Effect of Sintering Temperature on Hydroxyapatite Yield of Cuttlefish (*Sepia sp.*) Using the Wet Deposition Method

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Abstract. Hydroxyapatite, abbreviated as HAP ($Ca_{10}(PO_4)_6(OH)_2$), is the main component in bone structure which is osteoconductive. The HAP either synthetic or natural source, is widely used in biomedicine especially orthopedics or dental repair. In this study, HAP was synthesized using calcium and phosphate. Calcium was isolated from local cuttlefish shells (from Banggai Regency, Central Sulawesi) and phosphate in the form of a complex compound $(NH_4)_2HPO_4$ was obtained commercially. This study aims to produce hydroxyapatite and to determine the best sintering temperature with the purity obtained. Production of HAP from cuttlefish shells yields $Ca(OH)_2$ of 113.47% . Meanwhile, the best sintering results were obtained at 800 ℃ with HAP yield of 91.80% however there are no significant yields found at different temperatures of 900, 1000, 1100, and 1200 ℃. The results of FTIR characterization indicated that the functional groups refer to HAP. The X-RD characterization showed that HAP has a high-intensity crystallinity compared to amorphous.

Keywords: Cuttlefish Shell · Synthesis · Sintering Temperature · Hydroxyapatite

1 Introduction

Hydroxyapatite (HAP) $Ca_{10}(PO_4)_6(OH)_2$ is the main component of bone mineral that can be synthesized using calcium and phosphate precursors, HAP synthesis can utilize natural calcium sources such as those obtained from beef bones, fish bones, and cuttlefish shells. HAP is a bioceramic material that has been used as a substitute for artificial bones implanted into the human body.

Although human bone tissue has a very good ability to regenerate if the damage is severe enough, bone grafting is difficult, so the use of HAP can be a solution [1]. In this report, HAP was produced by using the wet deposition method, in which the reaction between Ca^{2+} ions and PO_4^{3-} ions also resulted in air and ammonium hydroxide as by-products. HAP products were characterized by X-RD to determine the phase and crystallinity; also by FTIR to determine the functional groups which may relate to HAP.

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The wet deposition method has been used by several researchers in research on the synthesis of HAP from waste materials, including research conducted in which the methy shell was calcined at a temperature of 900 ºC for 2 h and then obtained a calcium content of 48.5%, the HAP compound from the methyl shell produced the best yield of 59.12% which was produced at a sintering temperature of 1100 °C [2]. Then also using the wet deposition method with samples of cow bone waste, calcined at a temperature of 1000 ºC for 6 h and getting calcium from beef bone powder, which is 31.48%, HAP compounds from beef bones at a sintering temperature of 850 °C produced the highest yield of 48.05% [3].

2 Materials and Methods

Calcium elements were isolated from the shell of cuttlefish (*Sepia sp.*) which habituated around the Banggai Regency. Other materials are $(NH_4)_2HPO_4$ (Merck), NaOH 10% (*pa*)(Merck), distilled water, and filter paper.

The tools used in this research are Fourier Transform Infra Red (FTIR) Spectrophotometer, X-Ray Diffraction (XRD), sieve 100 mesh, furnace, stopwatch, mortar and pestle, the crucible, analytical balance, magnetic stirrer, desiccator, oven, burette 100 mL, stative, clamps, beaker glass 250 mL, measuring cylinder 100 mL, volumetric flask 100 mL, porcelain dish, petri dish, funnel and dropping pipet.

2.1 Isolation of CaO into Ca(OH)2 from Cuttlefish Shell

In the first step, the cuttlefish shells of as much as ±2 kg were washed and cleaned with water until the dirt attached to the shells was completely removed, dried at room temperature, then crushed and sieved through a 100 mesh sieve. In the next step, the sample was calcined at a temperature of 900 °C for 2 h to produce CaO. Product analysis was carried out by using FTIR and XRD. Afterward, the CaO compound was converted into $Ca(OH)_2$ by leaving it in direct contact with the air for a week at room temperature, then weighed again [4].

2.2 Synthesis of HAP

A powder of Ca(OH)2 as much as 3.7047 g was suspended in 100 ml to form Ca(OH)2 0.5 M. Then 100 mL (NH4)2HPO4 0.3 M slowly was added by titrating it into the 100 ml Ca(OH)2 0.5 M at 42 ºC and stirring speed at 300 rpm. After that, the mixture was left idle for 1 h at 90 ºC, then the mixture was stirred at the same temperature for 2 h. Furthermore, the mixture was adjusted to pH 10 by adding 10% NaOH, after that the mixture was left idle for 24 h at room temperature to form a precipitate. The precipitate was separated and washed with distilled water three times. Finally, the clean precipitate obtained was dried in an oven at 60 ºC for 24 h and then weighed [2].

2.3 Sintering Process

The sintering process was carried out at various temperatures; 800, 900, 1000, 1100, and 1200 °C for 6 h. The sintered HAP was cooled in a desiccator, then weighed to get the yield [2].

2.4 Determination of HAP Yield

The yield of the reaction from the synthesis of HAP with the wet deposition method are:

$$10Ca(OH)_2 + 6(NH_4)_2HPO_4 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 6H_2O + 12NH_4OH$$

$$(\%)\ \text{Yield} = \frac{\text{HAP actual}}{\text{HAP theoretical}} \times 100$$

2.5 Determination Yield of Sintering [4]

The calculation of the yield of HAP sintering using the wet method are:

$$(\%)\ \text{Rendemen} = \frac{\text{HAP actual}}{\text{Total mass of reactants}} \times \mathbf{100}$$

3 Results and Discussion

In the initial process, about 453.7560 g of cuttlefish shell powder was obtained, isolated by calcination and then hydrated so that the resulting color change was increasingly clean and the weight change was reduced compared to the weight before calcination. During one week of the hydration process, the yield of Ca(OH)2 was 113.47%. According to research from [9] a temperature and processing time calcination affects the yield of calcium oxide obtained, its level calcium oxide that is formed can be seen physically that calcium oxide calcination results have a high level of whitish color showed that the calcinations carried out were carried out perfectly which means almost all of the carbon dioxide present has evaporated (Fig. 1).

$$CaCO3 \xrightarrow{\Delta} CaO + CO_2 \quad (1)$$

$$CaO + H_2O \rightarrow Ca(OH)_2 \quad (2)$$

The calculation of HAp synthesis results uses two approaches namely yield (physical) and yield (scientific). Yield (physical) is a percentage comparison between the actual mass of HAp (the result of synthesis) with a mass of reactants, while yield (scientific) is the percentage comparison between actual HAp and theoretical HAp, where the theoretical HAp is calculated based on mole-based stoichiometry the limiting reagent that

Fig. 1. (a) Cuttlefish shell; (b) Powder of Cuttlefish shell samples before calcination; (c) Sample after calcination

is used up. The equation for the reaction that occurs in the HAp synthesis process is as follows:

$$10Ca(OH)2 + 6(NH4)2HPO4 \rightarrow Ca10(PO4)6(OH)2 + 6H2O + 12NH4OH$$

Ideally, the yield percentage of HAP result approached 100% (one hundred percent), but in this study, the yield ratio resulted in the discrepancy being quite large, which was around 19%. This discrepancy can be explained that the aspects of the transfer of reagents and products during the synthesis and post-synthetic weighing treatment processes. In the transfer process, it is suspected that the mass of the finished material is reduced due to the remaining mass in the previous container. The efficiency of the reaction during the stirring carried out in the synthesis process was assumed to be less than optimal because a solution of approximately 200 mL was stirred by using a medium-sized magnetic stirrer and the speed used was only 300 rpm; The time and temperature used for drying are less than optimal because it takes a lot of time so that the results obtained are too dry.

Based on the results of research conducted by [5] insufficient stirring will cause the formation of unwanted phases, and sufficient stirring will contribute to pH control and cause good interactions between reagents. This factor is also in accordance with [6] who said that a low rate of addition of precursors will lead to large crystallite size results, and a strong stirring rate was required to produce homogeneous HAP (Table 1).

The results of HAP sintering were carried out with temperature variations ranging from 800, 900, 1000, 1100 and 1200 ºC, from variations in sintering temperature the yields were sequentially as follows: 91.80; 89.67; 90.11; 89.64 and 89.42%. The yields obtained are not much different at each temperature, the decrease in yield at high temperatures is thought to occur due to the loss of water content in the sample.

According to [10] the decrease in yield that occurs during the sintering process is due to the loss of water content and organic matter contained in the sample (Table 2).

Table 1. Yield HAP

$Ca(OH)_2$ (g)	HAP Actual (g)	HAP Theoretical (g)	Yield (%)	Average Yield (%)
3,7020	4,3830	5,8701	74,67	81,21 ± 4,13
3,7040	4,8470	5,8730	82,53	
3,7040	4,6440	5,8715	79,09	
3,7050	4,5261	5,8715	77,09	
3,7100	4,4714	5,8756	76,10	
3,7082	4,4236	5,8830	75,19	
3,7050	4,9889	5,8730	84,95	
3,7052	5,1233	5,8782	87,16	
3,7049	4,8825	5,8707	83,17	
3,7044	4,9520	5,8761	84,27	
3,7058	4,9602	5,8733	84,45	
3,7072	4,9006	5,8725	83,45	
3,7051	4,9620	5,8731	84,49	
3,7030	4,9402	5,8748	84,09	
3,7060	4,5518	5,8795	77,42	

Table 2. Yield data on variations in sintering temperature.

Sintering temperature (ºC)	Yield (%)	Average yield (%)
800	90,76 90,46 94,17	91,80 ± 2,06
900	88,71 90,76 89,55	89,67 ± 1,03
1000	90,36 90,39 89,59	90,11 ± 0,45
1100	89,43 89,73 89,76	89,64 ± 0,18
1200	90,30 89,44 88,52	89,42 ± 0,89

FTIR characterization produces IR spectra as shown in Fig. 2(a) $Ca(OH)_2$ and (b) HAP synthesis. It can be seen that the results of the standard spectrum and after being synthesized showed a lot of peak changes which meant that the synthesis was successful, the spectrum of $Ca(OH)_2$ showed an absorption in the OH- group, which was indicated by the absorption band with the highest intensity, which was at 3460 cm^{-1}. While at wave numbers 1459 and 876 cm-1 an impurity group in the form of $CO_3{}^{2-}$. Research conducted by [11] explains that the decomposition of CaO compounds was identified in the long-range waves 1714, 1639, and 920 cm^{-1}.

The IR spectrum of HAP synthesis showed the absorption band of the OH- group at a wave of 3460 cm^{1}-. Meanwhile, the absorption bands for the PO $PO_4{}^{3-}$ group were 1036 cm^{-1}; 876 cm^{-1}, and 572 cm^{-1}. Where in the absorption band 1036 cm^{-1} and 876 cm^{-1} is a stretching vibration of the $PO_4{}^{3-}$ group, while in the absorption band, 572 cm^{-1} is the bending vibrations of the $PO_4{}^{3-}$ group. Then, the absorption band shown by the IR spectrum was not only the OH- group and the $PO_4{}^{3-}$ group but the presence of another functional group, namely $CO_3{}^{2-}$ in 1459 cm^{1-} absorption band. As well as the presence of other small peaks but not too prominent, it is possible that these small peaks are impurities that do not evaporate because the heating temperature is still standard (Fig. 3).

The results of the FTIR characterization analysis at sintering temperatures of 800 ºC, 900 ºC, 1000 ºC, 1100 ºC, and 1200 ºC found the presence of an OH^- group detected in waves 3448.72–3429.43 cm^{-1}. This indicates that the OH^- group is one of the constituents of HAP. According to [7] the absorption band of the OH^- group will be detected in the wave number range of 3000–3641.57 cm^{-1}, indicating that in that wave there is hydrogen bonding with the vibration of the OH^- functional group. In addition to the $PO_4{}^{3-}$ and OH^- groups which are read in the IR spectrum, the $CO_3{}^{2-}$ group is also

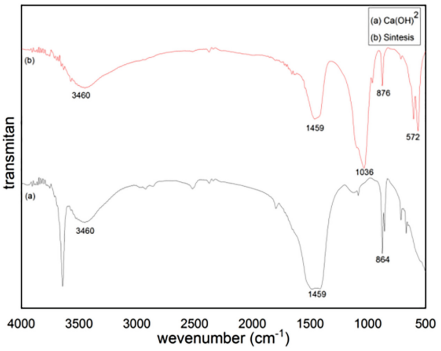

Fig. 2. Spectrum IR (a) Ca(OH)2 (b) Synthesis HAP

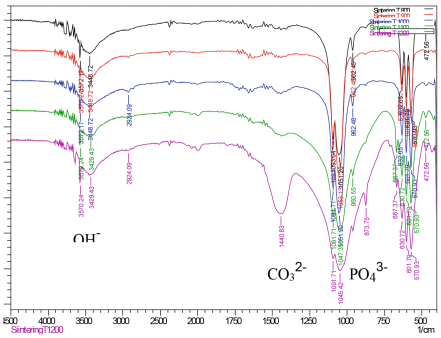

Fig. 3. IR Spectrum at Variation of Sintering Temperature

detected in the wave number spectrum of 1440.83–1490 cm^{-1} which is thought to be due to the presence of $Ca_{10}(PO_4)_6CO_3$ which has not changed to $Ca_{10}(PO_4)_6(OH)_2$ during the synthesis process which is considered as an impurity, in addition to impurities there are other factors that become one of the causes, namely at a temperature that is too high (1200 ºC) the electricity used is not in a stable state and the equipment used cannot reach a temperature that is too high. According to [1] the presence of impurities (CO_3^{2-}) cannot be avoided if the synthesis process is carried out openly with free air, so it is necessary to test in an environment that is slightly exposed to air.

The results of X-RD characterization showed changes in the peak of $Ca(OH)_2$ and HAP synthesis, in $Ca(OH)_2$ powder diffraction there were peaks with the three strongest intensities at angles of $2\theta = 17.94$; 30.58; 34.00º which are the phase pattern typical diffraction of $Ca(OH)_2$. The characterization results obtained are almost in accordance with JCPDS No. 04-0733 and the results obtained by [8] $Ca(OH)_2$ are characterized by its presence at an angle of $2\theta = 18.04$; 28.71; 34.13º. Diffraction of sintering temperature variations ranging from 800, 900, 1000, 1100, and 1200 ºC showed that the peak positions were almost the same overall, but differed at each intensity. It can be seen that the temperature of 900 ºC has a higher peak compared to other temperature variations, it can be stated that the level of crystallinity at that temperature is higher than the existing

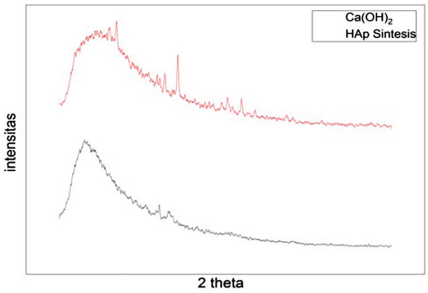

Fig. 4. Diffraction (a) Ca(OH)2; (b) Synthesis HAP

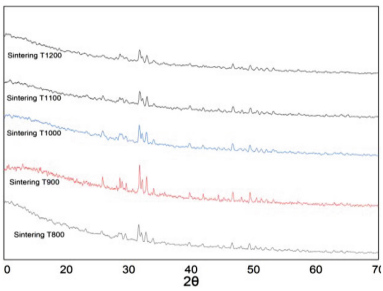

Fig. 5. X-ray diffraction sintering temperature variation

amorphous phase. The results obtained from the X-RD characterization showed that each sample was mostly present in the form of HAP, the identification of the HAP phase which was characterized by a distinctive diffraction peak between the angle of $2\theta = 20–80^{\circ}$. At sintering temperatures of 800, 900, 1000, 1100, and 1200 ºC, the typical phases of HAP are different at each angle of 2θ, each angle from temperature variations: At T800 ºC the angle of 2θ formed is 33.96º; 39.86º; 41.88º, T900 ºC angle $2\theta = 31.71^{\circ}$; 32.84º; 32.13º, At T1000 C angle $2\theta = 31.76^{\circ}$; 32.9º, At T1100 C angle $2\theta = 31.75$; 32.88; 8.84, At T1200 C angle $2\theta = 31$, 66º; 33.02º. The results of the sintering temperature with an angle of 2θ which is closest to the standard HAP data, namely a temperature of 900 ºC because the three strongest peaks are at that temperature and the highest intensity is at an angle of 2θ 31.71° (128), this proves that the level of crystallinity is higher than the amorphous (Figs. 4 and 5).

4 Conclusion

The yield of HAP from cuttlefish shells was 113.47%. The best sintering temperature was obtained at 800 °C with an HAP yield of 91.80% but not significantly different at temperatures of 900, 1000, 1100, and 1200 °C. The results of the FTIR characterization have obtained the HAP functional group and the X-RD characterization results have been obtained with high intensity compared to amorphous.

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