due to surface reactivity, powders are prone to agglomeration.



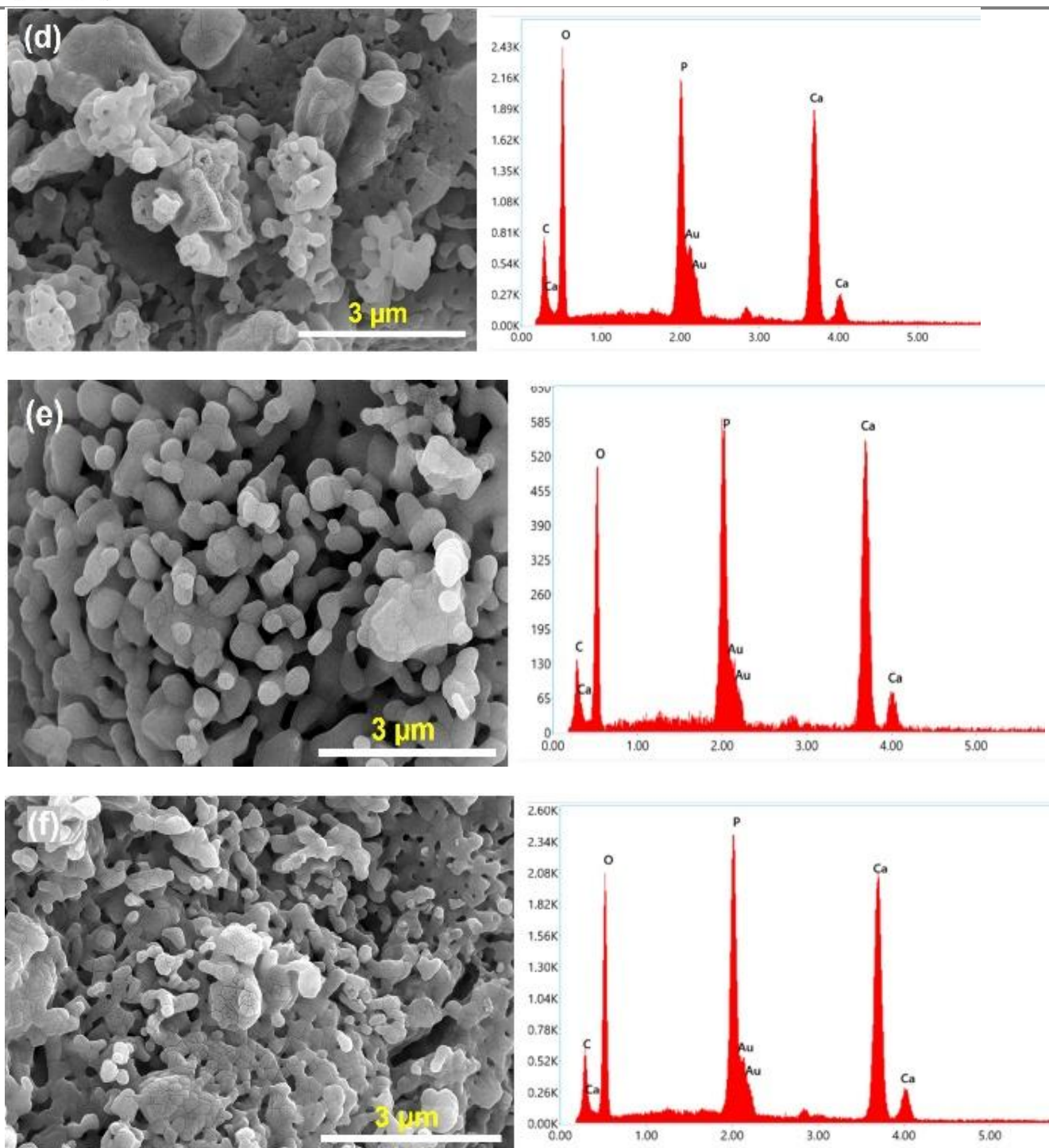


Fig. 3. FESEM micrographs and corresponding EDX spectra of HA powders milled at different rotational speeds: (a–c) before calcination and (d–f) after calcination at 800 °C. Samples were prepared at milling speeds of 200 rpm (a, d), 300 rpm (b, e), and 400 rpm (c, f). The micrographs reveal the influence of milling speed and calcination on particle morphology, agglomeration behaviour, and surface texture, while the EDX spectra confirm the presence of the major constituent elements (Ca, P, and O) characteristic of HA.

Figure 3(d-f) shows the FESEM micrographs of the samples heat treated to 800 °C. Increasing the milling speed gives rise to distinct changes in the microstructure. The sample milled at 200 rpm (Fig. 3d) shows large, agglomerated particles, a loosely packed structure with a high level of interparticle voids suggesting low level of densification. Such a microstructure is likely to adversely affect the mechanical properties of the sample. The sample milled at 300 rpm (Fig. 3e) has particles of more uniform morphology with improved grain packing and reduced porosity. This is likely the result of improved milling energy and the resulting increased precursor homogeneity. The sample milled at 400 rpm (Fig. 3f) shows the most refined and homogeneous microstructure of all the samples with a high level of packing and a further reduction in pore content. Higher hardness of this

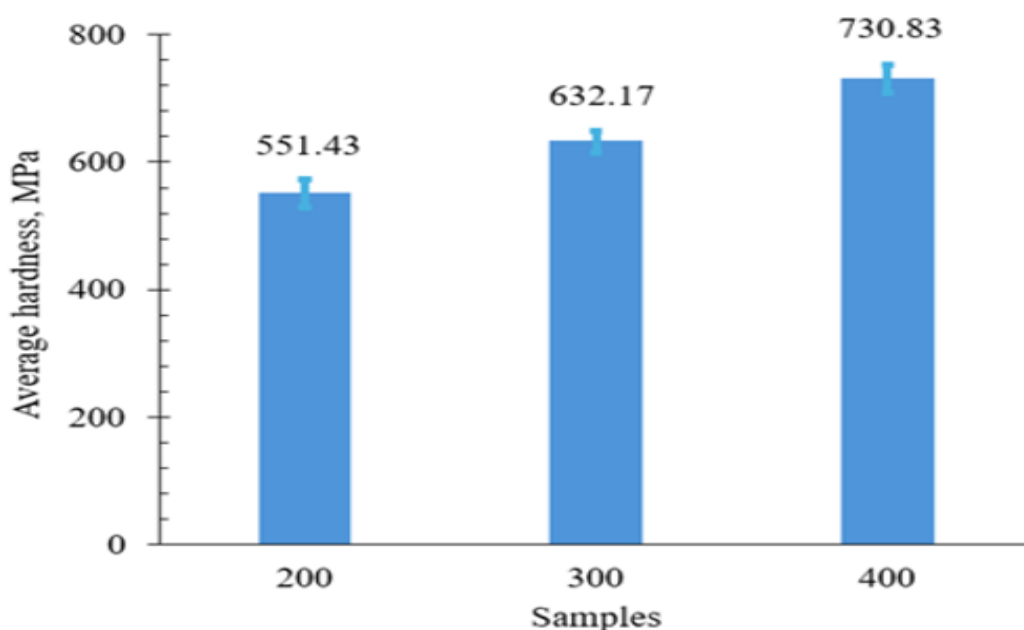
sample is associated with improved packing and homogeneity of the microstructure. The sample showed an increase in the milling speed which promoted microstructure refinement and improved the milling energy. Trzaskowska et al. [18] show a similar improvement in microstructural densification with reduced porosity and improved mechanical performance of hydroxyapatite-based materials, also noting a more compact microstructure. A more compact microstructure is also noted for increased hardness and structural integrity of materials for biomedical applications.

The existence of Ca, P, and O at all speeds without any change in HA stoichiometry at all speeds can be seen from the EDX results. This finding supports findings on moderate milling yielding optimal particle dispersion and excessive milling yielding structural disorder or shifts in composition [18, 19]. The Ca/P ratios indicated in Table 2 further support this finding, with the low ratios at 200–300 rpm (1.16–1.53) illustrating the prevailing phases of CaCO_3 or phosphates, in agreement with the thermal stability of biogenic CaCO_3 [6, 11]. However, the high ratio of 400 rpm (3.67) illustrates the introduction of Ca rich phases. Milling alone cannot achieve the Ca/P balance in eggshell derived CaO [8, 18]. After calcination at 800 °C, all samples align to a mean of 1.67–1.73, indicating the formation of HA as the dominant phase in response to Ca–P recombination, with slight increases attributed to minor β TCP, which are often found in biomedical calcium phosphate systems [5, 18].

Table 2, The calculated Ca/P ratios obtained before and after heat treatment.

Sample	Before heat treatment	After heat treatment
200	1.16	1.73
300	1.53	1.73
400	3.67	1.67

As shown in Figure 4, the hardness of HA pellets sintered at 1000 °C increases with milling speed, rising from 551.43 MPa (200 rpm) to 632.17 MPa (300 rpm) and reaching 730.83 MPa at 400 rpm, reflecting improved powder refinement and densification at higher milling energy. The FESEM images (Fig. 4) support this trend: the 200 rpm pellet (Fig. 4a) shows porous, loosely packed grains; the 300 rpm pellet (Fig. 4b) exhibits stronger grain coalescence and fewer pores; and the 400 rpm pellet (Fig. 4c) displays a dense, fine-grained microstructure with minimal porosity—consistent with the highest hardness value. These results agree with reports linking hardness enhancement in HA to pore elimination and improved intergranular bonding during sintering [10, 20]. Clinically, the 300–400 rpm pellets offer an optimal balance, combining wear resistance with microporosity beneficial for osteointegration, aligning with evidence that overly dense HA reduces bioactivity whereas excessive porosity weakens mechanical reliability [4, 5, 21].



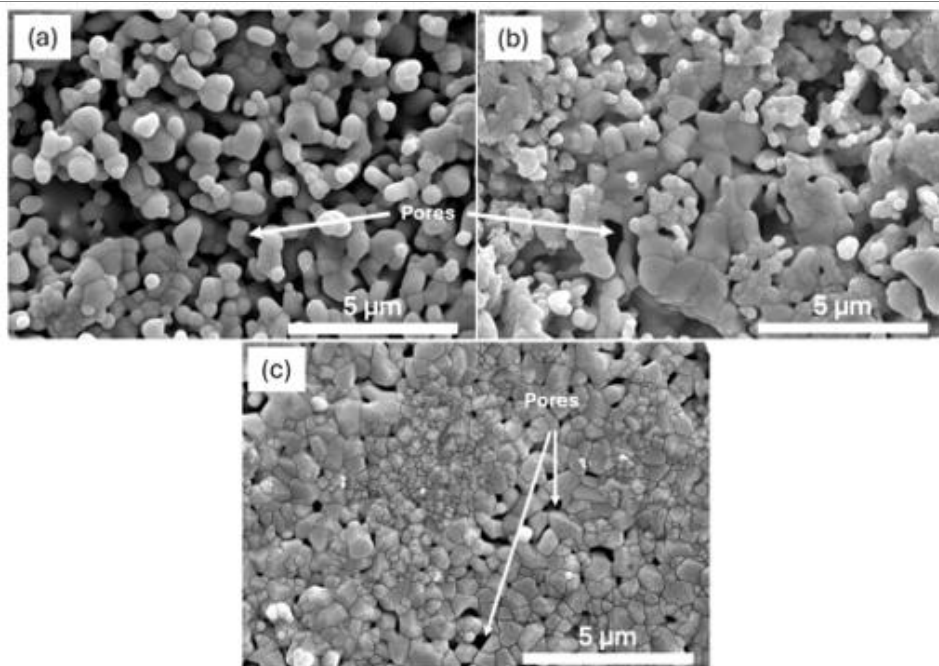


Fig. 4. Hardness of HA pellets sintered at 1000 °C (top) and FESEM microstructures of samples milled at 200 rpm (a), 300 rpm (b), and 400 rpm (c) (down), showing increasing densification and hardness with higher milling speed.

CONCLUSIONS

This study confirms that eggshell-derived CaO is a viable and sustainable precursor for producing HA. Milling speed played a major part in determining the final phase composition, crystallinity, and mechanical properties. Lower milling intensity at 200 rpm resulted in incomplete reactions, while 300 rpm improved HA formation but still produced secondary phases. The optimal condition was 400 rpm followed by heat treatment at 800 °C, yielding phase-pure, highly crystalline HA with refined particle morphology. Mechanical hardness increased with milling speed, reaching 730.83 ± 22.27 MPa at 400 rpm due to improved densification and reduced porosity. These results demonstrate that optimized milling—particularly at 400 rpm—produces high-quality HA suitable for biomedical applications. The successful use of eggshell waste also underscores the environmental and economic advantages of this green synthesis route.

Declaration

AI-assisted tools were used for language editing and manuscript writing refinement. All experimental data and research findings are original and were produced by the authors. The authors remain responsible for the accuracy and integrity of the manuscript.

ACKNOWLEDGEMENTS

This study is funded by the Universiti Teknikal Malaysia Melaka (UTeM) through the PJP/2024/FTKIP/PERINTIS/SA0025 research grant.

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