***JKPK (JURNAL KIMIA DAN PENDIDIKAN KIMIA)**, Vol. 7, No. 1, 2022*
Chemistry Education Study Program, Universitas Sebelas Maret
https://jurnal.uns.ac.id/jkpk

pp. 86-97
ISSN 2503-4146
ISSN 2503-4154 (online)

THE SYNTHESIS AND PHYSICOCHEMICAL CHARACTERIZATION OF HYDROXYAPATITE (HAP) FROM LOCAL DUCK EGG SHELLS

Agung Rimayanto Gintu[1*], Elizabeth Betty Elok Kristiani[1], and Yohanes Martono[2]

[1] Faculty of Biology, Satya Wacana Christian University
Jl. Diponegoro No. 52-60 Salatiga, Central Java 50711, Indonesia

[2] Faculty of Science and Mathematics, Satya Wacana Christian University
Jl. Diponegoro No. 52-60 Salatiga, Central Java 50711, Indonesia

* correspondence, tel/fax : 082138094517, email: agunggintu911@gmail.com; 422017002@student.uksw.edu

Received: September 28, 2021 Accepted: April 28, 2022 Online Published: April 30, 2022
DOI : 10.20961/jkpk.v7i1.58661

ABSTRACT

Hydroxyapatite (HAp) Biocheramics was the main component of remineralisation agent to the hard tissues; it is commonly synthesized from compounds rich with calcium. One of the compounds recommended as a HAp source was eggshells. This study aimed to synthesize the HAp from local duck eggshells containing Calcium 0,8631 ± 0,0015g/g and Phosphor 0,3118 ± 0,0016g/g. The synthesis process of HAp by Base Precipitation methods showed rendement 85,06 ± 1,0135%. The Physicochemical characterization to the HAp showed the Calcium contains 0,4760 ± 0,0027g/g; Phosphor 0,0897 ± 0,0023g/g; Porosity 82,0106 ± 0,4484%; Biodegradability 5,3506 ± 0,0295%; and Swelling ability 5,7678 ± 0,1897%. The results concluded that the local duck egg shells were recommended as HAp sources. Based on characterisation results, the HAp made from it was potentially applied as a remineralization agent.

Keyword: Bioceramics, Hydroxyapatite, Remineralisation agent, Local Duck Egg Shells.

INTRODUCTION

Hydroxyapatite bioceramic (HAp) is a type of apatite bioceramic that can be synthesized from calcium-rich compounds such as egg shells [1-5]. The HAp is potentially used as a remineralization agent for the hard tissues because it has a similar structure to the calcium complex that builds the bone and teeth [3-5]. The HAp has the chemical name [$Ca_{10}(PO_4)_6(OH)_2$] [3-6], also known as *Calcium Hydroxyphosphate* in trade name [2-4]. The research before a report that the HAp is used as a *Bone Implant, Bone Graft,* and *Bone Filer* to reparate and remineralization the damaged hard tissues in the human body [2,4] and replace the use of metallic pens (made from steel, iron, cobalt, titanium and platinat) because the metallic pen also caused the bad side effect to the human body [6,7]. In Indonesia, the main materials used to produce Biomaterials, including HAp still imported from abroad [1,7,8]. However, on another side, Indonesia has many raw materials for synthetic and production HAp [9,10], like limestones, animal bones (waste animals bones from markets), and *Molluscan* shells (snails and clams) from marine and lacustrine [1-4]. Those raw materials can be synthesized to produce HAp because it is rich in Calcium (Ca) and Phosphorus (P) contains [2,3] the main minerals that build HAp crystal structure [1-3]. Indonesia is one of the countries with the higher number of fractures in Asia [7,8];. At the same time, the bone donations (donors and stock) are very limited at once [7-10], then necessary need to produce the reparating (synthetic) compounds similar to the bone structure like HAp [9,10].

One of the raw materials suitable for becoming raw materials of the HAp synthetic process was the (waste) eggshells [9,10]. The eggshells were predicted to have minerals Ca and P contained in equal positions with another raw material (*Molluscan* shells, animal bones and limestones) recommended as main compounds in HAp synthetic process [1,4,9,10], but commonly used in HAp synthetic was the Chicken eggshells [9,10] not Duck eggshells [11]. Commonly in Indonesia, eggshells are just applied in simple ways, even potentially applied in advance [12]. The standard and most straightforward applications of the (waste) eggshells in Indonesia nowadays are using it as fertilizer for farming [13-16]. Especially in Salatiga city, those (waste) eggshells, including Duck eggshells, are easy to find in markets, traditional markets, cake shops, and restaurants and are commonly not used anymore than can be collected in large amounts at low prices, even free of the coast [11,17]. Availability and quantity of duck eggshell waste produced from the Salatiga Main Market. Duck egg shells have the same position as other eggshell wastes regarding mineral content. Duck eggshell waste has the appropriate mineral content. (same level) with chicken (waste) eggshells [11]. The Chicken waste eggshells from the central market of Salatiga have commonly been applied as fertilizer in farming or handicraft (based on eggshells) production [17-19]. However, duck eggshells are not used anymore[12], so they can collect without a coast.

Based on several preliminary research reports which report that the Duck eggshells can use as the main compounds of HAp synthetic because it rich in Ca and P compounds [11] so in, this study treated the synthesis of HAp from Local Duck (*Anas platyrhynchos javanica*) waste eggshells obtained from Central Market (Pasar Raya) of Salatiga city.

METHODS

1. Time and Place

The research was treated from June 2018 to June 2019 at CARC Laboratorium of Faculty of Biology, Satya Wacana Christian University. The eggshells were collected from 15 spots in Pasar Raya Salatiga (including cake shops and restaurants). The process obtained 5-7 kg of waste duck eggshells per day from the collection.

2. Research Instruments

The instruments used in this research were UV-Vis Spectrophotometer, Flame Photometer, HACH Photometer, Oven, Furnace, and magnetic stirrer. The chemical compounds used in this study were Hydrochloric Acid (HCl), Aquadest, Ammonium Solution, Kalium Dihydrogen Phosphate, Citric Acid, Ascorbic Acid, Murexide, Buffer PO_4 pH 10, and Etanol. The materials belong to Pro Analysis (P.A) classification.

3. Preparation of Eggshells

The eggshells were boiled and washed using water, and Hydrogen peroxide was then separated from the protein fiber inside; after being washed, the eggshells dried at 55^{o}C in the oven for 24 hours. After dried, the eggshells were crushed into powder form [11,17-19].

4. Characterization of the Eggshells

a. Water and Ash Contains

1.0055g of Eggshell powder was measured in the water automatically using Moisture Analyzer for 15 minutes [11,17-19]. 1.0055 g of eggshell powder was incinerated using a furnace for 600oC and 5 hours. The differences between the eggshell mass and the ash mass were calculated to determine the carbons and organics compounds [11,17-21]. The Silicone is determined by washing the ash using HCl 1% (v/v) and boiling water. After washing, the residual of the ash was dried and noticed as Silicone contains [20,21].

b. Eggshells Solutions

1.0055 g of Eggshells powder were dissolved using 50mL concentrated HCl [13,20,21].

c. Calcium Contains

10mL of Eggshells solutions were diluted to 100mL by aquadest then separated into 3 vessels:

The first vessel contains 20mL solutions, then titrating using EDTA with EBT indicators to measure Ca contains as hardness [14,15].

The second vessel contains 20mL solutions, measuring the absorption (Abs) using UV-Vis spectrophotometer in 515nm with Murexide reagent. The Abs of solution plotting in the standard curve to measure the Ca contains $CaCO_3$ [11,20,21]. The third vessel contains 20mL solutions, measuring the absorption (Abs) using a Flame photometer to measure the Ca contained as CaCO3 and confirm the measurements using a UV-Vis spectrophotometer [11,20,21].

d. Phosphorus Contains

10mL of Eggshells solutions were diluted to 100mL by aquadest then separated into two vessels:

The first vessel contains 20mL solutions, measured the P contains as PO_4 using UV-Vis spectrophotometer in 860nm with Ammonium Molybdate and Ascorbic Acid reagents [11,17-21]. The second vessel contains 20mL solutions, measured the P contains as PO_4 using HACH Photometer in 880nm with Ammonium Vanadate reagents [11,17-21].

5. HAp Synthetic Process from Eggshells

50mL of eggshell solutions were filtered and fulfil it to 100mL using aquadest, the added 25mL Citric Acid 25% (w/v). After the solution homogeny, add the ammonia solution until the pH of the solutions reaches 9.5. The solution turns white; then drops the solutions by K_2HPO_4 0.8 M until the solutions are saturated and incubated at room temperature for 15-30 minutes. After incubation, add the concentrated HCl into the saturated solutions until the pH reaches 1, sinter the solutions at 70^oC until the white *Brushite* precipitation [1-6]. In the next step, the brushite precipitation was incinerated at 900^oC using a furnace for 6 hours, then obtained the white crystal of Hydroxyapatite (HAp) [1-6,17-21]

6. The FTIR Scan

The chemical structure change from eggshells to HAp is proved by FTIR scanning by scanning 1.0015 of each eggshell and HAp [1-6].

7. Characterization of HAp

a. HAp Solutions

1.0055g of HAp were dissolved into 50mL aquades using a sonicator [20,21]. The HAp solutions are characterized by measuring the density, viscosity by centrifugation viscometer, and refraction index by refractometers [22].

b. Calcium Contains

10mL of HAp solutions were diluted to 100mL by aquadest then separated into three vessels: The first vessel contains 20mL solutions, then titrating using EDTA with EBT indicators to measure Ca contains as hardness [20,21].

The second vessel contains 20mL solutions, measuring the absorption (Abs) using UV-Vis spectrophotometer in 515nm with Murexide reagent. The Abs of solution plotted in the standard curve to measure the Ca contains $CaCO_3$ [13,20,21].

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c. Phosphorus Contains

10mL of HAp solutions were diluted to 100mL by aquadest then separated into two vessels: The first vessel contains 20mL solutions, measured the P contains as PO_4 using UV-Vis spectrophotometer in 860nm with Ammonium Molybdate and Ascorbic Acid reagents [20,21].

The second vessel contains 20mL solutions, measured the P contains as PO_4 using HACH Photometer in 880nm with Ammonium Vanadate reagents [20,21].

d. Physical Characterization

The physical characterization of HAp was measured by macerating the HAp in 3 methods. First, the Porosity of HAp was measured by macerating the HAp in Aquades for 24 hours. The porosity was calculated before and after maceration [20,21,23-25].

The biodegradability of HAp was measured by macerating the HAp in buffer pH 10 for 24 hours. The biodegradability was calculated based on the before and after maceration [20,21,23-25].

The swelling ability of HAp was measured by macerating the HAp in Etanol 70% in 24 hours. The swelling was calculated based on the before and after maceration [20,21,23-25].

RESULTS AND DISCUSSION

The biomaterial structure is built on the Ca, and P (including HAp) produced. Due to the need for a biomaterial similar to hard tissue to repair or remineralize damaged hard tissue [2,3,23]. The biomaterials are commonly named Bioceramics, and one of the famous bioceramics was Hydroxyapatite (HAp) [1-5]. The HAp can be synthesized from raw materials rich in Ca and P compounds like eggshells (waste eggshells) [4,5]. In Indonesia, the waste eggshells produce 178,599.33 tons a year [26]. Among those waste eggshells were the duck eggshells [11]. Especially in Salatiga, the waste eggshells are easy to find at Local Central Market, Pasar Raya Kota Salatiga [11,17-19]. Especially for the waste of Local Duck Eggshells can be collected from the food street shops that sell the salty eggs made from local duck (*Anas platyrhynchos* javanica) or the street food shops other products based on duck eggs [11]. The sampling process can be obtained 5-7Kg dry duck eggshells per day (dry mass free of contaminants and proteins fibers). The characterization results of the eggshells showed in Table 1.

Table 1 The Characterization of Local Duck Eggshell Powder

Parameters			Units	Results
Water			%	1.10 ± 0.10
Ash			g/g	0.9729 ± 0.0041
Organic			g/g	0.0321 ± 0.0047
Carbon			g/g	0.0186 ± 0.0026
SiO			g/g	0.2666 ± 0.0015
Si			g/g	0.2167 ± 0.0024
Ca	Titrimetric		g/g	0.9128 ± 0.0128
	UV-Vis Sp		g/g	0.8631 ± 0.0015
	Flame		g/g	0.9091 ± 0.0024
P	UV-Vis Sp	PO_4	g/g	0.3118 ± 0.0016
		P_2O_5 Av	g/g	0.0050 ± 0.0002
		P_2O_5 Sol	g/g	0.0023 ± 0.0001
		P_2O_5 Tot	g/g	0.0072 ± 0.0004
		P Sol	g/g	0.0032 ± 0.0002
		P Tot	g/g	0.0156 ± 0.0008
	HACH	PO_4	g/g	0.2027 ± 0.0002
		P_2O_5	g/g	0.1523 ± 0.0002
		P 490	g/g	0.0068 ± 0.0001
		P 496	g/g	0.0086 ± 0.0011
Ratio of Ca/P		Olsen	%	1.802 ± 0.291
		Bray	%	0.7874 ± 0.0784

The Ca and P measurements must be treated carefully because those two minerals were the main minerals in the synthesis process[20,21]. In this research, the Ca is measured three times to the eggshells and the HAp. The titrimetric methods can not describe the Ca contains because the titrimetric by EDTA with EBT indicators showed the total hardness contained, while the hardness was the mix of Ca and Mg [20,21]. Because of it, the Ca measurements need to confirm by a spectrophotometric method using UV-Vis Spectrophotometer and Flame Photometer. The three methods showed that the Ca contained in close ranges means that the measuring was carefully done with accuracy. The Ca contained by spectrophotometric was slightly lower than titrimetric. The spectrophotometric results showed that Ca contains (pure) CaCO3 or CaO, different from the titrimetric results, which showed the Ca as hardness (Mix of Ca ad Mg). Phosphorus (P) was carefully measured by two spectrophotometric methods, namely UV-Vis spectrophotometer and HACH photometer [20,21]. The results show that P contains close distances, meaning that the measurements are carried out carefully and thoroughly.

Based on Table 1, it can be concluded that the waste eggshells of the local duck potentially became one of the raw materials to synthesize became HAp because the Ca and P contained in the eggshells belongs to high [11]. The Minerals Ca and P contained in the eggshells are synthesized biochemically from the food consumed by the Ducks. The food has a high content of Proteins, Fats, Ca, and P will cause the eggshells to increase to hard and thick. The harness increasing means the height contains Ca and P [16,30,31].

The Ca and P contained in the eggshells are commonly $CaCO_3$ and $CaPO_4$ compounds [4,13,16]; those compounds will be restructured along the chemical reactions in HAp synthetic process to become $[Ca_{10}(PO_4)_6(OH)_2]$ [1,3-6] known as HAp. The chemical reaction during the HAp synthesis process from the eggshells show as follows:

$$10CaO + 6(NH_4)_2H_3PO_4 + 2H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2\downarrow + 12NH_4OH \quad \text{eq.1 [4,27]}$$

Or

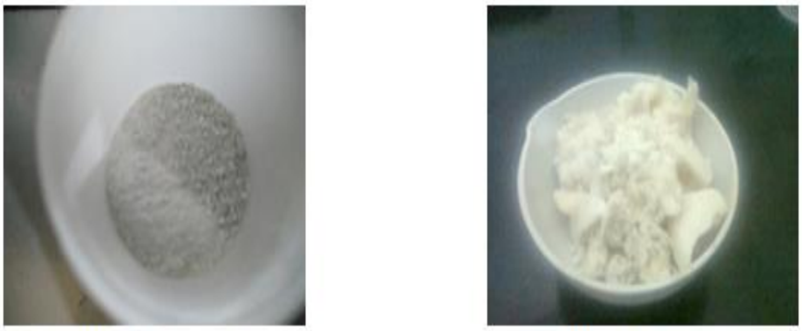
$$10CaO + 6H_3PO_4 \rightarrow Ca_{10}(PO_4)_6(OH)_2\downarrow + 8H_2O \quad \text{eq.2 [4,27-29]}$$

Those two chemical reactions showed that to produce the HAp (from its raw materials), the Ca and P contain the necessary need [26,27], then the characterization of the local duck eggshells showed that the contains of Ca, and P potentially synthesized became HAp [11]. Therefore, the following steps were to produce the HAp from local duck eggshells. The results of synthesis and characterization showed in Table 2.

Table 2. Physicochemical Characterization of HAp from Duck Eggshells

Parameters			Units	Results
Rendement			%	85.0640 ± 1.0134
Water			%	0.49 ± 0.01
Ca	Titrimetric		g/g	0.5440 ± 0.0103
	UV-Vis Sp		g/g	0.4760 ± 0.0027
	Flame		g/g	0.4852 ± 0.0037
P	UV-Vis Sp	PO_4	g/g	0.0897 ± 0.0023
		P_2O_5 Av	g/g	0.0014 ± 0.0004
		P_2O_5 Sol	g/g	0.0007 ± 0.0002
		P_2O_5 Tot	g/g	0.0021 ± 0.0005
		P Sol	g/g	0.0009 ± 0.0002
		P Tot	g/g	0.0045 ± 0.0012
	HACH	PO_4	g/g	0.0764 ± 0.0001
		P_2O_5	g/g	0.0057 ± 0.0001
		P 490	g/g	0.0026 ± 0.0001
		P 496	g/g	0.0013 ± 0.0001
Ratio of Ca/P		Olsen	%	1.191 ± 0.157

Parameters		Units	Results
	Bray	%	0.7778 ± 0.1055
Porosity		%	82.0106 ± 0.4484
Biodegradability		%	5.3506 ± 0.0295
Swelling		%	5.7678 ± 0.1897
Density		g/mL	0.9981 ± 0.001
Viscosity	Absolute	P.a	0.96 ± 0.0245
	Kinetic	CTs	0.9615 ± 0.0219
	MW	g/mol	0.001 ± 0.000002
Homogeneity	Brix	%	0.22 ± 0.02
	Dissolved Solids	%	0.2885 ± 0.0286
	Solubility	%	99.7112 ± 0.0286
Potentiality	Voltage	mV	1.00 ± 0.00
	Intensite	mA	15 ± 0.00
	Retention	O	60 ± 0.00



2a. Powder of Duck Eggshells

2b. HAp from Duck Eggshells

Figure 1. The Duck Eggshells Powder and It HAp

To prove the prediction about the change in the chemical structure during the synthesis of eggshells into HAp. FTIR Spectroscopy scanned both materials (eggshell and HAp). This method aims to approximate the method for describing changes by comparing the scattering of the FTIR spectra. these two materials [1,4,27–29]. For example, the FTIR scatter is shown in Figure 2.

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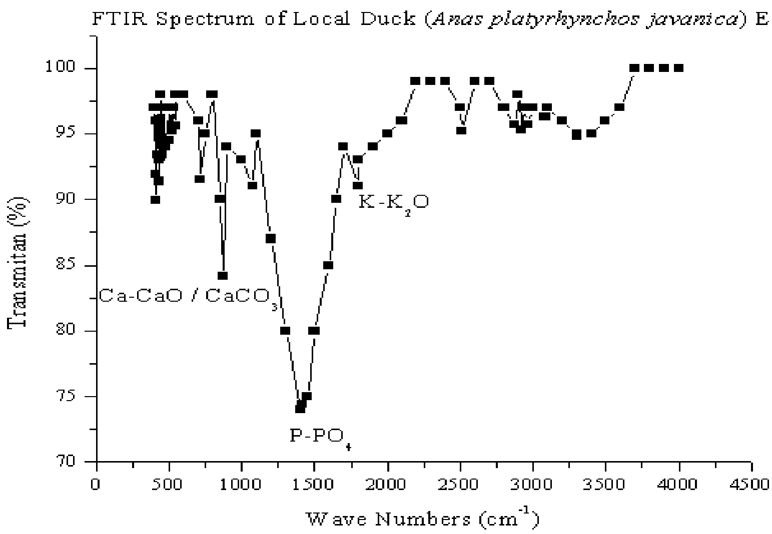

Figure 2a. The FTIR Spectrum Scater of the Duck Eggshells

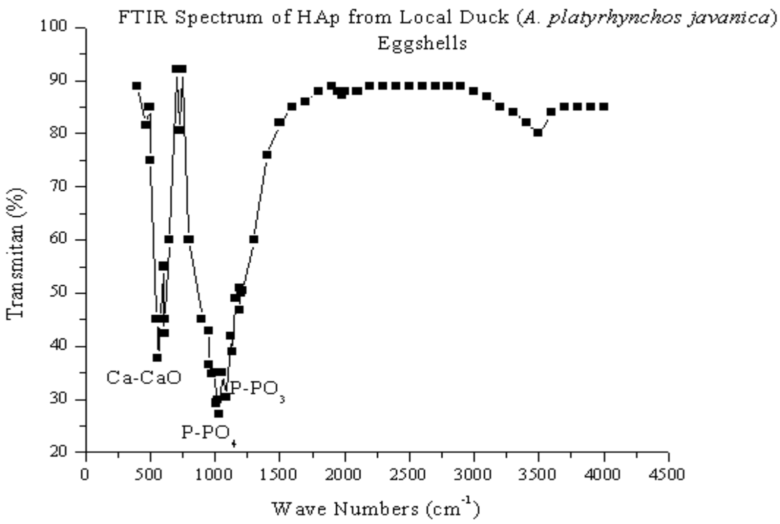

Fig.2b. The FTIR Spectrum Scatter of the HAp Synthesized from Duck Eggshells

The description of the FTIR scatter of Eggshells, and the HAp synthesized from it showed in Table 3.

Table 3. The Minerals Position and Intensity Based on FTIR Scatter

Minerals		Peak Position (cm^{-1})	Peak Intensity (%)
Eggshells	Ca	875.72	84.19
	P	1415.81	74.402
HAp	Ca	607.6	42.345
		560.35	37.825
	P	949.02	42.961
		979.88	34.844
		1006.89	29.283
		1030.03	27.287
		1058.97	30.189
		1082.11	30.426
		1134.19	38.936
		1187.24	46.869
		1212.31	50.509

The FTIR Scatter of the eggshell and its HAp showed differences throughout the peak position and intensity of Ca, and P contains. Those two components have the same minerals building the structure but are already different in the chemical position [1,6]. This case showed that the synthetic process's success in changing the eggshells became another different material predicted as HAp. In the eggshells, the Ca and P only showed one peak at each other, but in HAp, the Ca showed two peaks. The two peaks represent one of Ca at the bottom of the crystal structure, and the other peak belongs to Ca in the crystal wall (which binds to PO3). The Phosphorus (P) peak in HAp is also separated into nine small but significant peaks. One peak belongs to PO_4, which bond with Ca at the bottom of the crystal structure, and eight peaks belongs to PO_3, which bond with Ca in the wall of crystals. The chemical structure of the HAp showed in Figure 3.

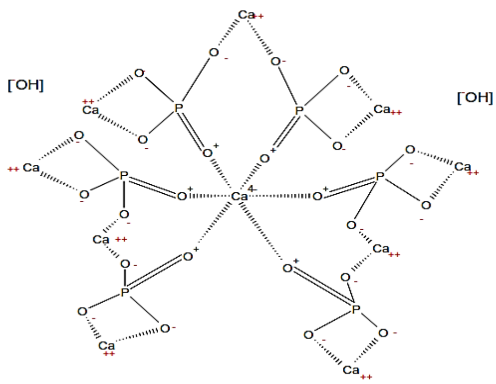

Figure 3. The Chemical Structure of the HAp [1-6]

Based on Figure 3, it can be seen that Ca (as CaO) and P (as PO4 and PO3) are separated at two positions, namely the bottom and the crystal wall [1-6]. Furthermore, the similarity of the scattering was shown from the FTIR HAp scattering of the duck eggshells in this study. The P peaks, in this case, are separated into nine peaks, whereas the ideal HAp generally has P peaks separated in 3-5 peaks. These results can predict that the HAp synthesized in this study is classified as Carbonate Apatite type II (CAp II) [1,4,32,33]. Nevertheless, this prediction is still rewiewed because it needs the XRD and SEM test analysis to be proved, and those two tests will be observed in the next part of this research.

The high Ca and P contained in duck eggshells cause the amendment of the HAp synthesis process to reach a high amount of 85,06%, which means the duck eggshells can become one of the HAp sources in Indonesia. However, the Ca and P contained in HAp decrease to the middle level; this is caused by the synthetic process, which uses concentrated (strong) acid combined with the incinerating temperature (higher than burning temperature), probably making the decay of minerals in the entire process [20,21]. Therefore, although the HAp gets the minerals decay, it still belongs to the ideal HAp based on the Ratio of Ca/P [1,4,6,19,20,21,23]. The ideal HAp showed the Ratio of Ca/P in the range of 0,67-1,67% with a crystal size of 250-50nm [1,6]. The Ca/P ratio in this study was 0.78% and 1.19%, meaning that the ratio is still classified as a standardized HAp ratio.

The Physics characterization, including Density, Molecular Weight (M.W.), Biodegradability, and Swelling ability of HAp, showed that the HAp belongs to ideals of Biocheramics or Biomaterials [23-25], but the porosity still in large amount, 82,011%. The porosity larger than the ideal that 45-65% of Porous, the porosity higher than the ideal range probably can fragility the biomaterials then it not ready to apply [34,35]. HAp synthesized from duck eggshell is not yet ready to be applied as a biomaterial to manage hard tissue damage. The reason is the high porosity, and research development is still needed to make HAp more at reducing porosity and increasing the hardness level of the structure [34,35]. If considering the obstacles of porosity, the possible application of the HAp from this research was to use the HAp as active compounds in toothpaste formula [17,19,22,32]. In the formula, HAp's role as a remineralization agent also includes abrasive compounds to handle the damage to the tooth enamel layer caused by caries bacteries [18]. The minerals contained in the eggshells are potentially observed deeply [13] to produce the biomaterials like HAp [32,33], and the minerals are the potential to increase the quality and quantity of the HAp [19-21].

CONCLUSION

Waste local duck eggshells potentially applied as raw materials to synthesic become Hydroxyapatite , one of biocheramics or biomaterials used to manage the damage of hard tissues in the human body. This study treated the synthesis of HAp from local duck eggshells from the Central Market of

Salatiga. The reason to select the local duck waste eggshells is that it is so easy to find in the market with a large amount and low cost. The characterization of the eggshells also showed that the local duck eggshells contain high Ca and P, the main minerals in HAp synthetic. Therefore, eggshells are very recommended as HAp sources. The HAp synthesized from the duck eggshells by *Base Precipitation* method then the rendements of synthetic belong to high because the eggshells have hight contains Ca and P. The HAp obtain from this synthetic were close with the references in the physicochemical characterizations. However, if the porosity is still in large amounts, it needs continuity research to reduce the porosity and produce the HAp, which is more hard in structure. Because the porosity is still significant, the HAp is recommended to apply as active compounds in toothpaste.

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