The characterization of hydroxyapatite from blood clam shells and eggs shells: Synthesis by hydrothermal method

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Abstract. Hydroxyapatite is a bioceramic that can be used as a denture material. Hydroxyapatite can be produced by synthesizing blood clam shells and egghells as a source of calcium and \( \text{(NH}_4\text{)}_2\text{HPO}_4 \), phosphate through hydrothermal methods. The hydroxyapatite samples were characterized by XRD and FTIR. The results of XRD analysis showed that the hydroxyapatite produced contained calcium carbonate \( (\text{CaCO}_3) \) and apatite carbonate type A. Hydroxyapatite has a hexagonal structure with lattice parameters \( a = 9.404 \) Å and \( c = 6.675 \) Å the resulting crystal size ranges from 10.19 nm to 57.29 nm. The results of the FTIR spectrum of the hydroxyapatite sample contained functional groups \( \text{PO}_{4}^{3-} \), \( \text{OH}^- \) and \( \text{CO}_3^{2-} \) which are functional groups of hydroxyapatite.

1. Introduction

Hydroxyapatite has been widely used in medicine and dentistry [1-3]. Hydroxyapatite is non-toxic, bioactive, biocompatible with surrounding tissues, and osteoconductive in nature, so it can be used as the rehabilitation of bone and tooth tissue [4,5]. Hydroxyapatite compounds are apatite and inorganic minerals that contain calcium and phosphate [6,7]. Hydroxyapatite has a monoclinic and hexagonal crystal structure. The monoclinic structure is due to the fact that \( \text{OH}^- \) is arranged in the order \( \text{OH}^- \text{OH}^- \text{OH}^- \text{OH}^- \) so that the lattice parameter \( b \) is doubled. The hexagonal structure occurs under stoichiometric conditions with an irregular \( \text{OH}^- \)-arrangement [8]. Hydroxyapatite material can be made through the synthesis of chemical compounds and from natural materials [9]. Hydroxyapatite from synthetic chemical compounds such as synthesizing calcium nitrate compounds \( \text{Ca(NO}_3\text{)}_2 \) with ammonium hydrogen phosphate \( \text{(NH}_4\text{)}_2\text{HPO}_4 \) and calcium hydroxide \( \text{Ca(OH)}_2 \) with phosphoric acid \( \text{H}_3\text{PO}_4 \) [10] while hydroxyapatite from natural ingredients contains \( \text{CaCO}_3 \) (calcium carbonate) such as blood clam shells \( (\text{Anandara granosa})[11] \), egg shells [7,12], beef animal bones [13] and tuna fish bones [14].

Research on the synthesis of hydroxyapatite from natural ingredients has been carried out such as from egg shells [7,12,15], coral [16], seashell [15,17] and clam shells [18]. The shells as a source of calcium carbonate have pores that are able to absorb other substances into the surface pores. The composition of the shells consists of \( \text{Ca}, \text{Mg}, \text{Na}, \text{P} \) and other minerals [19]. The blood clam shell contains 98% calcium carbonate which can be used as a hydroxyapatite material [20]. Research on the synthesis of hydroxyapatite from various clam shells has been carried out by [11] who synthesized blood clam shell to produce \( \text{CaCO}_3 \) by the precipitation method. Arrafique et al [21] synthesized hydroxyapatite from waste shell fish \( (\text{geloina expansa}) \) by hydrothermal method which resulted in a \( \text{Ca/P} \) ratio of 1.58, Muntamah [20] has synthesized hydroxyapatite from shells of blood clams using two
methods, namely the wet method and the dry reaction method which yielded Ca/P mole ratios of 1.64 and 1.84, respectively. Bharatham et al [22] synthesized hydroxyapatite from blood clams using sol gel precipitation method which resulted in a Ca/P ratio of 1.67. Khoirudin [23] synthesized hydroxyapatite from blood clam shells using the hydrothermal method which produced PO₄³⁻ and OH⁻ functional groups, while Siswanto [24] utilized dry scallop shell waste to produce CaO and Ca (OH)₂.

The hydroxyapatite from egg shells are composed of calcium carbonate (CaCO₃), calcium phosphate (CaPO₄), magnesium carbonate (MgCO₃) and magnesium phosphate (MgPO₄) compounds [25,26]. The synthesis of hydroxyapatite material from egg shells (chicken, duck and quail) has been carried out by [27] with single drop and wise drop precipitation methods, where the hydroxyapatite content in chicken eggshells is 70.84%, while the calcium for quail egg shells is 55, 46%, and duck egg shells is 53.60%. Tyas [28] synthesized eggshells of broilers and native chickens using the wise drop precipitation method which resulted in a Ca/P ratio below 1.67. The synthesis of hydroxyapatite from eggshells of native chickens and broilers has been carried out by Cahyati [29] with the precipitation wise drop method, the Ca/P ratio was obtained between 1.5-1.62.

The synthesis of hydroxyapatite material can be carried out by various methods, namely the wise drop precipitation method [30], the sol gel method [31], the mechanochemical method [32], and the hydrothermal method [33]. The use of different methods for the synthesis of the hydroxyapatite material can affect the particle size and character of the hydroxyapatite material [34]. The results of the synthesis of the hydroxyapatite material by the hydrothermal method obtained particles that have high crystallinity [23,29], high purity [35] and homogeneous particle distribution [36].

This article discusses the synthesis and characteristics of hydroxyapatite material from blood clam shells and egg shells using the hydrothermal method. The effect of mixed variations of blood clam shells and egg shells on the structure and size of the crystals and functional groups of the hydroxyapatite material will be discussed.

2. Methods

Hydroxyapatite has been synthesized from blood clam shells and egg shells using the hydrothermal method. Hydroxyapatite was synthesized by the ratio of the mass percentage of powder between clam shells and egg shells (i.e. A=100%;0%; B= 0%;100%; C=25%;75%; D=50%;50%; and E=75%;25%) in 5 grams. Both powders were dissolved with 15 ml (NH₄)₂HPO₄ until homogeneous and formed a gel. After that, the gel is dried in an oven at 160 °C for 5 hours, then ground into a very soft powder. The hydroxyapatite powder was calcined using a furnace for 1 hour at a temperature of 900 °C. The hydroxyapatite material was characterized by X-Ray Diffraction (XRD) analysis (merk Shimadzu, Type: MAXima_X XRD-7000) using Cu Kα radiation to determine the structure and size of the crystal, while the Fourier Transform Infra Red (FTIR) spectroscopy technique (merk Shimadzu, Type: IRPrestige 21) to define the information functional groups contained in the hydroxyapatite material.

3. Results and Discussion

The result of hydroxyapatite synthesis from blood clam shells and egg shells with various compositions using hydrothermal method were characterized by XRD analysis were presented in Figure 1. In Figure 1, shows the presence of diffraction peaks in each of the hydroxyapatite samples (A, B, C, D and E). These results are in accordance with the diffraction pattern of Joint Committee on Powder Diffraction Standards (JCPDS) 09-432 (hydroxyapatite), where the sample contains a hydroxyapatite compound characterized by three main peaks. The diffraction pattern and JCPDS hydroxyapatite data (09-432) are detailed in Table 1. The diffraction pattern of hydroxyapatite samples (A, B, C, D and E) is a shift of the diffraction peak (break angel) from an angle of 25° - 35°. This shift causes the crystal lattice to be stretched due to the effect of the calcination process which forms pores so that the spacing between the lattices increases [37] and the presence of other materials (see Figure 2). In Figure 2, there is another diffraction pattern which is the diffraction pattern of calcium carbonate (CaCO₃) and apatite carbonate of type A. The CaCO₃ compound is sampled B (100% egg shell) at angle of 2θ = 237.359° and the crystal plane [0 3 1], due to the calcination process which is not homogeneous and the presence of CO₂.
interaction from the air, then the CO$_2$ reacts with CaO to form CaCO$_3$ compounds. These results are consistent with the research of Khorudin [23] that the compound CaCO$_3$ can inhibit the formation of hydroxyapatite crystals. The presence of CaCO$_3$ compounds affects the 2θ shift in angle towards a larger angle, so it can cause the lattice of the hydroxyapatite crystals to widen [37]. The compounds of CaCO$_3$ are not found in hydroxyapatite samples A, B, C, D, and E, this is because the CaCO$_3$ compound has decomposed in the calcination process to become CaO. Compound Ca$_{10}$(PO$_4$)$_6$(CO$_3$)$_2$(OH)$_2$ (carbonate apatite of type A) was found in sample C (at an angle of 32.221°), sample D (at an angle of 32.251°) and sample E (at an angle of 32.236°). This is because there is a carbonate ion (CO$_3^{2-}$) which replaces the hydroxyl ion (OH$^-$) and is substituted for hydroxyapatite [38,39].

The resulting structure of the hydroxyapatite material is hexagonal with a lattice value of a = 9.404 Å and c = 6.675 Å. The crystal size of the hydroxyapatite of sample was calculated using the Debye-Scherrer Equation [40], as shown in Table 2. The crystal size of the hydroxyapatite samples (A, B, C, D and E) was obtained between 10.10 nm - 57.30 nm.

![Figure 1](image_url)

**Figure 1.** The diffraction pattern of the sample from the hydroxyapatite materials
Figure 2. Angle of 2θ hydroxyapatite sample in the plane [0 0 2]

| Sample | 20° | h k l | Phase          |
|--------|-----|------|----------------|
| JCPDS  | 25.879 | 0 0 2 | HA (09-432)    |
|        | 31.773 | 2 1 1 | HA             |
|        | 32.196 | 1 1 2 | HA             |
|        | 32.902 | 3 0 0 | HA             |
|        | 34.048 | 2 0 2 | HA             |
| A      | 25.996 | 0 0 2 | HA (100%: 0%)  |
|        | 31.932 | 2 1 1 | HA             |
|        | 34.209 | 2 0 2 | HA             |
| B      | 25.797 | 0 0 2 | HA (0%:100%)   |
|        | 32.032 | 1 1 2 | HA             |
|        | 34.279 | 2 0 2 | HA             |
|        | 37.359 | 0 3 1 | CaCO$_3$       |
| C      | 25.897 | 0 0 2 | HA (25%:75%)   |
|        | 31.813 | 2 1 1 | HA             |
|        | 32.221 | 2 0 2 | AKA            |
|        | 34.109 | 2 0 2 | HA             |
| D      | 25.921 | 0 0 2 | HA (50%:50%)   |
|        | 31.844 | 2 1 1 | HA             |
|        | 32.251 | 2 0 2 | AKA            |
|        | 34.129 | 2 0 2 | HA             |
| E      | 25.906 | 0 0 2 | HA (75%:25%)   |
|        | 31.836 | 2 1 1 | HA             |
|        | 32.236 | 2 0 2 | AKA            |
|        | 34.144 | 2 0 2 | HA             |

HA : Hydroxyapatite
CaCO$_3$ : Calcium Carbonate
AKA : Carbonate apatite of type A
Table 2. Crystal size and d-spacing of hydroxyapatite

| Sample | $2\Theta$ (°) | FWHM | d-spacing | D (nm) | $D_{\text{average}}$ |
|--------|---------------|------|-----------|--------|---------------------|
| A      | 25.996        | 0.120| 1.786     | 67.94  | 57.29               |
| 100%:0%| 31.932        | 0.140| 1.490     | 59.02  |                     |
|        | 34.209        | 0.185| 1.406     | 44.92  |                     |
| B      | 25.797        | 0.945| 1.798     | 8.62   |                     |
| 0%:100%| 32.032        | 0.945| 1.486     | 8.74   | 10.19               |
|        | 34.279        | 0.630| 1.404     |        | 13.20               |
| C      | 25.897        | 0.140| 1.792     | 58.22  |                     |
| 25%:75%| 31.813        | 0.159| 1.495     | 51.95  | 47.89               |
|        | 34.109        | 0.248| 1.409     | 33.50  |                     |
| D      | 25.921        | 0.137| 1.790     | 59.50  |                     |
| 50%:50%| 31.844        | 0.147| 1.494     | 56.19  | 51.63               |
|        | 34.129        | 0.212| 1.409     | 39.19  |                     |
| E      | 25.906        | 0.126| 1.791     | 64.69  |                     |
| 75%:25%| 31.836        | 0.140| 1.494     | 59.00  | 55.44               |
|        | 34.144        | 0.195| 1.408     | 42.61  |                     |

The OH$^-$ group in the sample of hydroxyapatite (A, B, C, D and E) is not sharp, which means that the H$_2$O content in the hydroxyapatite sample was small [29]. PO$_4^{3-}$ and OH$^-$ functional groups appear with sharp peaks, this indicates that the results of hydroxyapatite synthesis have good crystals [21]. The CO$_3^{2-}$ functional group in the hydroxyapatite compound becomes an inhibitor of crystal growth. The FTIR spectrum of the hydroxyapatite sample (see Figure 3) has a sloping peak of the CO$_3^{2-}$ functional group, this shows that the CO$_3^{2-}$ element in the hydroxyapatite sample is minimal [21,26]. The presence of the CO$_3^{2-}$ group in the hydroxyapatite of samples (A, B, C, D and E) indicates the presence of carbon apatite compounds of type A. The results of FTIR spectrum analysis (Figure 3) and XRD analysis (Figure 2) showed that the hydroxyapatite samples (C, D, and E) contained apatite carbonate of type A.

Figure 3. The FTIR spectrum of hydroxyapatite sample
4. Conclusion
The hydroxyapatite material from red clam shells and egg shells has been successfully prepared using the hydrothermal method which is used as a candidate for denture materials. The results of the XRD diffraction peaks analysis showed the diffraction pattern of the hydroxyapatite material according to the hydroxyapatite database JCPDS 9-0432 and has a hexagonal crystal structure with lattice parameters a= 9.404 Å c = 6.675Å. The crystal size of the hydroxyapatite of all samples were obtained between 10.10 nm - 57.30 nm. While the results of the FTIR spectrum, in each hydroxyapatite sample there are functional groups PO$_4^{3-}$, OH$^-$ and CO$_3^{2-}$ which are functional groups of hydroxyapatite.

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References

[1] Priyadarsini S, Mukherjee S and Mishra M 2018 J. Oral Biol. Craniofacial Res. 8 58
[2] Nobre C M G, Pütz N and Hannig M 2020 Scanning 2020 12
[3] Hendi A A 2017 J. Alloys Compd. 712 147
[4] Affandi 2015 Jom FTEKNIK 2 1
[5] Kumar K. V et al 2021 Saudi J. Biol. Sci. 28 840
[6] Unabia R, Piagola J C, Guerrero J R, Vequizo R, Gambe J, Odarve M K and Sambo B R 2015 Phys. Status Solidi C 12 572
[7] Goloshechapov D L, Kashkarov V M, Rumyangtseva N A, Seredin P V, Lenshin A S, Agapov B L and Domashevskaya E P 2013 Ceram. Int. 39 4539
[8] Mutmainah 2013 J. Chem. Inf. Model. 59 1689
[9] Mohd Pu’ad N A S, Koshy P, Abdullah H Z, Idris M I and Lee T C 2019 Heliyon 5(5) e01588
[10] Miranda M, Torrecillas R and Fernández A 2020 Ceram. Int. 46 27860
[11] Asmawati A, Thalib B, Thalib A M, Reni D S and Hasyim R 2018 J. Dentomaxillofacial Sci. 3 162
[12] Ronan K and Kannan M B 2017 ACS Sustain. Chem. Eng. 5 2237
[13] Sobczak A, Kowalski Z and Wzorek Z 2009 Acta Bioeng. Biomech. 11 23
[14] Latif A F A, Mohd Pu’Ad N A S, Ramli N A A, Muhamad M S, Abdullah H Z, Idris M I and Lee T C 2020 Mater. Sci. Forum 1010 MSF 584
[15] Lee S-W, Balázsi C, Balážs K, Seo D, Kim H S, Kim C-H and Kim S-G 2014 Tissue Eng. Regen. Med. 11 113
[16] Ripamonti U, Crooks J, Khoali L and Roden L 2009 Biomaterials 30 1428
[17] Ariffin M M, Yatim N I and Hamzah S 2017 Malays. J. Anal. Sci. 21 571
[18] Khiri M Z A, Matori K A, Zainuddin N, Abdullah C A C, Alassan Z N, Baharuddin N F and Zaid M H M 2016 SpringerPlus 5(1) 1
[19] Abdel-Salam Z A, Abdou A M and Harith M A 2006 Int. J. Poult. Sci. 5 35
[20] Muntamah 2011 Sintesis dan karakterisasi hidroksiapatit dari limbah cangkang kerang darah (Anadara granosa, sp) (Bogor: Institut Pertanian Bogor)
[21] Arrafiqie M, Aziz Y and Zultiniar 2016 Jom FTEKNIK 3 1
[22] Bharatham H, Hamid Za A, Musa M F, Ahmad N H and Perimal E K 2017 Malays. J. Health Sci. 15 1
[23] Khoirudin M 2015 Jom FTEKNIK 2 1
[24] Siswanto 2013 Sintesis Dan Pencirian Hidroksiapatit Dari Limbah Cangkang Kerang Hijau Dengan Metode Kering (Institut Pertanian Bogor)
[25] Cahyaningrum S E 2017 UNESA J. Chem. 6
[26] Yahya M 2016 Jom FTEKNIK 3 1
[27] Putri A A M 2012 Metode Single Drop Pada Pembuatan Hidroksiapatit Berbasis Cangkang Telur (Bogor: Institut Pertanian Bogor)
[28] Tyas R 2014 *Studi karakteristik hidroksiapatit dari cangkang telur ayam ras dan ayam kampung* (Bogor: Institut Pertanian Bogor)

[29] Cahyati C 2014 *Observasi morfologi dan komposisi hidroksiapatit yang terbuat dari cangkang telur ayam kampung dan ayam ras* (Bogor: Institut Pertanian Bogor)

[30] Mobasherpour I, Heshajin M S, Kazemzadeh A and Zakeri M 2007 *J. Alloys Compd.* **430** 330

[31] Bezzi G, Landi E, La T M G and Torretta C G 2002 *Mater. Chem. Phys.* **78** 816–24

[32] Rhee S-H 2002 *Biomaterials* **23** 1147

[33] Alqap A and Sopyan I 2009 *Indian J. Chem. - Inorg. Phys. Theor. Anal. Chem.* **48** 1492

[34] Muhara I 2015 *Jom FTEKNIK* **2** 1

[35] Castro M A M, Oliveira T P, Correia G S, Oliveira M M, Rangel J H G, Rodrigues S F and Mercury J M R 2020 *Bol. Soc. Esp. Ceram. Vidr* **232** 1

[36] Harahap A W 2015 *Jom FTEKNIK* **2** 1

[37] Fadila R, Fadli A and Yenti S R 2019 *Jom FTEKNIK* **6** 1

[38] Herawaty L 2014 *Sintesis Nano Hidroksiapatit dari Cangkang Tutut (Bellamya javanica) dengan Metode Presipitasi dan Hidrotermal* (Bogor: Institut Pertanian Bogor)

[39] Riyanto B, Akhiruddin M and Nurrahman 2014 *J. Pengolah. Has. Perikan. Indones.* **16** 119

[40] Kusuma H H, Ibrahim Z and Othaman Z 2020 *J. Ilm. Pendidik. Fis. Al-Biruni* **9** 295