

The Properties of Hydroxyapatite Derived from Carbonate Eggshell Waste Through Ball Milling and Heat Treatment Method

R. M. Ghouse¹, A. N. Natasha^{1,2*}, N. F. Shahedan¹, S. Ramesh³, L. T. Bang⁴

¹ School of Mechanical Engineering, College of Engineering,
Universiti Teknologi MARA, 40450 Shah Alam, Selangor, MALAYSIA

² Fracture Mechanics and Materials Integrity Research Group, College of Engineering,
Universiti Teknologi MARA, 40450 Shah Alam, Selangor, MALAYSIA

³ Faculty of Engineering, University of Malaya,
50603 Kuala Lumpur, Wilayah Persekutuan Kuala Lumpur, MALAYSIA

⁴ School of Materials Science and Engineering,
Hanoi University of Science and Technology, Hai Ba Trung, 113000, Hanoi, VIETNAM

*Corresponding Author: natashanawawi@uitm.edu.my

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Abstract

Synthetic calcium phosphate, particularly hydroxyapatite (HA), has been used in various medical applications due to its biocompatibility and bioactivity. With time, increased interest in the development of HA from natural resources has started to emerge to produce more sustainable biomaterial precursors. HA has been successfully prepared from biogenic waste as the calcium precursor via extraction of calcium oxide from eggshells, seashells, and animal bones (cow, chicken, fish) via the wet method. The dry process, particularly solid-state reaction, has been least preferred due to the tendency of powder contamination and non-homogeneous powder mixing. However, with the proper processing regime, this method can produce pure HA on a mass scale with improved properties as it does not require precise control conditions. In this work, HA was synthesised from eggshell wastes through mechanochemical activation and heat treatment processes. The calcium precursor in pure calcium carbonate (CaCO_3) was extracted from eggshells through calcination. Then, the Calcium Hydrogen Phosphate Dihydrate (DCPD) as phosphorus precursor was added to the calcined eggshells via a simple mechanochemical route for 2 hours, and the obtained powder mixture was subjected to heat treatment at 800°C to obtain pure HA. XRD analysis confirmed the formation of phase pure HA in the synthesised powder with an average crystallite size of 26.35 ± 0.1 nm and an average agglomerated particle size of 520 nm. Energy Dispersive X-ray (EDX) analysis has validated the elemental composition of synthesised HA with a Ca/P ratio of 1.76, which closely resembles pure HA with a Ca/P ratio of 1.67. Hence, through calcination, eggshell waste in pure CaCO_3 form has been successfully utilised as a sustainable calcium precursor in producing HA via a dry method approach with high potential as a biomaterial for biomedical applications and has indirectly contributed to cost reduction of materials processing.

1. Introduction

Calcium Phosphate (CaP) has been an interesting substance in many branches of study including biology, chemistry, geology and medicine due to its abundance in nature and existence in living organisms [1]. Note that the majority of calcium in the human body is stored in bone tissue as nanometric crystals of a calcium phosphate compound with properties resembling those of hydroxyapatite (HA). Hence, HA with the chemical composition of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, is the most significant among the calcium phosphate compounds as its composition is found close to the dominant inorganic component of human body hard tissues such as bone, dentin, and enamel [2]. Furthermore, it is the most promising material for replacing bone grafts because of its excellent adhesion to the surface of bodily tissue [3]. However, it should be mentioned that calcium phosphate in bone is similar but not identical to the mineral HA due to the presence of significant amounts of carbonate ions and traces of other ions substituted into its structure [4].

Various techniques have been developed over the years to prepare HA. Generally, the raw materials used to synthesise HA originate from an inorganic precursor or natural organic-based resources, including conversion from marine coral derivatives to natural HA with acceptable porosity [5]. Nevertheless, both types of biomaterials are equally bioactive and biocompatible.

HA can be converted from natural bio-sources such as bovine bones, fish bones, cuttlefish shells, oyster shells and coralline via solid-state processing. These biological compounds are generally denoted as calcium-deficient and contain a wide variety of relatively small amounts of substitute atoms or groups such as carbonate, Na, Mg and Sr ions [5], [6]. The primary advantage of using bones from animal species is their inherent morphology and pore structure, similar to human bone. However, problems do arise due to the variable nature of the bone's physical and chemical properties. Unlike eggshells, the continuous use of some of these resources may lead to their extinction, for example, in the case of corals, as they have a slow growth rate [6].

HA and its precursors can be converted from biological resources and grouped into three types: bone, plants and biogenic. In this case, bones present mammalian (bovine, sheep, pig), chicken and fish bones, while biogenic sources are eggshells, seashells and conversion from marine coral derivatives to natural HA with acceptable porosity. These biological compounds denoted as calcium deficient, contain a wide variety of relatively small amounts of other substitute atoms or groups such as carbonate, Na, Mg and Sr ions [6], [7]. It was reported that the biomolecule amount of these waste materials influenced the latter product's shape, size and crystal morphology, reflecting their applications.

To date, eggshell waste composed predominantly of CaCO_3 (~ 94 %) have been used as a raw material or direct source of calcium precursor to synthesise HA. In addition, the remnants of several trace elements from the eggshells' natural biological origin, e.g. K, Na, Mg, Si and Sr, in the crystalline structure of synthesised HA make its composition similar to human bone. Generally, HA is produced using commercial analytical grade calcium and phosphorus compounds that are relatively expensive. However, growing interest in using more sustainable precursors to produce biomaterials is pushing towards reusing and recycling biogenic waste. Thus, these drawbacks justify using natural resources as precursors in the synthesis of HA, as it is both sustainable and cost-effective. Besides that, the HA synthesised from eggshells demonstrated superior sinterability and better morphology when compared with HA synthesised from other sources [8]-[10]. Regarding cell culture, HA synthesised from eggshells showed a better cell response because the different elements inherent in eggshells could induce a better guide signal for the cells to adhere and migrate [11].

HA derived from eggshell can be synthesised by microwave irradiation precipitation, ball milling and attrition milling processes and solid-state reactions. However, it is reported that this milling process induced the risk of contamination that affects the purity of the end product [12]. As a result, the dry method, particularly the solid-state method, has received the least attention, probably because of the difficulty in obtaining homogeneous HA composition and the small diffusion of ions within the solid phase. However, this method is suitable for the mass production of highly crystalline HA powders with good morphology [13] and mechanical properties as it does not require precise control conditions [14].

In previous research, by modifying the milling process to minimise contamination, the eggshell-derived HA powders were fabricated using a solid-state reaction using eggshell in CaO as the source of calcium precursor [6]. In another research, nanostructured flower-like agglomerated particles of eggshell-derived HA have successfully been prepared via a simple solid-state route through attrition milling [15]. Based on these studies, mixing the starting materials is vital for homogenisation before the structural transition occurs during heat treatment. With a proper processing regime, the dry method can produce pure HA on a mass scale with good morphology [10] and mechanical properties as it does not require precise control conditions [14].

Raw eggshells are rich in calcium carbonate and can be easily converted into HA through environmentally sustainable processes. Therefore, this research aims to produce HA using eggshell waste as a source of Ca precursor in the form of CaCO_3 as an alternative to conventional HA bioceramics produced by using 100% synthetic chemicals, besides exploring the potential of the dry method in preparing eggshell-derived HA with better mechanical properties through the optimisation of its mechanochemical and sintering parameters. This

work synthesises HA from eggshell waste through mechanochemical activation (ball milling) and heat treatment processes. Ball milling is proposed as the mechanochemical activation method. It is efficient in grain size reduction, and this technique is reasonably straightforward, with low-temperature processing and easy composition control.

2. Methodology

In this work, HA was synthesised via a solid-state reaction method through mechanochemical activation (ball milling) and heat treatment. The waste chicken eggshell was collected from the University of Technology Mara (UiTM) student canteen in Shah Alam, Selangor. The waste eggshells were initially cleaned, and the inner membrane layer was removed by rinsing with distilled water. After that, it was dried in an oven at 60 °C for 24 hours to remove the residue and odour from the waste eggshell. Before calcination, dried eggshells were crushed and ground into a fine powder consistently with a pestle and mortar. The eggshell was then calcined in a furnace at a rate of 5°C/min at the temperature of 700 °C for 2 hours to obtain pure calcium carbonate, CaCO_3 which was then used as a calcium precursor to synthesis HA. To initiate the solid-state reaction, the calcined eggshell powder in the form of CaCO_3 was mixed with phosphate precursor, dicalcium phosphate dihydrate (DCPD), and ethyl alcohol at the existing calcium and phosphorus (Ca/P) ratio of 1.67 under magnetic stirring conditions. The eggshell was then ball milled for 2 hours to induce a homogeneous starting material and fine particle mixture. The ball mill was set to 400 rpm, with one repetition. The ball and the mixture weight ratio were kept at 2:1. After the milling process, the eggshell powder was sieved through a 212 mm mesh sieve to ensure the homogeneity of the milled powder. The mixture was then heat treated at a rate of 5°C /min for 5 hours at a temperature of 800 °C to obtain pure HA powder. Fig. 1 shows the process flowchart in pure HA preparation.

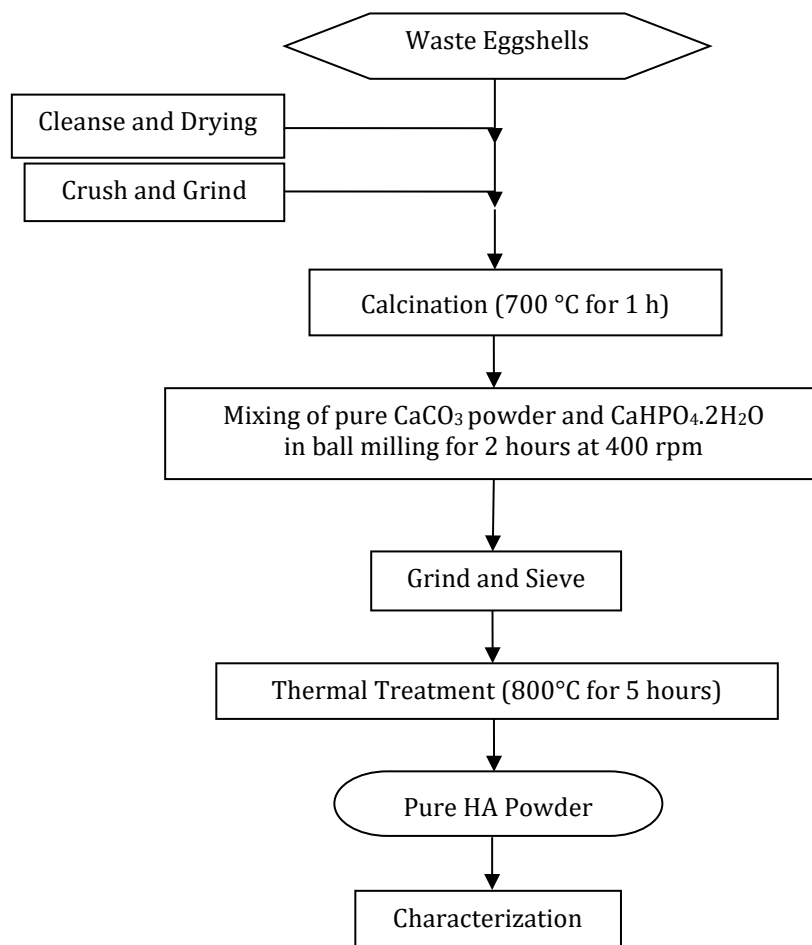


Fig. 1 Flowchart of preparation of HA powder using CaCO_3 from eggshell well as calcium precursor

For characterization, thermogravimetric analysis (TGA) is used to measure the material's thermal stability, reaction temperature, and the proportion of volatile components by measuring the weight change. At the same time, a sample is heated at a constant rate. In this study, approximately 23 mg of eggshell powder was heated at a room temperature of 30-1000°C. In a nitrogen atmosphere, a heating rate of 5°C/min was applied at a 60 mL/min

flow rate. The TGA data was used to determine the purity and composition of materials, drying and ignition temperatures, and compound stability temperatures.

The phase composition of HA powder was determined using X-ray diffraction (XRD) analysis to indicate the formation of highly crystalline pure HA powder. The parameter employed rate is 2°C/min for the diffraction range of 2θ° at 10° to 70°. The XRD was done on the powder mixture before and after heat treatment for 2 hours, 4 hours and 6 hours to examine the phase difference before heat treatment.

The average crystallite size for each sample was then calculated by applying Scherer's equation.

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

where t is the average crystallite size, λ is the wavelength of the XRD radiation, β is the full width at half maximum (FWHM) value of the measured diffraction peak, and θ is the diffraction angle of the investigated peak.

Scanning Electron Microscope (SEM) analysis was conducted to visualise the particle size [16] and the surface morphology of the synthesised HA powder. Meanwhile, Energy Dispersive X-ray (EDX) analysis helps to determine the calcium and phosphorous (Ca/P) ratio based on atomic percentage. The sputter coater coats the sample's surface to lower the charging effect for better imaging. Previous studies' literature reported that the sputter coating prevents the specimen from charging due to the concentration of static electric fields, which would otherwise occur [17].

3. Results and Discussion

Fig. 2 shows the TGA curve of the calcined eggshell and DCPD powder mixture. The first step showed a 1.53% weight loss at the midpoint of 39.57°C. The weight loss was correlated to the elimination of moisture from the sample. According to other researchers, it happened because of the evaporation of adsorbed water [18]. Next, the weight loss of 55wt% at midpoint 452.07°C was due to reduced organic matter. The prominent weight loss over the temperature range 650-710°C indicates that the partial reaction between CaCO₃ of the eggshell and CaHPO₄·2H₂O has occurred [15]. Eventually, the completed reaction between CaCO₃ and DCPD was indicated by negligible weight loss beyond 762.17°C. Hence, it is confirmed that the phase transformation to pure HA took place beyond this temperature, and this finding justifies the chosen calcination temperature of 800 °C that was carried out in this work.

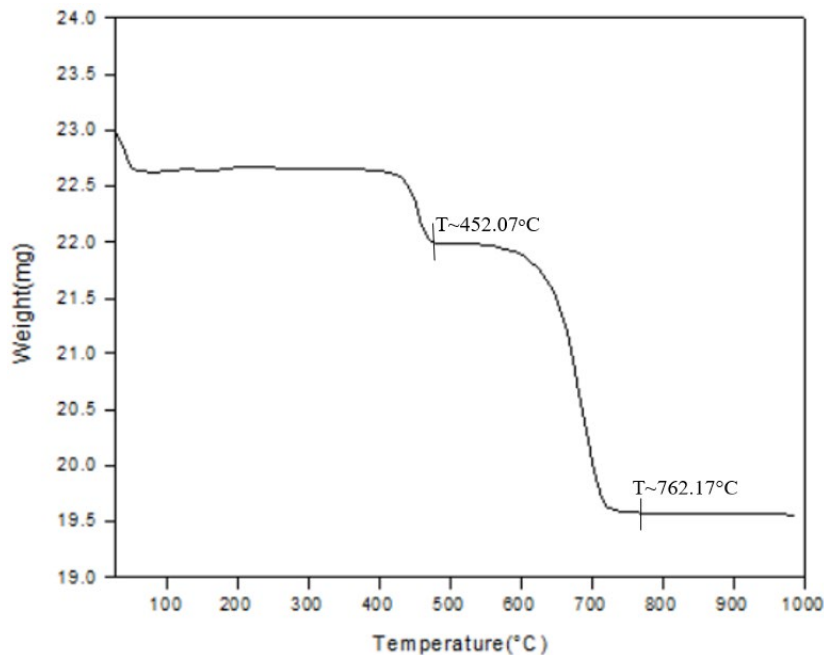


Fig. 2 TGA graph of eggshell powder mixture (CaCO₃ and DCPD)

Fig. 3(a) illustrates the XRD pattern for raw eggshell with the presence of predominant peaks of Calcium Carbonate (CaCO₃) known as Calcite, which is well-matched with the Joint Committee on Powder Diffraction Standards (JCPDS) Card Number 01-085-1108. Calcite is the form of CaCO₃ which contains a high percentage of calcium in waste eggshell [6], [10], [36] whereby the particles are highly crystalline with a trigonal structure of crystal system (recheck). Some diffraction peaks of raw eggshells also matched with Whitlockite Ca₃(PO₄)₂ (JCPDS

Card Number 00-055-0898). Fig. 3(b) shows the XRD peaks of eggshells upon calcination at 700 °C. The XRD analysis indicated that the only inherent crystalline phase of pure CaCO_3 remained at a 2θ angle of 29.7°. This phase stability suggests that complete decomposition to CaCO_3 has taken place.

Meanwhile, the XRD results in Fig. 3(c) showed that high phase purity of HA was observed in the heat-treated synthesised powder as demonstrated by the prominent peaks that belong to the HA group which are (002), (211), (112), (300) and (202) planes. These XRD peaks matched the peak position well with the standard HA phase from JCPDS card number 01-074-9944. In previous research by Ramesh et al. [14], which also used CaCO_3 from eggshells as a calcium precursor, pure HA was also obtained after the combination of 2 hours of attrition milling (400 rpm) and calcination at 800°C. Munira Khalid et al. [19] reported a similar result stating that heat treatment contributes to the formation of high purity of HA in the literature for the effect of heat treatment on the synthesis of HA from eggshell powders. Besides that, the sharp and intense XRD peaks of HA planes prove that the produced HA powder was crystalline and corresponds to the hexagonal crystal structure of HA.

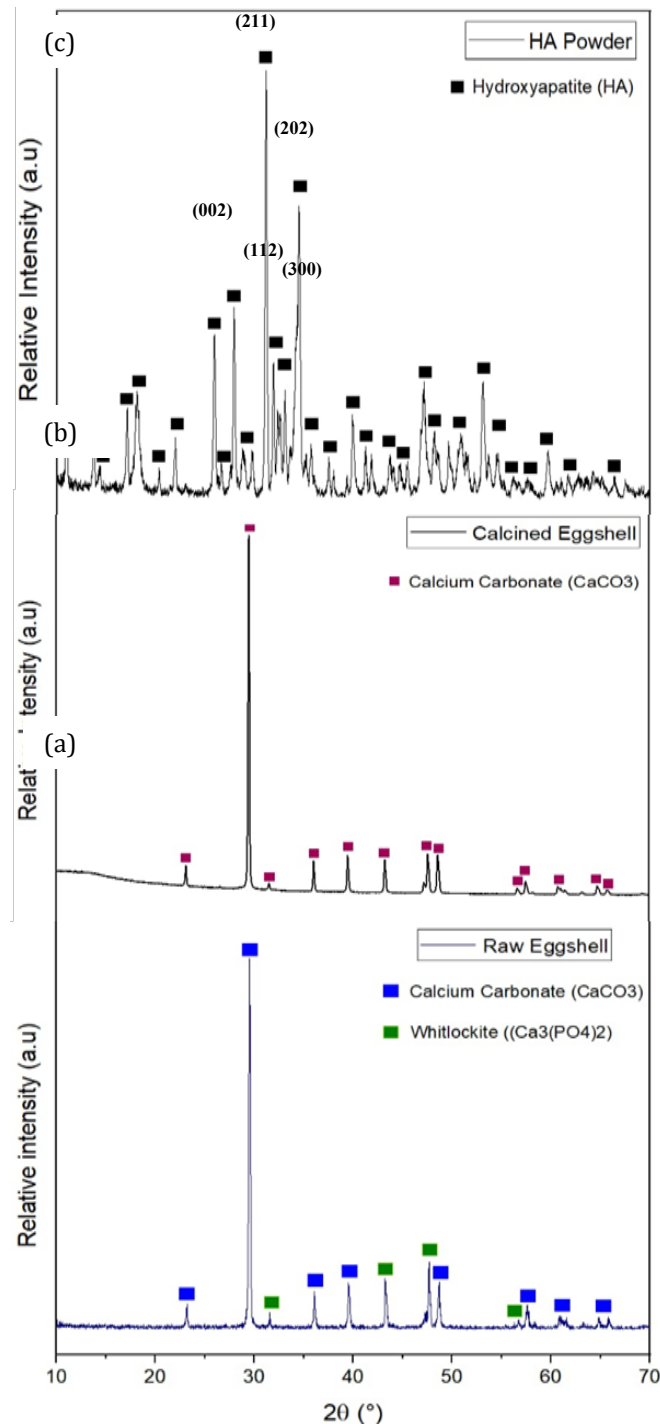


Fig. 3. XRD patterns of (a) raw eggshell; (b) calcined eggshell; and (c) HA powder

In addition to the phases present, three principal planes that corresponded to pure HA which are (211), (300), and (112) planes, were used to calculate the average of HA crystallite size (Fig. 3(c)). The average HA crystallite size was found to be 26.35 ± 0.1 nm. The crystallite size was smaller than in past studies using eggshells as a Ca precursor. In contrast, the evaluated crystallite size of HA was 54.6 nm via attrition milling at a similar milling time [6]. According to Poppy Puspitasari et al. [20], the larger crystallite sizes may be caused by agglomeration and influenced by the sintering process [15], [20]. However, in this work, the finding must be attributed to the ball milling process, resulting in a smaller crystallite size than manual grinding [21].

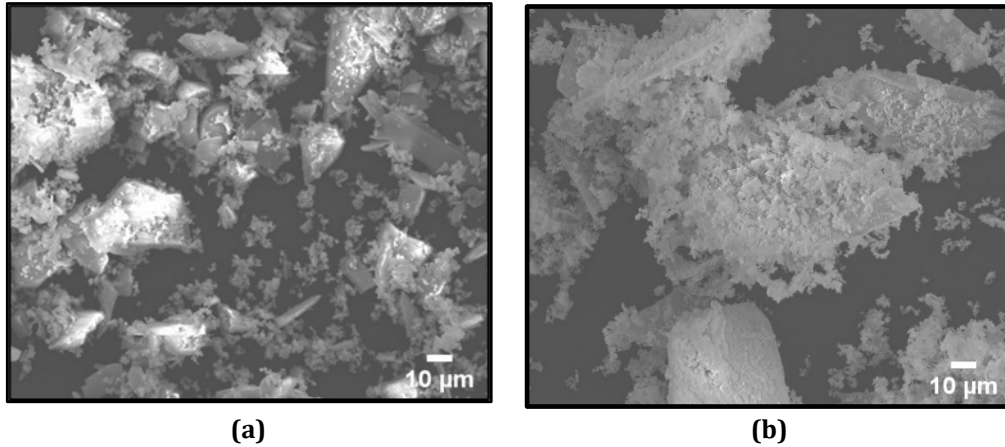


Fig. 4 SEM images of (a) Calcined eggshell and DCPD mixture; and (b) HA powder at 300x magnification

Fig. 4(a) shows the SEM image of the HA powder sample (after heat treatment at 800 °C), while the powder mixture (CaCO₃ and DCPD) sample (before heat treatment) SEM image is shown in Fig. 4(b) under a magnification of 300x. It was observed that after 2 hours of ball milling, the calcined CaCO₃ with DCPD mixture showed dispersed powder and soft agglomerates of powder particles. SEM images in Fig. 4(b) reveal the agglomeration of loosely packed smaller particles. It is known that the heat treatment process promotes the agglomeration in particle size, forming a bigger particle due to the rise of crystallinity degree [22]. A. A. Hamidi et al. described similar results and reported that the morphology shape of the powder tends to form agglomerate, contributing to the particles' non-uniform size. ImageJ software was used in this study to calculate the average particle size for both samples. As the results, this study justified the obtained average particle size of HA was bigger than the powder mixture, which is approximately 0.52 ± 0.02 µm and 0.14 ± 0.02 µm, respectively. This finding agrees with other works claiming that further heat treatment will result in bigger particle sizes of HA powder [19]. Besides that, it is expected that as the crystallite size of particles increases, the particle sizes observed by SEM analysis will also increase, as observed by Guihun Jiang [22].

The elemental composition and the weight and atomic percentages of present elements were tabulated in Table 1. Validation of HA synthesis had been proven with the presence of Ca, P, O, and C. The same elemental composition was reported in the previous studies where the HA represents the appearance of Ca, P, O, and C [19], [24], [25]. These elements are also the essential mineral constituents for bone growth [26]. Ca helps to reduce osteoporosis [27], P assists in bone resorption [28], and O helps in accelerating bone fracture healing [29]. The ca/P ratio for the observed HA powder was 1.76, which closely resembles the HA for human bone as the Ca/P ratio for perfect HA is well-known to be 1.67 [26], [30], [31]. Previous studies show that a high deviation [32] in the Ca/P ratio can be due to the lack of proper heat distribution [33], [34]. According to Rameez Saeed et al. [35], the oxidising conditions and fluctuating calcination temperature in the furnace were thought to be the causes of the impurity phases. Generally, this finding has indirectly supported that homogeneous HA could be attained via the solid-state method via a proper and sufficient mixing of the starting materials before the structural transition takes place during heat treatment.

Table 1 The elemental composition of eggshell-derived HA samples at different milling times

Ball milling (hours)	C	O	P	Ca	Ca/P ratio
2	5.89	56.61	13.14	24.36	1.85

4. Conclusions

Pure HA powder was successfully synthesised using sustainable calcium precursor, CaCO₃ which is derived from calcination of raw eggshells at 700 °C via the combination of mechanochemical activation for 2 hours and heat treatment at 800°C for 5 hours. XRD peaks that belong to the pure HA phase were observed for the synthesised

powder. The synthesised HA crystallite size was found to be 26.35 nm, which is smaller than HA derived by the attrition milling process at similar parameters. This finding confirmed the viability of the dry method through ball milling in producing HA with small particle sizes. SEM images revealed that the CaCO₃ with DCPD mixture before heat treatment showed dispersed and soft agglomerates of powder particles, while the obtained HA powder had the agglomeration of loosely packed smaller particles. This observation has justified heat treatment's influence in inducing the densification of produced powder particles. The average particle size for HA is 520 nm, while for the powder mixture, the value of particle size observed is 140 nm. Besides that, the presence of the significant constituents of HA, which are Ca, P, O, and C, were detected in the obtained HA powder and yielded the stoichiometric ratio for Ca/P of 1.76, which is closest to the theoretical value of 1.67. The results supported the idea that this straightforward method of 2 hours of mixing and heat treatment can produce HA powder with good physical properties. This result is significant in expanding the potential of HA powder from a sustainable precursor and straightforward approach as an ideal alternative for cost-effective, biocompatible biomaterials for biomedical applications.

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Conflict of Interest

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

Author Contribution

*The authors confirm contribution to the paper as follows: **study conception and design:** A. N. Natasha, S. Ramesh, L. T. Bang; **data collection:** R. M. Ghouse, A. N. Natasha; **analysis and interpretation of results:** A. N. Natasha, R. M. Ghouse; **draft manuscript preparation:** A. N. Natasha, R. M. Ghouse, N. F. Shahedan. All authors reviewed the results and approved the final version of the manuscript.*

References

- [1] Nawawi, N. A., Azmi, F. A., & Azizan, A. (2023). Densification of calcium phosphate from biogenic waste for biomedical application. *Malaysian Journal of Medicine and Health Sciences*, 19, 9-14. <https://doi.org/10.47836/mjmhs19.s18.2>
- [2] Oliveira Jr, P. H., Santana, L. A. B., Ferreira, N. S., Sharifi-Asl, S., Shokuhfar, T., Shahbazian-Yassar, R., & dos Santos, E. A. (2020). Manganese behavior in hydroxyapatite crystals revealed by X-ray difference Fourier maps. *Ceramics International*, 46, 10585-10597. <https://doi.org/10.1016/j.ceramint.2020.01.062>
- [3] Natasha, A. N., Ramesh, S., Bang, L. T., & Koay, M. H. (2021). Influence of calcination temperature in synthesizing eggshell-derived calcium phosphate. *Materials Today: Proceedings*, 48, 1915-1919. <https://doi.org/10.1016/j.matpr.2021.09.388>
- [4] Rajabnejadkeleshteri, A., Kamyar, A., Khakbiz, M., Bakalani, Z. L., & Basiri, H. (2020). Synthesis and characterization of strontium fluor-hydroxyapatite nanoparticles for dental applications. *Microchemical Journal*, 153, 104485-104488. <https://doi.org/10.1016/j.microc.2019.104485>
- [5] Ho, W. F., Hsu, H. C., Hsu, S. K., Hung, C. W., & Wu, S. C. (2013). Calcium phosphate bioceramics synthesized from eggshell powders through a solid state reaction. *Ceramics International*, 39, 6467-6473. <https://doi.org/10.1016/j.ceramint.2013.01.076>
- [6] Wu, S. C., Hsu, H. C., Hsu, S. K., Chang, Y. C., & Ho, W. F. (2015). Effects of heat treatment on the synthesis of hydroxyapatite from eggshell powders. *Ceramics International*, 41, 10718-10724. <https://doi.org/10.1016/j.ceramint.2015.05.006>
- [7] Fihri, A., Len, C., Varma, R. S., & Solhy, A. (2017). Hydroxyapatite: A review of syntheses, structure and applications in heterogeneous catalysis. *Coordination Chemistry Reviews*, 347, 48-76. <https://doi.org/10.1016/j.ccr.2017.06.009>
- [8] Ahmad Nawawi, N., Ahmad, N. N. A., Noor Hamdan, N. H. I., & Le Thi Bang, L. T. B. (2023). The densification of hydroxyapatite from eggshell waste at different sintering temperature. *Journal of Mechanical Engineering*, 12, 269-281. <https://doi.org/10.24191/jmeche.v12i1.24650>
- [9] Kumar, G. S., Thamizhavel, A., & Girija E. K. (2012). Microwave conversion of eggshells into flower-like hydroxyapatite nanostructure for biomedical applications. *Materials Letters*, 76, 198-200. <https://doi.org/10.1016/j.matlet.2012.02.106>

- [10] Abdulrahman, I., Tijani, H. I., Mohammed, B. A., Saidu, H., Yusuf, H., Ndejiko Jibrin, M., & Mohammed, S. (2014). From garbage to biomaterials: An overview on egg shell based hydroxyapatite. *Journal of Materials*, 2014, 1-6. <https://doi.org/10.1155/2014/802467>
- [11] Bang, L. T., Ramesh, S., Hamdi, N., & Sopyan, M. (2015). Effects of silicate and carbonate substitution on the properties of hydroxyapatite prepared by aqueous co-precipitation method. *Materials & Design*, 87, 788-796. <https://doi.org/10.1016/j.matdes.2015.08.069>
- [12] Sadat-Shojai, M., Khorasani, M. T., Dinpanah-Khoshdargi, E., & Jamshidi, A. (2013). Synthesis methods for nanosized hydroxyapatite with diverse structures *Acta Biomaterialia*, 9, 7591-7621. <https://doi.org/10.1016/j.actbio.2013.04.012>.
- [13] Ezerskyte-Miseviciene, A., & Kareiva, A. (2019). Everything old is new again: a reinspection of solid-state method for the fabrication of high quality calcium hydroxyapatite bioceramics. *Mendeleev Communications*, 29, 273-275. <https://doi.org/10.1016/j.mencom.2019.05.010>
- [14] Cho, J. S., & Rhee, S. H. (2013). Formation mechanism of nano-sized hydroxyapatite powders through spray pyrolysis of a calcium phosphate solution containing polyethylene glycol. *Journal of the European Ceramic Society*, 33, 233-24. <https://doi.org/10.1016/j.jeurceramsoc.2012.08.029>
- [15] Ramesh, S., Natasha, A. N., Tan, C. Y., Bang, L. T., Ching, C. Y., & Chandran, H. (2016). Direct conversion of eggshell to hydroxyapatite ceramic by a sintering method. *Ceramics International*, 42, 7824-7829. <https://doi.org/10.1016/j.ceramint.2016.02.015>
- [16] Luthfiyah, S., Soegijono, B., Susetyo, F. B., & Notonegoro, H. A. (2022). Comparing properties of bovine bone derived hydroxyapatite and synthetic hydroxyapatite. *Journal of Applied Science and Engineering (Taiwan)*, 25, 1045-1051. [https://doi.org/10.6180/jase.202212_25\(6\).0015](https://doi.org/10.6180/jase.202212_25(6).0015)
- [17] Mohd Pu'Ad, N. A. S., Koshy, P., Abdullah, H. Z., Idris, M. I., & Lee, T. C. (2019). Syntheses of hydroxyapatite from natural sources, *Heliyon*, 5, e01588-e01590. <https://doi.org/10.1016/j.heliyon.2019.e01588>
- [18] Razali, N., Jumadi, N., Jalani, A. Y., Kamarulzaman, N. Z., & Pa'ee, K. F. (2022). Thermal decomposition of calcium carbonate in chicken eggshells. *Malaysian Journal of Analytical Sciences*, 26, 347-359.
- [19] Khalid, M., Jikan, S. S. B., Adzila, S., Murni, Z., Badarulzaman, N. A., Rosley, R., & Hameed, M. U. (2022). Synthesis and characterizations of hydroxyapatite using precursor extracted from chicken egg shell waste. *Biointerface Research in Applied Chemistry*, 12, 5663-5671. <https://doi.org/10.33263/BRIAC124.56635671>
- [20] Puspitasari, P., Iftiharsa, M. A., Zhorifah, H. F. N., & Gayatri, R. W. (2021). Analysis of physical properties and compressibility of avian eggshell nanopowders in solid state reaction. *EUREKA: Physics and Engineering*, 5, 110-120. <https://doi.org/10.21303/2461-4262.2021.001670>
- [21] Mobarak, M. B., Hossain, M. S., Yeasmin, Z., Mahmud, M., Rahman, M. M., Sultana, S., Masum, S. M., & Ahmed, S. (2022). Probing the photocatalytic competency of hydroxyapatite synthesized by solid state and wet chemical precipitation method. *Journal of Molecular Structure*, 1252, 132142. <https://doi.org/10.1016/j.molstruc.2021.132142>
- [22] Jiang, G., Ramachandraiah, K., Wu, Z., Li, S., & Eun, J. B. (2020). Impact of ball-milling time on the physical properties, bioactive compounds, and structural characteristics of onion peel powder. *Food Bioscience*, 36, 100630. <https://doi.org/10.1016/j.fbio.2020.100630>
- [23] Hamidi, A. A., Salimi, M. N., & Yusoff, A. H. M. (2017). Synthesis and characterization of eggshell-derived hydroxyapatite via mechanochemical method: A comparative study. *AIP Conference Proceedings*, 1835, 020045-10. <https://doi.org/10.1063/1.4981867>
- [24] Lala, S. D., Deb, P., Barua, E., Deoghare, A. B., & Chatterjee, S. (2019). Characterization of hydroxyapatite derived from eggshells for medical implants. *Materials Today: Proceedings*, 15, 323-327. <https://doi.org/10.1016/j.matpr.2019.05.012>
- [25] Ma, L., Li, M., Komasa, S., Yan, S., Yang, Y., Nishizaki, M., Chen, L., Zeng, Y., Wang, X., Yamamoto, E., Hontsu, S., Hashimoto, Y., & Okazaki, J. (2022). Characterization of hydroxyapatite film obtained by Er:YAG pulsed laser deposition on sandblasted titanium: An in vitro study. *Materials*, 15, 2306. <https://doi.org/10.3390/ma15062306>
- [26] Agbabiaka, O., Oladele, I., Akinwekomi, A., Adediran, A., Balogun, A., Olasunkanm, O., & Olayanju, T. (2020). Effect of calcination temperature on hydroxyapatite developed from waste poultry eggshell. *Scientific African*, 8, e00452. <https://doi.org/10.1016/j.sciaf.2020.e00452>
- [27] Dashnyam, K., Bayaraa, O., Mandakhbayar, N., Park, J. H., Lee, J. H., Jang, T. S., Luvsan, K. & Kim, H. W. (2020). Nanoscale calcium salt-based formulations as potential therapeutics for osteoporosis. *ACS Biomaterials Science & Engineering*, 6, 4604-4613. <http://dx.doi.org/10.1021/acsbiomaterials.0c00219>
- [28] Bello, A., Dersjant-Li, Y., & Korver (2020). Effects of dietary calcium and available phosphorus levels and phytase supplementation on performance, bone mineral density, and serum biochemical bone markers in aged white egg-laying hens. *Poultry Science*, 99, 5792-5801. <https://doi.org/10.1016/j.psj.2020.06.082>
- [29] Vorland, C. J., Stremke, E. R., Moorthi, R. N., & Gallant, K. M. H. (2017). Effects of excessive dietary phosphorus intake on bone health. *Current Osteoporosis Reports*, 15, 473-482. <https://doi.org/10.1007/s11914-017-0398-4>

- [30] Gokcekaya, O., Ergun, C., Webster, T. J., Bahadir, A., Ueda, K., Narushima, T., & Nakano, T. (2021). Effect of precursor deficiency induced CA/P ratio on antibacterial and osteoblast adhesion properties of AG-Incorporated hydroxyapatite: reducing AG toxicity. *Materials*, 14, 3158. <https://doi.org/10.3390/ma14123158>
- [31] Mohammadi, M., Tulliani, J., Montanaro, L., & Palmero, P. (2021). Gelcasting and sintering of hydroxyapatite materials: Effect of particle size and Ca/P ratio on microstructural, mechanical and biological properties. *Journal of the European Ceramic Society*, 41, 7301–7310. <https://doi.org/10.1016/j.jeurceramsoc.2021.07.025>
- [32] Obada, D., Dauda, E., Abifarin, J., Dodoo-Arhin, D., & Bansod, N. (2020). Mechanical properties of natural hydroxyapatite using low cold compaction pressure: Effect of sintering temperature. *Materials Chemistry and Physics*, 239, 122099. <https://doi.org/10.1016/j.matchemphys.2019.122099>
- [33] Wati, R., & Yusuf, Y. (2019). Effect of sintering temperature on carbonated hydroxyapatite derived from common cockle shells (cerastodermaedule): Composition and crystal characteristics. *Key Engineering Materials*, 818, 37–43. <https://doi.org/10.4028/www.scientific.net/KEM.818.37>
- [34] Chen, P., Wang, S., Chien, R., Tu, C., Feng, K., Chen, C., Hung, K., & Schmidt, V. H. (2019). Evolution of the microstructural and mechanical properties of hydroxyapatite bioceramics with varying sintering temperature. *Ceramics International*, 45, 16226–16233. <https://doi.org/10.1016/j.ceramint.2019.05.144>
- [35] Wasim, M., Malik, R. S., Tufail, M. U., Jutt, A. U., Ahmad, R., & Deen, K. M. (2014). Synthesis and characterization of hydroxyapatite powder from natural bovine bone. *Journal of Biomimetics, Biomaterials, and Tissue Engineering*, 19, 35–42. <https://doi.org/10.4028/www.scientific.net/jbbte.19.35>