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CHARACTERIZATION AND SINTERING PROPERTIES OF HYDROXYAPATITE BIOCERAMICS SYNTHESIZED FROM CLAMSHELL BIOWASTE

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ABSTRACT: Hydroxyapatite (HA) is a type of calcium phosphate-based bioactive ceramic that resembles the mineral phase of bone and teeth with great potential for bone substitution and biomedical implants. Biogenic-derived HA emerges as a cheap and eco-sustainable alternative to improve waste utilization. However, hydroxyapatite has limited applications due to its apparent brittleness, thus prompting investigation for enhanced sintering properties. In the present study, the combination of calcination and chemical precipitation technique was used to extract hydroxyapatite (HA) from ark clamshells (*Anadara granosa*). The method successfully produced HA powder with a Ca/P ratio of 1.6 and characteristic bands corresponded to pure HA via Fourier Transform Infrared Spectroscopy (FTIR). The synthesized HA powder was then sintered at temperatures ranging from 1200 °C to 1300 °C, followed by mechanical evaluation of the density, Vickers hardness, fracture toughness and grain size. It was revealed that the samples sintered at 1250 °C achieved a relative density of ~88%, Vickers hardness of 5.01 ± 0.39 GPa, fracture toughness of 0.88 ± 0.07 MPa.m$^{1/2}$ and average grain size of ~3.7 µm. Overall, the results suggest that ark clamshell synthesized HA (ACS) had the potential to be used as functional bioceramics for biomedical applications.

ABSTRAK: Hidroksiapatit (HA) adalah sejenis seramik bioaktif berasaskan kalsium fosfat yang menyerupai fasa mineral tulang dan gigi, berpotensi besar mengantikan tulang dalam implan bioperubatan. HA yang berasal dari biogenik muncul sebagai alternatif yang murah dan eko-lestari dalam menambah baik pengurusan sisa. Walau bagaimanapun, hidroksiapatit mempunyai aplikasi yang terhad kerana mempunyai kerapuhan yang ketara, menyebabkan penyelidikan diperlukan bagi meningkatkan sifat sintering. Gabungan teknik kalsinasi dan pemendakan kimia telah digunakan dalam kajian ini, bagi

mengekstrak hidroksiapatit (HA) dari kulit kerang (*Anadara granosa*). Kaedah ini telah berjaya menghasilkan serbuk HA dengan nisbah 1.6 Ca/P dan jalur puncak sepadan dengan HA tulen melalui Spektroskopi Inframerah Transformasi Fourier (FTIR). Serbuk HA ini kemudian disinter pada suhu antara 1200 °C hingga 1300 °C, diikuti penilaian mekanikal pada ketumpatan, kekerasan Vickers, kerapuhan dan ukuran bijirin. Hasil ujian menunjukkan bahawa sampel yang disinter pada suhu 1250 °C mencapai ~88% ketumpatan relatif, kekerasan Vickers 5.01 ± 0.39 GPa, kerapuhan pada 0.88 ± 0.07 MPa.m$^{1/2}$ dan purata ukuran butiran ~ 3.7 µm. Secara keseluruhan, dapatan menunjukkan bahawa kulit kerang HA yang disentisis (ACS) berpotensi sebagai bioseramik bagi aplikasi bioperubatan.

KEYWORDS: *hydroxyapatite; bioceramics; chemical synthesis; calcination; sintering*

1. INTRODUCTION

Hydroxyapatite (HA) with a chemical formula of $Ca_{10}(PO_4)_6(OH)_2$, is a type of calcium phosphate-based ceramic that comprises the main mineral constituent to human bones and teeth, which is widely used in dental and orthopedic applications. The conventional methods to synthesize HA are solid-state, mechanochemical, chemical precipitation, hydrolysis, sol-gel, hydrothermal, emulsion, sonochemical, high-temperature processes or a combination of a few techniques [1]. Among these methods, wet-chemical precipitation is the most promising and low-cost technique [2,3].

In recent years, biowaste-derived HA has attracted attention as numerous food wastes such as bones, eggshells and seafood shells had piled up in landfill globally. Specifically, shell wastes such as oyster, mussel, scallop, clam and cockle are discarded in an abundant amount. Million tons of shell wastes have been discarded and piled up in landfills in China, Taiwan, Spain, South Korea, Peru, Indonesia, Nigeria, and Malaysia [4-7]. Instead, these shell wastes could be utilized to synthesize HA owing to the rich calcium carbonate ($CaCO_3$) content [8-10]. Typically, HA has been successfully synthesized via hydrothermal synthesis method by utilizing various species of clamshell such as *Strombus gigas*, *Tridacna gigas* [11], *Venerupis* [12], *Corbicula* [13,14], *Mercenaria* [15], and *Anadara granosa* [16]. However, the majority of studies did not report the mechanical properties of sintered HA. In the current study, *Anadara granosa* clamshell will be used as the calcium precursor to synthesize natural HA powder, followed by characterization of its properties at various sintering temperatures.

2. MATERIALS AND METHODS

2.1 Synthesis of Powder

Biogenic sources such as seashells or eggshells are good natural sources of calcium precursor for the synthesis of HA bioceramics. In this study, the *Anadara granosa* clamshells collected from peninsular Malaysia were used as the starting materials to synthesize HA. The as-received clamshells were washed thoroughly, rinsed with distilled water, and dried in an oven at 80 °C for one hour. The dried clamshells were then crushed, ground and sieved through a 300 µm test sieve. This was followed by calcination at 1000 °C for four hours in an electrical furnace (Carbolite Gero, UK), to transform the calcium carbonate ($CaCO_3$) into calcium oxide (CaO).

Figure 1 depicts the flow chart of the HA synthesis process via wet chemical precipitation technique. First of all, 0.25 M of calcium precursor to 0.15 M of phosphorus precursor was employed to achieve stoichiometric HA with the calcium/phosphorus (Ca/P)

concentration ratio of 1.67. 2.8 g of CaO powder was then added in 200 ml of distilled water to formulate the $Ca(OH)_2$ solution as the calcium precursor. The solution was subsequently magnetic stirred at 400 rpm for an hour and maintained at pH 12. On the other hand, the 2.05 ml concentrated H_3PO_4 (phosphorus precursor) was diluted into 200 ml of distilled water, stirred and kept at about pH 2. Subsequently, the prepared H_3PO_4 solution was then added dropwise into $Ca(OH)_2$ solution to begin the titration process, continued with a vigorous stirring at 700 rpm for 30 minutes. The NH_4OH solution was then added to adjust the pH to 10. This was followed by magnetic stirring at 500 rpm for an hour after the titration process and aging for 21 hours (to form white precipitate). Vacuum filtration was subsequently performed on the precipitates using an electrical aspirator pump (Jerio Tech, Korea). Finally, the precipitate was dried in an oven at 100 °C for 16 hours and then crushed and sieved through a 300 µm test sieve to obtain ark clamshell synthesized HA (ACS) powder.

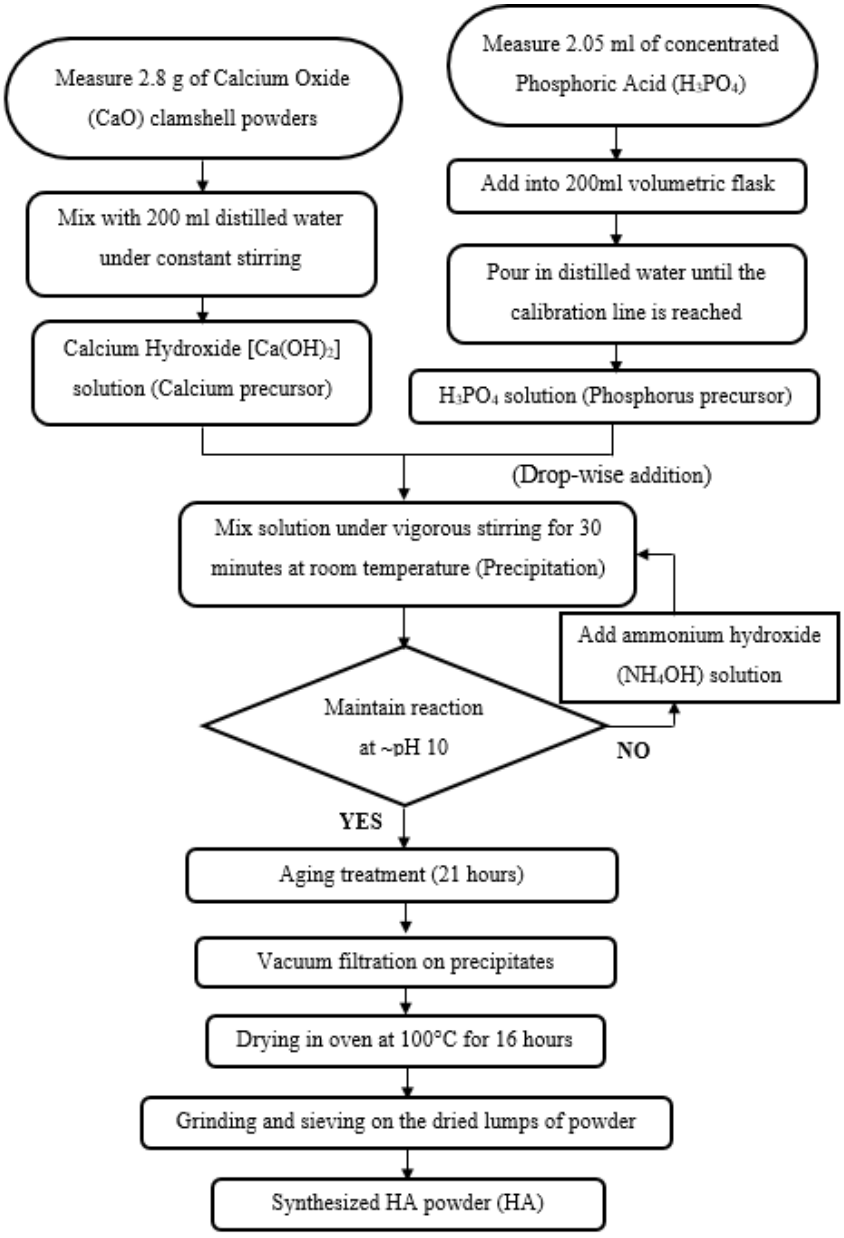

Fig. 1: Flow chart of the HA synthesis process via wet chemical precipitation technique.

2.2 Sample Preparation

The ACS synthesized HA powders were compacted into 20 mm disc samples (Fig. 2) by a hydraulic press machine (Enerpac, USA) at 1000 psi, which was set at a pressure lower than 3000 psi, as recommended by Mel et al. [17]. The green samples were then conventionally sintered (Carbolite Gero, UK) at 1200 °C, 1250 °C and 1300 °C for two hours with a ramp rate of 10 °C/min. The dimension of the green and as-sintered samples was recorded with a digital Vernier caliper (Mitutoyo, Japan) for the shrinkage measurements. Prior to characterization, the sintered disc samples were ground with silicon carbide (SiC) sandpapers and polished to achieve a 1 μm optical reflective surface.

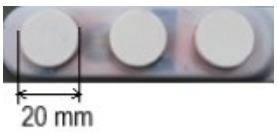

Fig. 2: HA green samples.

2.3 Characterization and Mechanical Property Evaluation

A Fourier transform infrared (FTIR) Spectrum 65 Spectrometer (Perkin Elmer Inc., USA) was used to identify the functional groups and composition present in the synthesized powder, at the scan range of 650 to 4000 cm^{-1}. Differential scanning calorimetric (DSC)/ Thermogravimetric analysis (TGA) (TA Instruments, USA) was employed to determine the weight loss and phase change of synthesized HA powder, from room temperature to 1400 °C, with a heating rate of 10 °C/min under nitrogen gas environment. Bulk density measurement was also performed on the sintered samples using the Archimedes' principle, by taking the theoretical density of HA as 3.156 g/cm^3. The microstructure of synthesized and sintered samples was examined via scanning electron microscopy (SEM) (SEC Co. Ltd., Korea). Energy dispersive X-ray (EDX) spectroscopy was used to determine the Ca/P ratio of the synthesized and sintered samples. The grain size of the sintered samples was measured using the linear intercept method according to ASTM E112-96. Vickers hardness of the sintered samples was evaluated via micro-hardness tester (Bowers ESEWAY, UK), with an applied load of 200 gf at a loading time of 10 seconds based on ASTM E384-99. For each sample, at least five indentations were used to obtain the average hardness and to calculate the standard deviation value. Fracture toughness was also obtained via the relationship derived by Niihara et al. [18].

3. RESULTS AND DISCUSSION

3.1 FTIR Analysis of ACS Synthesized HA Powder

The functional groups in the ACS synthesized HA powder were identified using FTIR and the spectrum is shown in Fig. 3. The result confirms that the synthesized powder exhibited the typical spectrum of pure HA powder, with the chemical groups of phosphate group (PO_4^{3-}), hydroxyl groups (OH^-) and carbonate groups (CO_3^{2-}). The distinctive peaks at 1025 cm^{-1} and 1087 cm^{-1} are corresponding to the PO_4^{3-} (v_3), while the peak at 962 cm^{-1} corresponds to the PO_4^{3-} (v_1). On the other hand, weak characteristic peaks observed at 3350 cm^{-1} and 3570 cm^{-1} could be related to the OH^- group. The FTIR peaks exhibited key characteristics of HA phase. The peaks at 1456, 1420 cm^{-1} and 874 cm^{-1} show the presence of CO_3^{2-} in the samples. Similar carbonate bands were also reported in previous studies [19-20].

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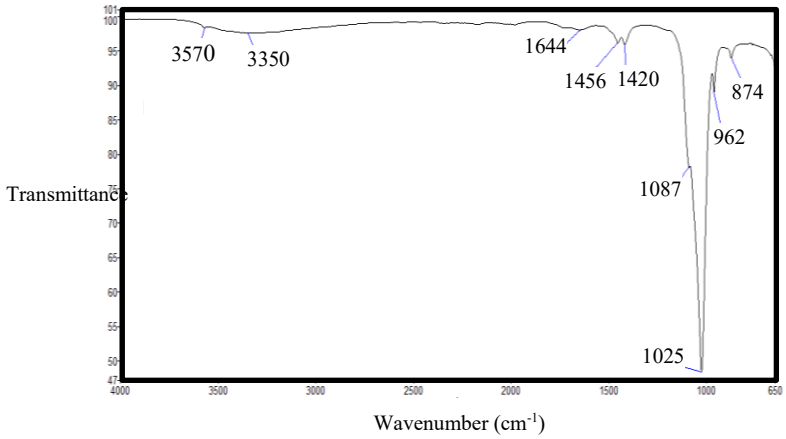
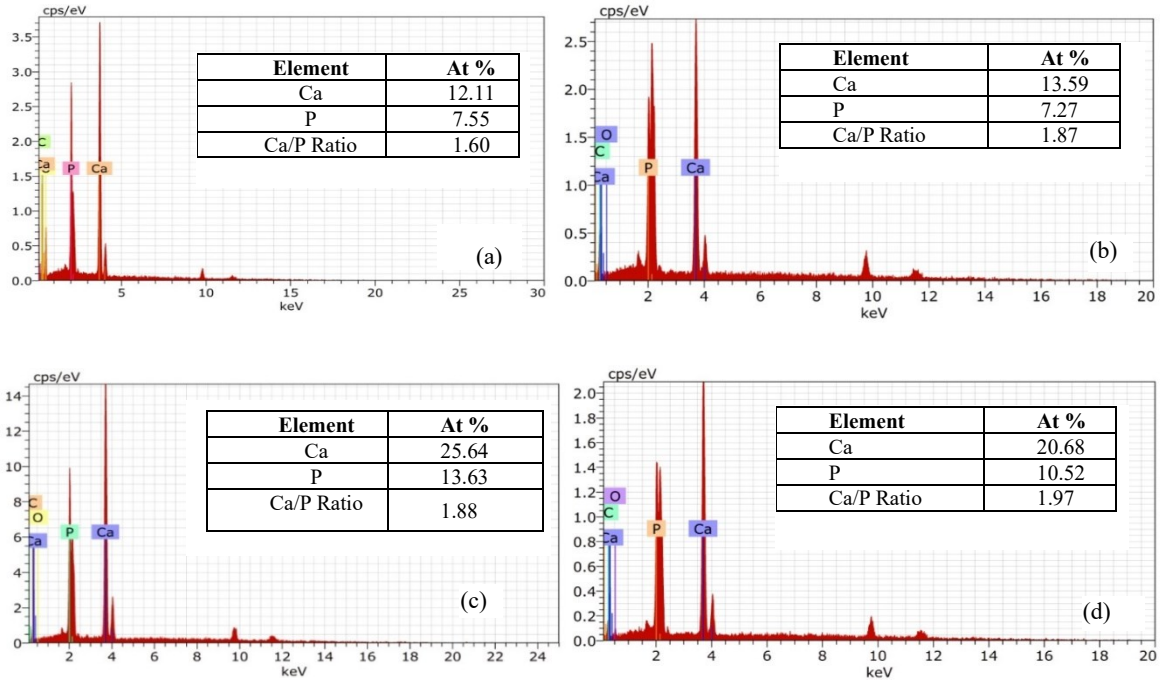


Fig. 3: FTIR spectrum of ACS synthesized HA powder.

3.2 EDX Analysis

Figure 4 shows that the EDX spectrums of ACS synthesized and sintered HA samples consist of three main elemental constituents of HA bioceramics, which are calcium (Ca), phosphorus (P) and oxygen (O). From the atomic percentage (At %), the calculated Ca/P ratio of synthesized biogenic HA powder is 1.60, while the sintered HA bioceramics increased to ~1.88 when sintered at 1200 °C and 1250 °C, and reached at 1.97 when sintered at 1300 °C. The obtained Ca/P ratio from this work deviated from the theoretical value for pure stoichiometric HA of 1.67. Similar observation was reported by Ramesh et al. [21]



Element	At %
Ca	12.11
P	7.55
Ca/P Ratio	1.60

Element	At %
Ca	13.59
P	7.27
Ca/P Ratio	1.87

Element	At %
Ca	25.64
P	13.63
Ca/P Ratio	1.88

Element	At %
Ca	20.68
P	10.52
Ca/P Ratio	1.97

Fig. 4: EDX spectrum ACS (a) synthesized HA powder, sintered HA at (b) 1200 °C, (c) 1250 °C, and (d) 1300 °C.

3.3 Microstructural and Grain Size Analysis

Figure 5 (a) shows the SEM micrograph of the ACS synthesized HA powder, which consists of large agglomerates. The microstructural evolution of the ACS sintered HA ceramics is presented in Fig. 5 (b)-(d). The SEM investigation revealed that the average grain size for the sintered HA samples increases with the increase of sintering temperatures. The results show a gradual increase in grain size from 2.14 µm at 1200 °C to 3.70 µm at 1250 °C. As the sintering temperature increased to 1300 °C, the grain size dramatically increased to 6.44 µm. Accelerated grain growth in HA samples sintered beyond 1250 °C implied a change in phase stability of HA.

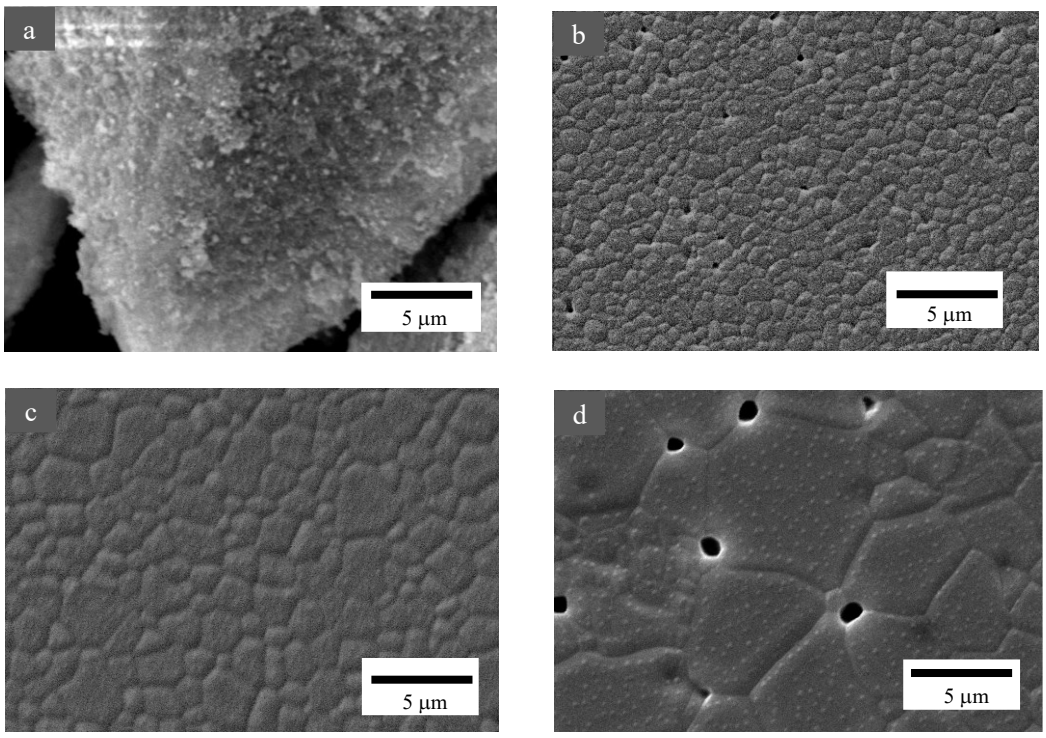


Fig. 5: SEM images of (a) ACS synthesized HA powder and sintered HA at (b) 1200 °C, (c) 1250 °C, and (d) 1300 °C.

3.4 Thermal Stability Analysis

Figure 6 shows the differential scanning calorimetric (DSC)/ thermogravimetric analysis (TGA) measurement of the ACS synthesized HA powder. At 300 °C, a pronounced weight loss of ~5.3% was observed from total weight loss of 7.6% of the HA sample. The weight loss could be ascribed to the evaporation of physically adsorbed water molecules. With additional heating upon 500°C, the insignificant weight loss (~0.75%) is attributed to the release of interstitial water molecule in the crystal lattice of HA. An endothermic peak at ~1000 °C can be postulated as dehydroxylation and decarboxylation of the HA powder. Weight loss at higher temperatures may be due to the dissociation of HA to tricalcium phosphate (TCP) and tetracalcium phosphate (TTCP). The decomposition of HA phase is believed to cause the increased in the average grain size and reduced the mechanical properties of the HA.

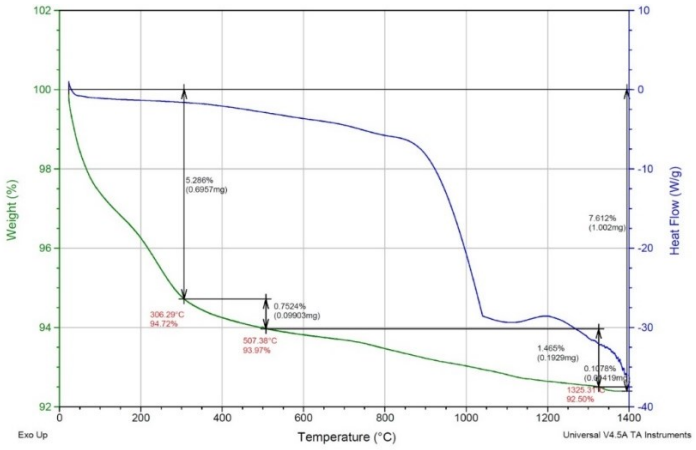


Fig. 6: DSC/ TGA plot of the ACS synthesized HA powder.

3.5 Mechanical Properties

The effect of sintering temperature on the relative density, Vickers hardness, and fracture toughness of HA ceramics are presented in Table 1. The density of ACS sintered HA increased from 83.8% at 1200 °C to 88% at 1250 °C. A slight decrease in density (86.7%) was observed when the sintering temperature increased to 1300 °C. The result is in agreement with the grain growth of the sintered HA as shown in the SEM images. On the other hand, the Vickers hardness of the sintered HA increased from 4.35 ± 0.43 GPa at 1200 °C to a maximum of 5.01 ± 0.39 GPa at 1250 °C. When the sintering temperature reached 1300 °C, the Vickers hardness was reduced to 4.03 ± 0.35 GPa, which is corresponding with the reduction in relative density and the grain growth of the sintered HA. This is in agreement with Aminzare and co-authors, where the Vickers hardness of the sintered biomimetic-synthesized HA decreased from 2.52 GPa to 2.23 GPa when sintered at 1250 °C and 1300 °C, respectively [22].

In the current study, the fracture toughness exhibited similar trends as the hardness, i.e. the fracture toughness increased from 0.67 ± 0.20 MPa.m$^{1/2}$ to a maximum of 0.88 ± 0.07 MPa.m$^{1/2}$, when sintered at 1200 °C and 1250 °C, respectively. This was followed by a decrease to 0.71 ± 0.10 MPa.m$^{1/2}$ when the sintering temperature reached 1300 °C. Similarly, the decreased fracture toughness at 1300 °C is related to the decrease of relative density. It is postulated that the reduced density and mechanical properties at 1300 °C was due to the decomposition of HA phase at a high sintering temperature regime (>1250 °C) [22-23]. The results also show that calcium-rich HA with the Ca/P ratio of 1.88 possessed an overall higher density and mechanical properties as compared to HA with Ca/P ratio of 1.97.

Table 1: The properties of ACS sintered HA

Sintering temp/ holding time	Grain size (µm)	Relative density (%)	Vickers Hardness (GPa)	Fracture toughness (MPam$^{1/2}$)
1200 °C/ 2 hrs	2.14	83.8	4.35 ± 0.43	0.67 ± 0.20
1250 °C/ 2 hrs	3.70	88.0	5.01 ± 0.39	0.88 ± 0.07
1300 °C/ 2 hrs	6.44	86.7	4.03 ± 0.35	0.71 ± 0.10

4. CONCLUSION

The present study revealed that ACS synthesized HA samples were successfully synthesized via the calcination and wet chemical precipitation using ark clamshells (*Anadara granosa*) as the calcium precursor. The results also show that ACS sintered HA with the Ca/P ratio of 1.88 possessed a higher density (~88%) and mechanical properties (Vickers hardness of ~5 GPa and fracture toughness of ~0.88 MPa.m$^{1/2}$) when sintered at 1250 ºC, which shows that the phase stability of HA was retained up to 1250 ºC and the decomposition of HA to TCP and TTCP occurred above 1250 ºC. The finding of this study would promote the recycling and reuse of the animal shells or bones to convert biowaste into value added biomedical products which would help in attaining Sustainable Development Goal targets. Future research would encompass further works on two-step or hybrid sintering routes to produce finer microstructure with enhanced mechanical properties.

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