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Synthesis of Hydroxyapatite from Egg Shells using Precipitation Methods for Bone Implant Application

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Abstract: Patients with fractures continue to increase from year to year. So that the need for biomaterials is very high and has had a considerable impact, especially in the field of orthopedic medicine. Hydroxyapatite (HAp) is one of the suitable biomaterials for bone implants. Hydroxyapatite (HAp) with the chemical formula ($Ca_{10}(PO4)_6(OH)_2$) is a calcium phosphate that contains hydroxide, which is classified as a mineral in the apatite group. HAp can be obtained from natural waste such as the eggshells of a chicken. This is because chicken eggshells have a very high CaO content (98,43%) which serves as a precursor in the synthesis of HAp. The purpose of this research the aim of observing the effect of stirring time on the purity of the HAp produced and to see the optimum stirring time used to produce good HAp. In this study, HAp was synthesized using a precipitation method by varying the stirring time. Based on variations in stirring time of 1 hour, 2 hours, 3 hours, 4 hours, and 5 hours, the purity of HAp obtained was 15%, 49%, 66%, 82%, and 50%, respectively. Therefore, it was concluded that the optimal stirring time to produce HAp with the highest purity is at a time interval of 4 hours.

Keywords: Bone implant, Broiler egg shells, Hydroxyapatite, Stirring time.

1. Introduction

Bioceramics have played a critical role in revolutionizing the healthcare industry over the past five decades for bone implant applications. As a subcategory of ceramics, bioceramics are biocompatible and possess anti-thrombogenic properties, making them ideal for filling bone and teeth defects, facilitating bone grafting, and replacing damaged tissues. Bone implants that are often used include stainless and ceramic biomaterials. Stainless steel and ceramic biomaterials are good materials for bones because they are suitable for tissues. However, this material is brittle so it cannot be impacted [1]. One solution to cover the weaknesses of bone implants is HAp.

Hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ is a calcium phosphate that contains hydroxide, which is classified as a mineral in the apatite group [2]. HAp is a naturally occurring calcium phosphate

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mineral phase that is commonly found in human bones. It is widely used in the medical field as a bioceramic material due to its biocompatibility and bioactivity. HAp has been found to be an effective material for bone grafting, as it promotes bone growth and can be easily incorporated into the natural bone structure. Additionally, HAp has been used in the development of dental implants and other orthopedic applications due to its unique properties and compatibility with the human body [3]. Researchers have synthesized HAp and used it to create various implant forms, including implant coatings [3]. Hydroxyapatite can be synthesized using calcium precursors and phosphate precursors. HAp is used in the field of dentistry as a metal coating material to increase the ability to bind bones, increase the ability to bind bones, to increase biocompatible properties, and artificial bone coatings that are inserted into the human body to provide hard properties to bone tissue [4]. The source of calcium in the synthesis of HAp is obtained from the eggshells of purebred chickens.

Eggshell waste in Indonesia is very high, amounting to 178,566.33 tons per year. Chicken egg shells cannot be decomposed by soil microorganisms, which will cause environmental pollution [5]. Eggshells are composed primarily of calcium carbonate, accounting for over 95% of their total mass. However, they also contain proteins that can be extracted and used as a substitute for limestone-based calcium carbonate [6]. Since it consists of higher hydroxyapatite than other poultry eggshells [7], So broiler eggshells have the potential as a calcium precursor in hydroxyapatite synthesis. Calcium carbonate contained in chicken egg shells can be used as a basic ingredient in the manufacture of (HAp). However, eggshells are often discarded as waste, creating an environmental issue. On the other hand, eggshells which be converted into hydroxyapatite powders or calcium compounds, can reduce unused waste [8]. This material has also high value [9], because synthesis of HAp from this material is expensive. Calcium, being the primary component of eggshells, plays a crucial role in determining their hardness.

HAp was synthesized using precipitation method. In this method, hydroxyapatite is synthesized using two precursors, in the form of calcium phosphate $Ca(OH)_2$ and phosphoric acid (H_3PO_4). Optimal mixing time will produce a better HAp product and minimize the formation of impurity compounds. The use of these precursors minimizes the risk of failure in hydroxyapatite synthesis, making it a reliable method for producing this important biomaterial [10]. In the HAp synthesis process using the precipitation method, because it is a simple method that costs not too much and does not use a lot of organic solvents [11]. There are several factors that can affect the purity of HAp produced. One important factor is the long stirring time, which affects the increase in the resulting hydroxyapatite. This is because the longer the reaction time, the longer the collision time between reactant particles, resulting in more HAp products. Vigorous stirring produces a homogeneous HAp precipitate. Insufficient stirring will cause the formation of monetite ($CaHPO_4$) and brushite ($CaHPO_4.2H_2O$) phases [12]. Therefore, we need to investigate the truth of this statement by varying the stirring for 1, 2, 3, 4, and 5 hours on HAp synthesis. The pure HAp synthesis process was carried out at a temperature of 900^{o}C and pH 10. In this work, the resulting HAp crystals were then tested using X-Ray Fluorescence (XRF) and X-Ray Diffraction (XRD).

2. Materials and Method

The experimental method was used in this study which aims to determine the effect of stirring time on the purity of the hydroxyapatite produced from the basic ingredients of purebred chicken egg shells. Variations in stirring time were 1 hour, 2 hours, 3 hours, 4 hours, and 5 hours. Prepare tools and materials to be used for the manufacture of hydroxyapatite powder. The tools used are a furnace, oven, sieve, X-ray Fluorescence (XRF), X-Ray Diffraction (XRD), whatman filter paper, magnetic stirrer, thermometer, beaker glass, measuring cup, volumetric flask, cup, funnel, erlenmeyer, pipettes, spatulas, electronic scales. The materials used were egg shells as a source of CaO, (H_3PO_4) as a source of phosphate, Sodium Hydroxide (NaOH) as a pH regulator, and Aquadest (H_2O) as a solvent. The diffraction pattern of each crystalline solid is very typical, which depends on the crystal lattice, the parameter unit, and the wavelength of the X-Ray used [13]. The method used is the precipitation method, which consists of two stages, namely sample preparation and calcination, then the second stage is the synthesis of HAp.

2.1 Sample preparation and calcination

Egg shells of purebred chickens are washed and soaked in aquadest for 30 minutes to clean the remaining dirt. Soaked broiler eggshells were dried at room temperature for 24 hours. Then eggshells were crushed using a mortar and pestle. Dried eggshells were calcinated by using a furnace at 900°C for 5 hours. Weigh the sample and grind again using a mortar and pestle. The synthesis of hydroxyapatite with the basic ingredients of purebred chicken egg shells can be seen in Figure 1.

(a) (b) (c)

Figure 1. Chicken egg shells (a) Egg shells of purebred chickens before calcination, (b) Cao powder from eggshells of purebred chickens after calcination, (c) Hydroxyapatite powder

Figure 1 (a) is the waste of purebred chicken egg shells, Figure 1 (b) is the result of the synthesis of purebred chicken eggshells before being characterized, Figure 1 (c) is the result of the synthesis of purebred chicken eggshells after being characterized using a furnace.

2.2 Hydroxyapatite Synthesis

Hydroxyapatite based biogenic, the properties like pore structure and chemical composition are preserved as in precursor material [14]. Hydroxyapatite synthesis was carried out by the precipitation method. Make 100 mL of 0.3 M H_3PO_4 precursor solution, then make a solution ion of 200 mL of 0.5 M CaO precursor. Gently mix the phosphate solution into the CaO solution. Stir the two solutions until homogeneous with a magnetic stirrer temperature operation

at 37°C at 700 rpm with time variations for 1 hour, 2 hours, 3 hours, 4 hours, and 5 hours. Maintain the pH value of the solution to pH 10 by dropping NaOH. Leave the solution for 24 hours. Wash the filter with use aquadest 3 times to remove the remaining solution. The precipitate was dried in an oven at 110℃ for 3 hours. Give heat treatment with sintering temperature variations of 900℃ for 5 hours [15]. Weigh the resulting powder and the powder is ready to be used for testing.

$$10Ca(OH)_2 + 6H_3PO_4 \rightarrow Ca_{10}(PO4)_6(OH)_2 + 18H_2O$$

Calcium oxide reacts with phosphoric acid to produce hydroxyapatite and water.

3. Results and Discussion

XRF characterization tests were carried out at the Chemistry Laboratory of the Faculty of Mathematics and Natural Sciences, Universitas Negeri Padang. Tests were carried out to determine the content of calcium oxide (CaO) obtained from calcined eggshells of purebred chickens. Characterization with X-RF begins by placing an X-ray tube in front of the CaO powder sample [16]. X-Ray beam is then emitted to the sample, which absorbs the photons from the beam. The components that make up the CaO powder will then re-emit the photons themselves, and in the process, they will fluoresce. The photon energy or wavelength emitted by each component will then be used to identify the component itself. Table 1 is the obtained from the XRF characterization of CaO powder made from purebred chicken egg shells.

Table 1. Characterization of CaO using XRF

Chemical content	Composition (%)
Al_2O_3	0.4 %
P_2O_5	1.09 %
Cl	0.006 %
CaO	97.96 %
Mn	0 %
Zn	0.002 %
Sr	0.038 %
Zr	0.001 %
Ag	0.464 %
Cd	0.023 %
Ba	0.015 %
Nd	0 %
Sm	0 %
Re	0.001 %

Table 1 can be seen that the eggshells of purebred chickens have a high CaO content of 97.96% and there are other impurities of around 3%, from the results of the characterization by XRF it was concluded that HAp has the potential as a precursor of calcium in the synthesis of HAp. The mass resulting from the calcination process is smaller than the mass before calcination. The reduction in the mass of the hen's eggshell is due to the retention of CO_2 from the $CaCO_3$ molecule. Various characterization techniques have confirmed that hydroxyapatite (HAp) can be synthesized from CaO, which is extracted from eggshell waste. This method has been found to

have high reproducibility, simplicity, and economic benefits, making it an attractive option for large-scale industrial production. One of the main advantages of this method is the use of eggshell waste as a precursor, which is abundantly available and relatively inexpensive. Additionally, the only by-product generated during the preparation process is water, which makes the method environmentally friendly. Overall, the use of eggshell waste to synthesize hydroxyapatite offers an innovative and sustainable solution to both waste management and the need for this important biomaterial. Biological HAp obtained from these sources showed that these products are considered as natural calcium resources which contain high amount of calcium as carbonate and oxide [17]. Besides cattering, events that could have happened is the photon to be absorbed, emitted and reflect as it passes through a medium (material) [18]. Characterization of hydroxyapatite using XRF aims to determine the compounds it contains in the sample with 5 variations of stirring time, namely 1 hour, 2 hours, 3 hours, 4 hours, and 5 hours as shown in the Table 2.

Table 2. Characterization of hydroxyapatite using XRF

Chemical content	Composition (%)				
	1 hour	2 hour	3 hour	4 hour	5 hour
Al_2O_3	1.261 %	0.849 %	1.118 %	0.625 %	1.938 %
P_2O_5	37.601 %	30.186 %	40.494 %	33.697 %	35.288 %
Cl	0.008 %	0.041 %	0.004 %	0.023 %	0.026 %
CaO	61.08 %	68.315 %	57.804 %	64.819 %	62.186 %
Zn	0.006 %	0.011 %	0.017 %	0.01 %	0.01 %
Sr	0.024 %	0.023 %	0.024 %	0.029 %	0.03 %
Zr	0.001 %	0.001 %	0.002 %	0.002 %	0.002 %
Re	0.001 %	0 %	0%	0 %	0 %

Table 2 it was found that the elemental content of CaO from the variation of stirring time 1 hour was 61.08%, while the phosphate content of (P_2O_5) was 22.758%. Calculations using the V/V formula, the CaO content was 66.6%, while the phosphate content was 33.3%. There is a reduction in the percentage of CaO levels by 5.5% during synthesis. The CaO content of the 2-hour stirring time variation was 68.315%, while the phosphate content (P_2O_5) was 30.186%. There was an increase in the percentage of CaO levels by 1.7% during synthesis. This is due to when dissolving CaO using a 250 ml volumetric flask. While the CaO solution used for the synthesis of hydroxyapatite was 200 ml. The CaO content of the 3-hour stirring time variation was 57.804%, while the phosphate content (P_2O_5) was 40.494%. There was a reduction in the percentage of CaO levels of 8.8% during synthesis. The CaO content the 4-hour stirring time variation was 64.819%, while the phosphate content (P_2O_5) was 33.697%. There is a reduction in the percentage of CaO content of 1.8% and 0.3% during synthesis. The CaO content from the 5-hour stirring time variation was 62.186%, while the phosphate content (P_2O_5) was 35.288%. There was a decrease in the percentage of CaO content of 4.414% during synthesis. Characterization with XRF it can be interpreted that CaO before synthesis and after synthesis with the proportion of phosphoric acid produced are not much different. The reduction in CaO and phosphoric acid levels is caused by several factors such as the presence of contaminated substances in the calcination process and the presence of wasted substances during the synthesis stage. Characterization using XRD was carried out qualitatively, namely by comparing the peaks formed on the diffractogram with COD database 1011242 as shown in Figure 2.

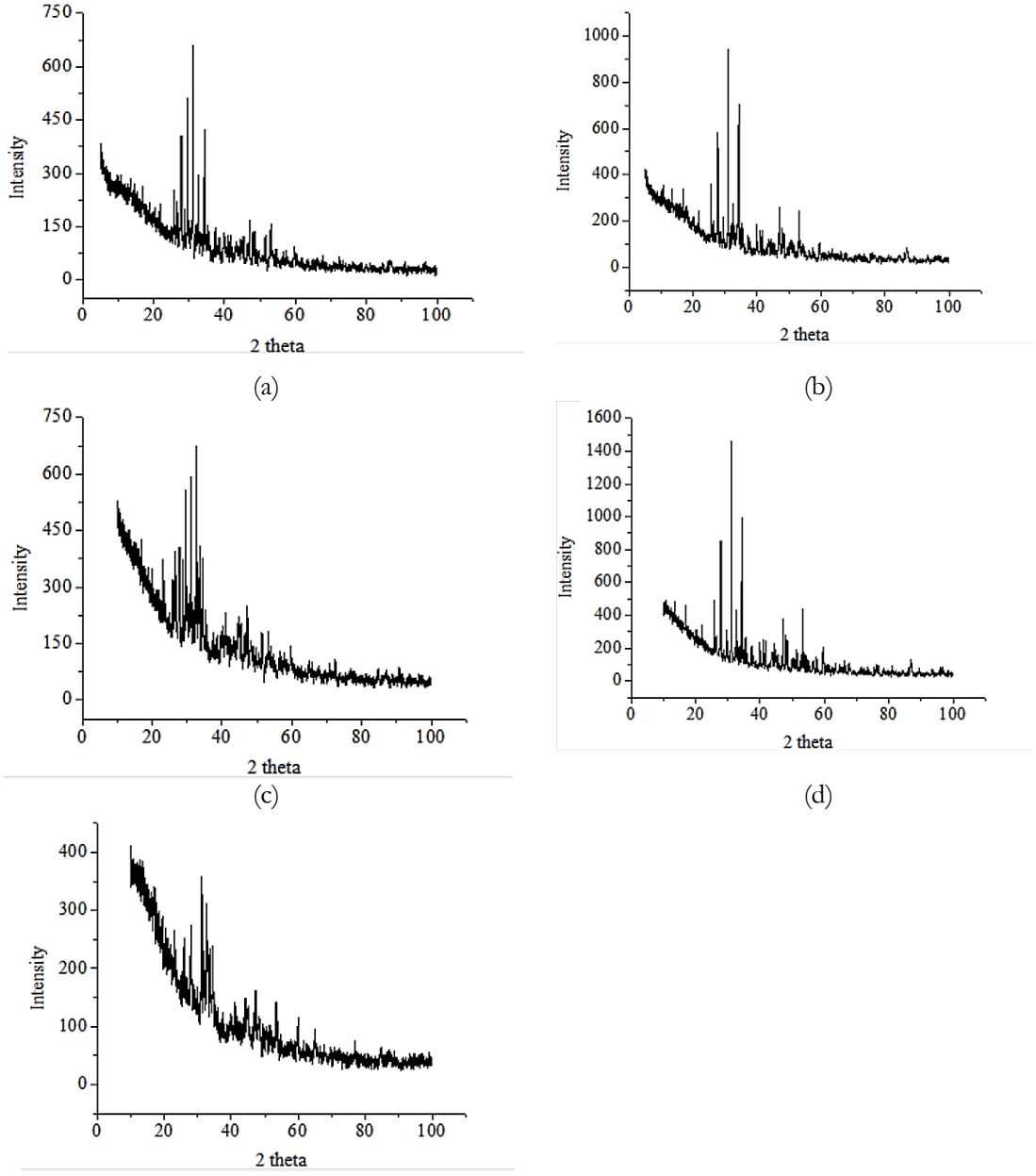

Figure 2. XRD HAp characterization results with variations in stirring time (a) 1 hour, (b) 2-hour, (c) 3-hour, (d) 4-hour, (e) 5-hour

Figure 2 is the result of characterization with XRD, which is used to strengthen the correctness of the FTIR characterization results. From a time, variation of 1 hour the phase formed is Calcium diphosphate-beta. The diffraction pattern shows peaks with high intensity at, which successively have peaks of 27.963°, 28.997°, 32.066°, 33.950°, diffraction hkl (012), (210), (112), (202). CO_3 compounds can inhibit the formation of hydroxyapatite. The presence of the $CaCO_3$ compound affects the shift of the 2θ angle in a larger direction so that it can cause the hydroxyapatite crystal lattice to widen [19]. At a time, variation of 2 hours a HAp phase was formed by comparing it with COD database 9002213 the 2θ peak, namely 28.103°, 31.804°,

34.058°, hkl diffraction respectively (102), (211), (022). At a time, variation of 3 hours, the hydroxyapatite phase was obtained with COD database 9002213 an angle of 2θ, namely, 28.103°, 28.971°, 31.804°, 32.954°, 34.058°, 35.501°, the diffraction hkl fields were (102), (210), (211), (030), (022), (031). At a time, variation of 4 hours the phase formed is hydroxyapatite with COD database 1011242 an angle of 2θ, namely 25.689°, 27.963°, 31.795°, 33.950°, 53.185°, hkl diffraction respectively are (002), (012), (211), (202), (141). At a time, variation of 5 hours, the hydroxyapatite phase was obtained with COD database code 9002214 an angle of 2θ, namely 25.856°, 28.100°, 28.874°, 31.717°, 32.843°, 35.399°, hkl diffraction were (002), (012), (210), (211), (030), (031).Then, knowing the compounds produced, characterization using XRD was also carried out to determine the crystal structure of the HAp sample and the crystals obtained. X-ray analysis showed the presence of calcium oxide (CaO) [20]. Table 3 is the percentage of hydroxyapatite content with a stirring time of 1 hour, 2 hours, 3 hours, 4 hours, and 5 hours which was analyzed based on the results of characterization with XRD.

Table 3. Percentage of hydroxyapatite with variations in stirring time

Mixing time (hours)	Hydroxyapatite composition (%)
1	15 %
2	49 %
3	66 %
4	82 %
5	50 %

The percentage of HAp obtained from data analysis with XRD can be seen in Table 3, a stirring time of 1 hour produces 15% hydroxyapatite, a stirring time of 2 hours produces 49% hydroxyapatite, a stirring time of 3 hours produces 66% hydroxyapatite, a stirring time of 4 hours produces 82% hydroxyapatite, a long stirring time of 5 hours produced 50% hydroxyapatite. It was concluded that the length of time of stirring affects the purity of the hydroxyapatite produced. The longer the stirring time, the purer the hydroxyapatite produced, but the optimum stirring time is 4 hours with a calcination temperature of 900°C. Mixing time that is not optimal will cause the formation of other elements or impurities apart from HAp and affect the purity of the HAp produced from the synthesis process.

Figure 3 aims to explain the relationship between stirring time and the percentage of hydroxyapatite produced.

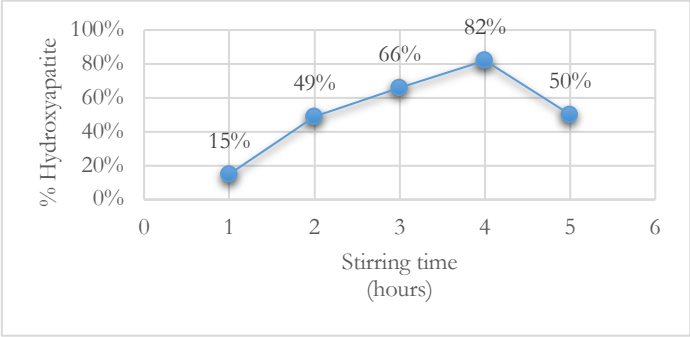

Figure 3. Stirring Time vs Hydroxyapatite Percentage

Figure 3 is the pattern of hydroxyapatite produced based on the length of stirring time. The hydroxyapatite content continued to increase from a stirring time of 1 hour to 4 hours and a decrease in the hydroxyapatite produced at a stirring time of 5 hours. This is because the optimal stirring time is 4 hours. Table 4 is the crystal size with a stirring time of 1 hour, 2 hours, 3 hours, 4 hours, and 5 hours which were analyzed based on the results of characterization with XRD.

Table 4. Relationship between the length of stirring time and the size of the crystals

Mixing time (hours)	Crystal size (nm)
1	66.9 nm
2	44 nm
3	54 nm
4	61 nm
5	41 nm

Crystal size was calculated using the Schrer equation. Table 4 it can be seen that the characterization by XRD showed that the resulting compound was in the form of hexagonal crystals with a crystal size of 66.9 nm each; 44 nm; 54 nm; 61 nm; and 41 nm. Can be seen concluded that the length of time of stirring has no effect on the size of the crystals. Crystal size is inversely proportional to FWHM. The size of the hydroxyapatite crystals is affected by pressure [21]. The powder particle size depends to a large extent on the reaction temperature, reaction time, and the aging time of the reaction solution before calcination [22]. The characterization with FTIR can be seen in Figure 4 to strengthen the results of the characterization with XRD that there is a hydroxyapatite compound in the synthesis carried out.

Based on the results of the FTIR spectrum shown in Figure 4 with a stirring time of 1 hour, clusters of CO_3 groups are detected which is intensity the highest is at wave number 1024,88 cm^{-1}. Then from the graph of the $PO4^{3-}$ detected at waves numbers 726.76 cm^{-1}, 548.59 cm^{-1}, 470.68 cm^{-1}, 410.70 cm^{-1}. Carbonate ions have peaks with the highest wavelengths so that carbonate ions can replace phosphate ions in the crystal structure [23]. At 2 hours of stirring, O-H groups were detected with the highest intensity at wave number 2162 cm-1. Then from the $PO4^{3-}$ graph it was detected at wave numbers 436.33 cm^{-1}, 554.88 cm^{-1}, 726.22 cm^{-1}, 966.75 cm^{-1}, 1026.89 cm^{-1}. These results indicated that the FTIR result contained $PO4^{3-}$ and OH^- which indicate the presence of hydroxyapatite. At 3 hours of stirring, O-H groups were detected with the highest intensity at wave number 3745.05 cm^{-1}, 2185.55 cm^{-1}, 2084.08 cm^{-1}. Then from the $PO4^{3-}$ graph it was detected at wave numbers 1026.18 cm^{-1}, 965.42 cm^{-1}, 726 cm^{-1}, 551.87 cm^{-1}, 471.41 cm^{-1}, 459.74 cm^{-1}, 419.59 cm^{-1}. These results indicated that the FTIR result contained $PO4^{3-}$ and OH^- which indicate the presence of hydroxyapatite. At 4 hours of stirring, O-H groups were detected with the highest intensity at wave number 3745.26 cm^{-1}, 2183.34 cm^{-1}, 2070.62 cm^{-1}. Then from the $PO4^{3-}$ graph it was detected at wave numbers 1020.27 cm^{-1}, 598.22 cm^{-1}, 545.38 cm^{-1}, 477.04 cm^{-1}, 460.38 cm^{-1}, 416.54 cm^{-1}. These results indicated that the FTIR result contained $PO4^{3-}$ and OH^- which indicate the presence of hydroxyapatite. At 5 hours of stirring, O-H groups were detected with the highest intensity at wave number 3745.31 cm^{-1}, 2186.37 cm^{-1}. Then from the $PO4^{3-}$ graph it was detected at wave numbers 1021.07 cm^{-1}, 559.13 cm^{-1}, 477.06 cm^{-1}, 414.50 cm^{-1}.

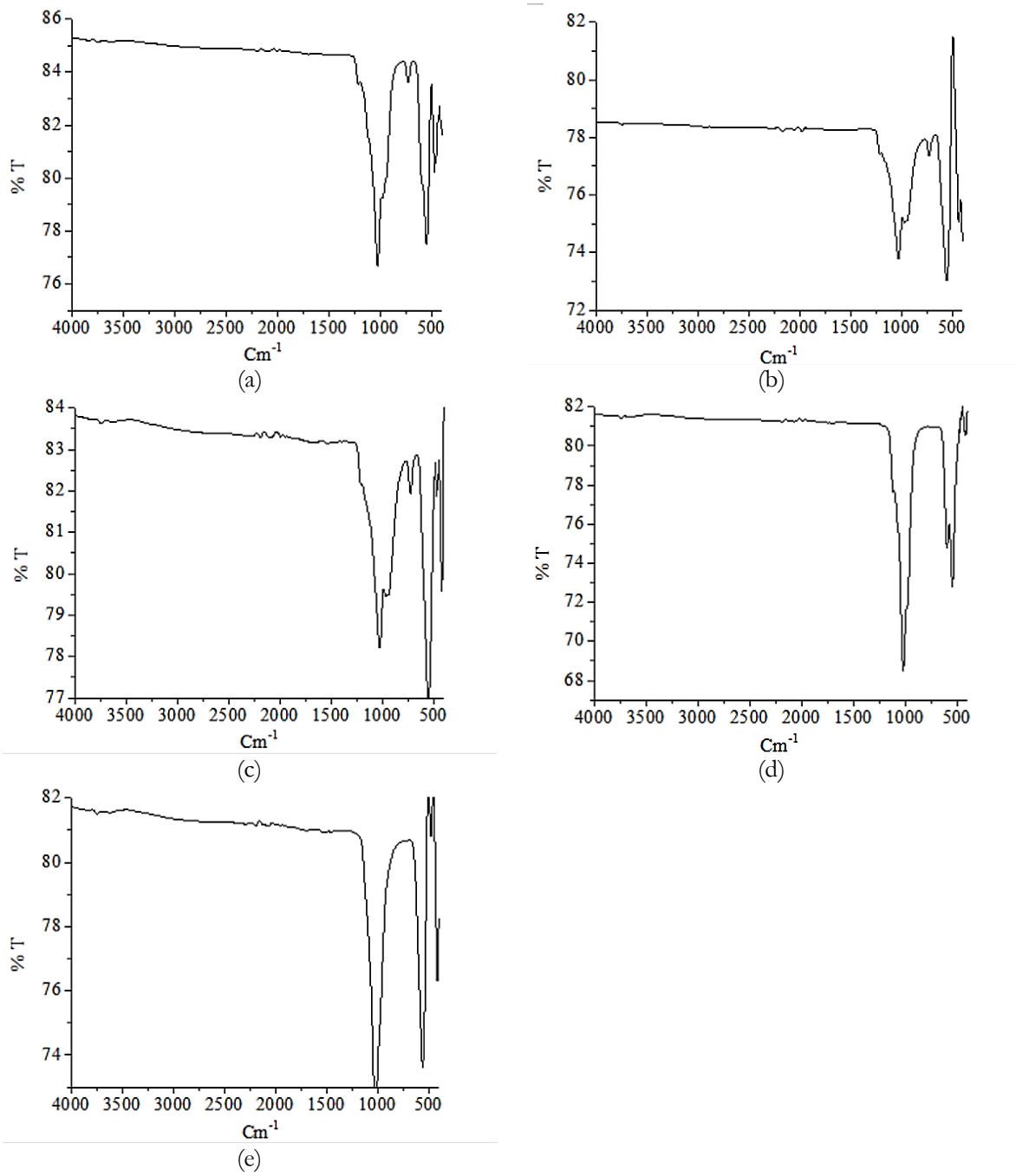

Figure 4. FTIR HAp characterization results with variations in stirring time (a) 1 hour, (b) 2-hour, (c) 3-hour, (d) 4-hour, (e) 5-hour

Based on the analysis results, information was obtained that mixing time that was not optimal would cause the PO_4^{3-} functional group to be replaced by the CO_3^{2-} functional group or carbonate ions which could interfere with the formation of hydroxyapatite. The stirring time was 1 hour, the formation of carbonate compounds was caused by the non-optimum stirring time, which inhibited the formation of hydroxyapatite. At 2 hours, 3 hours, 4 hours, and 5 hours, hydroxyapatite was formed which was indicated by the presence of the $PO4^{3-}$ functional group. One of the influencing factors in the synthesis of hydroxyapatite is the long stirring time. So it is

necessary to do strong (vigorous) stirring to produce a homogeneous HAp precipitate. Insufficient stirring will result in the formation of monetite ($CaHPO_4$) and brushite ($CaHPO_4.2H_2O$) phases [12]. Homogeneous stirring results in a better mixed pH and leads to better interactions between reagents.

4. Conclusion

Based on the research it can be concluded that the eggshells of purebred chickens have a high CaO content, namely 97.96%. Of the five variations of stirring time, the successive HAp was 15%, 49%, 66%, 82%, 50%. The length of time of stirring affects the purity of the hydroxyapatite produced. The optimum stirring time for the synthesis of hydroxyapatite was 4 hours. The hydroxyapatite produced is 82%. From the results of the FTIR spectrum, each sample contains hydroxyapatite with the functional groups $PO4^{3-}$, OH^- and $CO3^{2-}$ which are the hydroxyapatite functional groups.

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