Synthesis and characterization of hydroxyapatite bioceramics from shells of serai snail and mangrove crab in Tanjung Jabung beach: effect of milling process

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Abstract. Hydroxyapatite (HA) is a calcium phosphate compound that is used as a substitute for damaged body tissues, such as bone and dental implants. HA has been synthesized from the serai snail shells and the mangrove crab of west Tanjung Jabung, Jambi province using the dry method. Calcination of the shell at 1000 °C for 12 hours produces CaO, then contact of CaO with air produces Ca(OH)2 which is used as a source of calcium in HA synthesis. Milling time variations for 0 hours, 3 hours, and 6 hours. Sintering at 600 °C for 2 hours produces HA as characterized by using X-Ray Diffraction (XRD), Fourier Transform Infrared (FTIR), and particle size analysis using Particle Size Analyzer (PSA). HA which has the highest accuracy of lattice parameters is that which is milling for 3 hours. The diffraction pattern shows the formation of the HA phase accompanied by several other calcium phosphate phases at an angle of 2θ. The FTIR spectrum shows the presence of PO43− and OH− groups from HA. PSA analysis shows the longer the milling makes the smaller particle size.

Keywords: Serai snail shell, crab shell, hydroxyapatite, dry method, synthesis.

1. Introduction
Tanjung Jabung Regency is located in the mangrove ecosystem with an area of 5.009,82 km² or about ± 9.38% of the area of Jambi Province, Indonesia, that reaches about 53.435,72 km². The West Tanjung Jabung Regency located in a low-lying area with an altitude between 10-500 m above sea level. West Tanjung Jabung Regency especially the regency capital region, Kuala Tungkal, is a coastal area where the coastal waters of Tanjung Jabung Barat Regency reach 12 nautical miles from the outer coastline. In these vast waters living a wide variety of biological resources [5].
Many biological resources for the coastal area are natural resources from animals, especially fish. The types of fish resources that found in the coastal waters of Tanjung Jabung Barat Regency include pelagic fish, demersal fish, and non-pelagic fish. Serai snail and mangrove crabs are non-pelagic, they are large in number and also cause waste. The part that is consumed from the serai snail and the mangrove crab is the meat (the inside of the shell), while the serai snail shell and the mangrove crab are only disposed of by the community, and are said to be waste. Though the shell is composed of various minerals and the main mineral making up the shell is calcium. Based on Sasmita’s research, the content calcium in the snail shells was 28.7%, magnesium was 2.14%, and ferrum was 0.033% [12]. According to Siahaya, et al, crab shells contained protein 15.60-23.90%, calcium carbonate 53.70-78.40%, and chitin 18.70-32.20% [14]. One way to utilize and maximize the use-value of serai snail shells and mangrove crabs is to synthesize HA material from the shells of manogrove snails and mangrove crabs in the Kuala Tungkal area by utilizing calcium in the animal’s shell.

HA (Ca_{10}(PO_{4})_{6}(OH)_{2}) has been synthesized since the early 1970 and is one of the most widely used biomaterials today [8]. The physical properties of HA have a hexagonal crystal structure and lattice parameters a=b=9,418 Å and c = 6,884 Å so that the HA formed from the synthesis should have these physical properties. HA is proven to be biocompatible and can be tolerated by human oral tissue, has the osteoconductive ability and is proven to be able to stimulate osteoblast differentiation and bone formation [9]. HA prices on the market are very expensive because they are still imported from Japan and Korea. Therefore, it is necessary to innovate the development of HA material from local materials [1], such as by utilizing the shells of many snails and mangrove crabs that have not been utilized in the Kuala Tungkal area.

Much research has been carried out in synthesizing HA from various biomaterial, including chicken eggshell waste, shellfish, tutut shells, crab shells, golden snail shells, squid/cuttlefish bones, lamuru bone, and beef bones. Synthesized HA from cow bone waste using the wet method, the results of the characterization using XRF the most dominant HA content Ca and P, which was 88.88% and 8,78% [17]. Sintering temperatures, several studies state that the temperature of 900°C – 1200°C has the best HA results and the ball mill process makes the grain size smaller, but the processing time of the ball mill for 3 hours has not been able to make HA nano-sized [15].

Based on the description above, it is necessary to do research related to the synthesis of HA waste from serai snail shells and mangrove crabs’ shells. HA synthesis method can be done in several ways including wet, dry, precipitation, mechanochemical, hydrothermal and sol-gel methods [21]. In this study, the method used is the dry method. The advantages of the dry method compared to other methods are that it can produce HA powder with fine grains and a high degree of crystallinity [3], the process is simpler and produces more HA and has economic value.

2. Experimental Method

2.1 Materials and instrument

The materials used in this study are samples of snail shells and mangrove crabs shells obtained from the coast of West Tanjung Jabung, NaOH 1%, acetone 96%, H_{3}PO_{4}, and distilled water. The instruments used in this study are digits analytical balance, glassware, pans, furnace, planetary ball mill, X-Ray Fluorescence, X-Ray Diffraction (XRD), Fourier Transform Microscopy(FTIR), Particle Size Analyze (PSA).

2.2 Preparation of serai snail shells and mangrove crabs’ shells

1 kg serai snail shells and mangrove crabs’ shells are cleaned (washed) from dirt, boiled separately in a high-pressure pan, each shell is treated soaked in 1% NaOH solution for 24 hours and soaked in 96% acetone solution. The dried shell is calcined at 1000 °C for 12 hours and crushed using a mortar until smooth. After that, the levels of calcium (Ca) and other elements in serai snail shells and mangrove crabs’ shells were tested using XRF. To identify compounds formed using XRD, and to identify functional groups using FTIR.
2.3 Synthesis of Hydroxyapatite
Snail and crab shells were given each treatment mixed with H$_3$PO$_4$ then milling process was carried out with variations for 0, 3 and 6 hours at a speed of 300 rpm. The molar ratio of Ca(OH)$_2$ and H$_3$PO$_4$ used is 0.3:0.18. Furthermore, the mixture is allowed to stand for 24 hours in a closed container before sintering at a temperature of 600 °C for 2 hours.

\[10\text{Ca(OH)}_2 + 6\text{H}_3\text{PO}_4 \rightarrow \text{Ca}_{10}\text{(PO}_4)_6\text{(OH)}_2 + 18\text{H}_2\text{O} \quad (1)\]

2.4 Characterization of samples
The resulting products were characterized using FTIR, XRD, and PSA. FTIR analysis was applied to determine the functional groups that exist in the product. XRD analysis was undertaken to identify the crystal structure of the resulting products, and PSA analysis is used to determine the particle size distribution.

3. Result and Discussion
3.1 Shell Calcination Result
Based on XRF testing, the content of calcined snail and crab shells is dominated by calcium, namely 99.3% in snail shell and 98.4% in crab shells and there is other element in small amounts as can be seen in Table 1.

| No | Element | Snail (%) | Crab (%) |
|----|---------|-----------|----------|
| 1  | Ca      | 99.3      | 98.4     |
| 2  | P       | 0.0255    | 1.46     |
| 3  | Cd      | 0.602     | 0.077    |

Based on the analysis of XRD, the compound Ca(OH)$_2$ was formed because the calcined samples originally predicted as calcium oxide (CaO) was allowed to contact with water vapor contained in the air. The diffraction pattern is presented in Figure 1a and FTIR spectra is presented in Figure 1b. Diffraction pattern can be seen in Figure 1a in accordance with the equation:

\[\text{CaO + H}_2\text{O} \rightarrow \text{Ca(OH)}_2 \quad (2)\]

![Figure 1](image1.png)

Figure 1. (a) Diffraction pattern, (b) FTIR spectra of shell powder after calcinations

In the samples identified compounds CaCO$_3$ was allegedly due to having reached its equilibrium, so that a small amount of CaO react with CO$_2$ to form CaCO$_3$. Similar to the eggshell calcined at 900 °C for 8 hours had reached equilibrium to form the CaCO$_3$ phase [7]. Based on FTIR spectra, it was observed that the crab shell calcined for 12 hours had a peak in the area of 3641,006 cm$^{-1}$ which was stronger than the snail shell. In the crab shell spectra, there were also observed CO groups of carbonates reinforced with a strong peak at wave number 1043,318 cm$^{-1}$. This could indicate
more carbonate content in the calcium of the crab shell. The presence of carbonate in the FTIR results is thought to originate from carbon dioxide (CO$_2$) returning to the CaO when thermal equilibrium has been reached.

3.2 Result of XRD analysis

The diffraction pattern of the HA sample can be seen in Figure 2. Sample S0, S3 and S6 has a peak with high intensity at 2θ 31.8° hkl (211), 32.2° hkl (112), 25.9° hkl (002) indicating HA compound (COD 96-900-1234). Samples S3 and S6 look purer. This is caused by the sintering temperature needed by sample S6 being lower than other samples in forming HA compounds, because the particle size is smaller and homogeneous. Other studies mention that the smaller particles cause better particle homogeneity and high surface energy during the sintering process, this causes the sintering process to occur faster [19].

![Figure 2. The diffraction pattern HA synthesis product with a source of calcium (a) the shell of a snail on a variety of time milling 0 h (S0), 3 hours (S3), and 6 hours (S6), (b) the shells of crabs at the time variations milling 0 h (K0), 3 hours (K3) and 6 hours (K6).](image)

Sample K0, K3, K6 sample has a high-intensity peak at 2θ 31.3° hkl (0 2 10), 34.7° hkl (220), 28.1° hkl (124) indicating β-TCP (β-Tricalcium phosphate) compound (COD 96-901-2138). HA diffraction pattern with crab shell calcium source is dominated by β-TCP phase. So, milling time variation does not shift the peaks that can be seen in the enlarged area $2\theta$ 31-34 meaning that the variation of milling time does not change the phase formed, but decreases or increases the intensity of the peaks which will affect the crystallinity. Based on Nakano, et al, crystallinity also decreases with increasing length of time of milling [10]. Then other results showed that milling time affected pore size, porosity, compressive strength, and density of HA samples [11].

HA has a hexagonal structure with $a = b = 9.432$ Å and $c = 6.881$ Å [2]. The results of calculating lattice parameters and their accuracy can be seen in Table 2. The calculation results show that sample S0, S3 and S6 have lattice parameters with accuracy value of lattice $a$ ranging from 98.181 - 99.990% and accuracy of lattice $c$ ranging from 98.185 - 99.176%, these values are still within the range of lattice parameters for calcium phosphate HA compounds. This is consistent with matching the maximum peaks of the sample which shows that the sample is dominated by apatite compounds which are present in the form of HA. The sample that has the highest accuracy value is the sample S3.

The lattice accuracy $a$ of samples K0, K3, and K6 ranges from 89.220 - 90.436% while the lattice accuracy $c$ is 0%. The lattice parameter of the three HA samples originating from the crab shell is far from the HA reference lattice parameter value, but the value is close to the lattice parameter value of β-TCP. Based on Shi, 2003, β-tricalcium phosphate crystals are rombohedral with a lattice parameter $a = 10.439$ Å and $c = 37.375$ Å. If the lattice parameters of the samples K0, K3, and K6 are compared with
β-TCP, the accuracy of the values of lattice a is 99,413, 99,906, and 98,995 Å and the accuracy of lattice c are 99,614, 99,696, 99,328 Å which is still in the range of β-tricalcium phosphate (β-TCP) compounds.

The number of β-TCP phases and lattice parameter values that are similar to β-TCP in samples made with crab shells may be caused by the inaccurate Ca/P molar ratio for making HA which is 1,67. The lack of a molar ratio of 1,67 can lead to the formation of β-TCP because the molar ratio of Ca/P to make β-TCP is 1.5.

Table 2. Lattice Parameters and Accuracy Value of HA

| Sample | a (Å)   | Accuracy (%) | c(Å)  | Accuracy (%) |
|--------|---------|--------------|-------|--------------|
| S0     | 9,380   | 99,447       | 6,848 | 99,613       |
| S3     | 9,433   | 99,990       | 6,894 | 99,716       |
| S6     | 9,603   | 98,181       | 6,999 | 98,185       |
| K0     | 10,378  | 89,809       | 37,231| 0            |
| K3     | 10,448  | 89,220       | 37,489| 0            |
| K6     | 10,334  | 90,436       | 37,124| 0            |

β-TCP compounds that are formed are not a problem because these compounds are also widely used as bone replacement material. The most widely used calcium phosphate is HA and tricalcium phosphate. Although both have the same chemical composition, these two materials have differences like resorbability (absorbability) in biological tissue [16]. HA and TCP are bioactive and osteointegrative when implanted into the body, but HA tends to be non-resorbable. The ideal bioceramics is not only bioactive but also bioresorbable, to provide space for new bone tissue growth. The combination of HA bioactive properties and TCP bioresorbable properties with certain compositions can better control the process of resorption and substitution of bone biomaterials [2].

Crystallinity is a term relating to the degree of regularity of the structure of a solid, the degree of crystallinity influences the physical properties of the solid. The more orderly the arrangement of atoms in the material, the higher the level of crystallinity. This is indicated by the higher intensity and narrower width of the peak half [13]. The degree of crystallinity of the sample can be quantitatively calculated by comparison of the top peak area to the total peak area as follows:

\[
\text{Degree of crystallinity} = \frac{\text{area fraction of crystalline}}{\text{area fraction of crystalline} + \text{area fraction of amorphous}}
\]  

\[
\text{Area fraction of crystalline/amorphous} = \text{FWHM} \times \text{height}
\]

Crystal size is calculated using the Debye Scherrer formula. Measurement analysis was carried out in the field hkl (002). Table 3 shows the calculated crystallinity and crystal size and the chart is shown in Figure 3. The size of the calculated crystal results is inversely proportional to FWHM, in accordance with the scherrer equation where if the FWHM value is small then the size of the sample crystal will be large.

Variations time milling does not give effect linear concerning to crystallinity. HA made with snail shells has the highest crystallinity in S3 sample, that is, samples that are milling for 3 hours according to the diffraction pattern where the S3 sample has the highest intensity. In contrast to HA which is made with crab shells milling results for 3 hours (K3) makes the lowest degree of crystallinity seen in the diffraction pattern of the K3 sample has a low intensity. This is consistent with other studies that show the relationship between milling time, crystal size, and the degree of crystallinity [11].
### Table 3. HA Crystallinity Degrees and Crystal Size

| Sample | Crystallinity | $\beta$ (°) | $D_{002}$ (nm) |
|--------|--------------|-------------|---------------|
| S0     | 65.365       | 0.5862      | 15.140        |
| S3     | 73.494       | 0.4519      | 19.658        |
| S6     | 70.529       | 0.5682      | 15.158        |
| K0     | 74.521       | 0.3984      | 22.298        |
| K3     | 70.318       | 0.3892      | 22.827        |
| K6     | 77.937       | 0.5636      | 15.767        |

The shape of the curve is not linear but very volatile. The size of the crystals in the sample increased at 3 hours of milling and then decreased at 6 hours of milling. Decreasing the size of the crystals at 6 hours of milling could have occurred because samples that have passed the peak point will rupture resulting in a decrease in crystallite size [18].

#### 3.3 Result of FTIR analysis

The presence of carbonate phase in the sample was confirmed in the FTIR test results which showed carbonate absorption bands in the sample. FTIR spectra for all milling time variations can be seen in figure 4a and FTIR spectra of sample S3 is presented in Figure 4b. The FTIR spectra showed the peak absorption of the group of $\text{CO}_3^{2-}$, $\text{PO}_4^{3-}$, $\text{OH}^-$ in the synthesis of HA samples. The functional groups that were characteristic of HA is $\text{PO}_4^{3-}$, $\text{OH}^-$, $\text{CO}_3^{2-}$ and $\text{PO}_4^{2-}$ which characterizes nonstoichiometric of HA [4].

The wavenumbers of each sample are presented in Table 4.

![Figure 3. Graph of differences in (a) crystallinity and (b) crystal size](image)

![Figure 4. FTIR Spectra for (a) all variations (b) sample S3](image)
The HA spectra obtained by each researcher did show different results but were still in close proximity. Absorption at wavenumbers around 3506 cm\(^{-1}\) shows stretching vibrations OH\(^-\). The results of the analysis show that there are groups that should not be present in pure HA. The group is carbonate (CO\(_3^{2-}\)) and its presence is known in the range of wave numbers 1700-1400 cm\(^{-1}\) and at 875 cm\(^{-1}\). The presence of carbonate in HA synthesis results probably comes from sources of calcium snails and crabs that have been confirmed by XRD and FTIR indeed the presence of carbonate phases and groups in calcium sources and then the presence of carbonate groups may be derived from free carbon dioxide in the air. The presence of carbonate in HA synthesized results are suspected to originate from free carbon dioxide (CO\(_2\)) in the air because the mixing of the precursors is carried out in the open air. Carbonate that enters the crystal lattice will affect the value of the Ca/P ratio of HA and also the crystal fields. Carbonate ions into the HA crystal lattice will replace the hydroxyl ion (OH\(^-\)) or phosphate (PO\(_4^{3-}\)) and generate carbonate HA [6].

The PO\(_4^{3-}\) group is the highest intensity in all samples. In HA phosphate groups can be found in the four modes of vibration, namely: vibration stretching (v\(_1\)) at wave number of about 941 cm\(^{-1}\), the vibrational bending (v\(_2\)) at wave number of about 363 cm\(^{-1}\), vibration asymmetric stretching (v\(_3\)) at wave number of about 1020 cm\(^{-1}\), and vibration antisymmetric bending (v\(_4\)) at wave number of about 552-560 cm\(^{-1}\) this value indicates vibration P-O from the group PO\(_4^{3-}\). PO\(_4^{3-}\) group seen in all samples can be observed in the range 1100-550 cm\(^{-1}\). The varied milling time did not affect the FTIR test results. The sharpness of the peak FTIR spectra shows the same results, so also the wavenumbers do not show a striking difference.

The percent transmittance value is the amount of light transmitted (not absorbed) to the molecule. If all the light passes through the sample without the slightest absorption then the absorbance is zero, if all the light is absorbed then the percent transmittance is zero. In Table 5 we can see the percentage of transmittance of the PO\(_4^{3-}\) sample S0, S3, S6, K0, K3, and K6. The analysis was carried out on the group because it had the sharpest band. Based on Table 5 it can be seen that there is no linear effect between the percentage of transmittance and the length of time milling. A small transmittance value indicates the amount of absorbance in the sample so that the peak in the FTIR spectrum is sharp.

### Table 4. Absorption Tape of FTIR Result

| Group  | S0         | S3         | S6         | K0         | K3         | K6         |
|--------|------------|------------|------------|------------|------------|------------|
| \(\nu_1\)PO\(_4^{3-}\) | 941.83     | 959.88     | 957.91     | 960.74     | 987.43     | 875.65     |
| \(\nu_2\)PO\(_4^{3-}\) | 1024.91    | 1024.04    | 1024.43    | 1021.33    | 1022.76    | 1019.86    |
| \(\nu_3\)PO\(_4^{3-}\) | 598.98;    | 599.18;    | 598.99;    | 598.58;    | 598.23;    | 599.19;    |
| \(\nu_4\)CO\(_3^{2-}\) | 1451.03;   | 1451.49;   | 1452.00;   | 1420       | 1417.18    | 1422.28    |
| HPO\(_4^{3-}\) | 1415.02    | 1414.79    | 1414.79    |            |            |            |
| OH\(^-\) | 3506.34    | 3514.68    | 3523.98    | 2967.07    | 2980.23    | 2990.12    |

### Table 5. Value of \%\(T\) CO\(_2^{2-}\) and \(\nu_3\)PO\(_4^{3-}\) group Milling Time Variations

| Sample | CO\(_2^{2-}\) \(\%\ T\) | \(\nu_3\)PO\(_4^{3-}\) \(\%\ T\) |
|--------|--------------------------|----------------------------------|
| S0     | 86.060                   | 34.958                           |
| S3     | 89.424                   | 44.489                           |
| S6     | 89.572                   | 35.413                           |
| K0     | 94.193                   | 35.824                           |
| K3     | 93.768                   | 35.542                           |
| K6     | 96.217                   | 28.228                           |
3.4 Result of PSA Analysis

Based on the particle size distribution curve (Figure 5), the sample S0 the highest frequency is at 1523.5 nm, sample S3 the left-hand curve with frequency the highest is at 691.5 nm, and as the sample S6 the curve shifts to the left and narrower with the highest frequency at 670.6 nm.

While the particle size distribution curve sample K0 is highest frequency at 1012.5 nm, sample K3 looks curve shifts to the right with the highest frequency there at 2406.5 nm, and sample K6 the curve seemed to shift to the left narrowing with the highest frequency at 705.3 nm. The narrowing of the curve indicates the increasingly homogeneous particle size in the sample. The average particle size results can be seen in Table 6.

![Figure 5. Particle size distribution curve](image)

Table 6. Average Particle Size of HA

| Sample | \(D_{average}\) (nm) |
|--------|---------------------|
| S0     | 1726.6              |
| S3     | 727.3               |
| S6     | 704.9               |
| K0     | 1308.1              |
| K3     | 2778.3              |
| K6     | 895.5               |

The particle size of HA samples made with snail shells decreased with the increasing length of milling time. While the particle size of HA made with crab shells is non-linear, in the K3 the particle size increased to 2778.3 nm. The large particle size of the K3 sample may be due to the agglomeration of the particles due to imperfections during the process of dispersion of the sample to the liquid media. The average HA particles from each sample were not yet nano-sized, because the size was still above 100 nm.
4. Conclusion

Synthesis HA from the Serai snail shell and the Mangrove Crab Coast of Tanjung Jabung Barat, Jambi has been successfully carried out. Based on the results of the FTIR characterization, a functional group has formed which is a feature of HA. Lattice parameter calculation results show that the serai snail shell waste was successfully synthesized into HA using the dry method while the results of the synthesis of mangrove crab waste produced $\beta$-TCP. Based on testing using XRD, time measurement of the degree of crystallinity and crystal size of HA is also $\beta$-TCP. The degree of crystallinity of HA increases at 3 hours of milling, then decreases at 6 hours of milling, whereas $\beta$-TCP rises at 3 hours of milling, then increases at 6 hours of milling. Crystal size increases after 3 hours of milling and then increases at 6 hours of milling. Decreasing the size of the crystals at 6 hours of milling could have occurred because samples that have passed the peak point will break down in size. Then from tests conducted using PSA, it is known that the length of milling time affects the particle size, where the longer the milling makes the particle size smaller.

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