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Artikel

Synthesis of porous Hydroxyapatite (HAp) from Laying Chicken Waste Eggshells

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ABSTRACT: Hydroxyapatite (HAp) was the type of bioceramics synthesized from the compound which rich Calcium (Ca) contains, these bioceramics commonly used to repair the damage of hard tissues. In this study threatened the synthesis of HAp from laying chicken eggshells, the characterization of the eggshells showed that the contains of Calcium 0,9809 ± 0,0118g/g; Phosphate 0,0236 ± 0,0009g/g; and ratio of Ca/P 2,0840%. The synthetic process by base precipitation method showed the yield 77,5786 ± 0,3509%; and from the synthetic obtained the HAp with contains of Calcium 0,1522 ± 0,0047g/g; Phosphate 0,01354 ± 0,0019g/g; and ratio of Ca/P 0,9162%. The Biochemical characterization of the HAp showed that the Porosity 70,5940 ± 0,4104%; Biodegradability 16,1879 ± 0,1362%; and swelling Ability 28,0549 ± 0,1054%. The entire result of the research concluded that the sample eggshells potentially became one of the sources of HAp, and the HAp synthesized from the eggshells potentially to apply as remineralization agents.

INTRODUCTION

Hydroxyapatite (HAp) was one kind of Bioceramics compound commonly used to repair the damaged hard tissues, especially bone [2,7,10,23]. The HAp has chemical structure [$Ca_{10}(PO_4)6(OH)_2$] also known as Calcium Hydroxyphosphate in trade name [2,7,23]. HAp can be synthesized from the raw materials with high Calcium contained by Base Precipitation Method [23]. The raw materials referenced as HAp sourcer were Limestones [10,23], waste animals bones [23,41], Molluscan shells [1,13,14,25], and Eggshells [5,11,21,33,42,45]. Those raw materials selected as HAp sources because the main minerals build the materials were Calcium (Ca) and Phosphorus (P) [23,29,34,39,40], those two minerals need in the HAp synthesis process because those minerals also the main minerals build the chemical structure of HAp [29,30]. The HAp synthesized mainly because the needs of the compounds can repair the damaged bone tissues [30,36], HAp initiated as repairing compounds because it has similar structure and shape with the Calcium complex formation to build the hard tissues [10,23,40]. HAp is called bioceramics materials because it is used as Bone Filler, Bone Implant, Bone Graft, and remineralising agent [2,10,29,30]. Apart from similarity, HAp also initiated as reparation / remineralitation compounds for hard tissues [12,15,44] because it has biodegradability, biocompatible, and low toxicity characteristics [29,30,40]. The limited amount of bone donors in stock and supply was the main reason of synthetic and producing of HAp compounds [10,23,30]. The Chemical Structure of HAp showed at Figure 1.

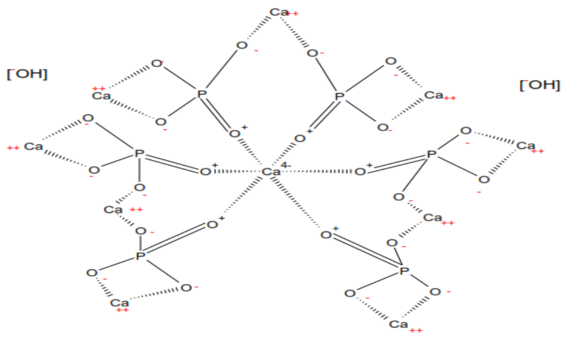

Figure 1. Chemical Structure of Hap [1,2,7,11]

In Indonesia, one of the main (or raw) material can be synthesized became HAp were eggshells (waste eggshells), the eggshells has high potentiality to become a source of HAp because it's easy to found, the price is cheap (even free because it's a kind of food or farm waste), and the abundance of mineral contains inside mainly Calcium [11,34,42]. Based on the references, reported that the eggshells containing Calcium (as CaO, $CaCO_3$ and $CaPO_4$) 50-90% and Phosphorus (as P or PO_4) 15-25% of it dry mass (depending on the aves species produce the shells) [3,5,11,21,33,42,50], the mineral contains in eggshells showed the potentiality in chemical side so can predict that with the Ca contains in that high will be directly proportional with the increase of yield of HAp synthetic process [33,42]. In Indonesia, reported that the produce of waste eggshells 178.599,33 tons a year [49] showed the big potential of availability, it will only has a price (economic value) if there is famer(s) buy it used to as fertilizer to enrich their farming land with minerals [6,8,9,22,24,26,27,28,38], and sometimes the shells buy to made as substitute materials for food [18,35], souvenirs or merchandises, but other than that for now the eggshells still not has economics values and can be obtained in cheap or free [11,31]. Based on the availability, mineral contains, and low economic value (cheap price) of the eggshells so initiated the use of Laying Hen waste eggshells to synthesized became HAp, the eggshells mainly obtained from the Central Local Market (Pasar Raya) of Salatiga and from the cake shop around. The aims of this study were to synthesize the HAp Laying Hen waste eggshells and Characterize the HAp to observe the chemical properties.

RESULT AND DISCUSSION

The results of characterization of shells, synthetic process and characterization of HAp synthesized from the shells showed at Table 1. The range of Ca measurements to the shells showed that the laying hen eggshells has potentially Ca contains to synthesized became HAp. The measurements are treated using 3 instruments / methods; Titration, UV-Vis Spectrophotometer, and Flame photometers aim to classify each type of Ca contains. The measurements using titration by EDTA and EBT indicators can detect the Ca contains as "Total Hardness" it means the results did not show the Ca pure but the mix of $CaCO_3$ and $MgCO_3$ [11]. The measurements using UV-Vis spectrophotometer by Murexide reagents can describe the Ca contains as $CaCO_3$ pure without interruptions from another cations and the measurements using Flame photometers can describe the entire kinds of Ca contains as $CaCO_3$ including CaO and $CaPO_4$. So to predict the potentiality based on Ca contains the detailed measurements.

Table 1. The Characterization of The Eggshells and the HAp

Method	Physicochemical Characteristics	Main Mineral	Conversion	Unit	Results	
					Shells	HAp
Gravimetric	Water			%	0,50 ± 0,00	-
	Ash			g/g	0,9763 ± 0,0008	-
		Organic		g/g	0,0275 ± 0,0009	-
			Carbon	g/g	0,0160 ± 0,0005	-
		SiO		g/g	0,2428 ± 0,0012	-
			Si	g/g	0,2199 ± 0,0002	-
Titrimetric		Ca		g/g	0,7485 ± 0,0001	0,1877 ± 0,0001
UV-Vis			CaO	g/g	0,7609 ± 0,0118	0,1522 ± 0,0047
Flame				g/g	0,9277	0,1945
UV-Vis (Ascorbic Acid) (Bray Method)		PO_4		g/g	0,0236 ± 0,0009	0,0135 ± 0,0019
			P_2O_5 Available	g/g	0,0037 ± 0,0001	0,0022 ± 0,0003
			P_2O_5 Soluble	g/g	0,0018 ± 0,0001	0,0010 ± 0,0002
			P_2O_5 Total	g/g	0,0055 ± 0,0002	0,0031 ± 0,0004
			P Soluble	g/g	0,0024 ± 0,0001	0,0014 ± 0,0002
			P Total	g/g	0,0118 ± 0,0005	0,0068 ± 0,0010
HACH (Blue Molybdenum) (Olsen Method)		PO_4		g/g	0,0210 ± 0,0002	0,01067 ± 0,0001
			P_2O_5	g/g	0,01577 ± 0,0002	0,0080 ± 0,0003
			P (490)	g/g	0,0070 ± 0,0001	0,0033 ± 0,0003
			P (496)	g/g	0,0117 ± 0,0002	0,0039 ± 0,0001
Flame		K		g/g	0,0027	(-)
HACH				g/g	0,0048 ± 0,00004	0,0017 ± 0,00003
AAS				g/g	(-)	(-)
Flame		Na		g/g	(-)	(-)
UV-Vis		SO_4		g/g	0,0037 ± 0,0003	0,0013 ± 0,0004
Gravimetric	Ratio Ca/P			% (Bray)	2,0840 ± 0,3545	0,9162 ± 0,0745
				% (Olsen)	2,29 ± 0,44	0,792 ± 0,331
	Porosity			%	-	70,5940 ± 0,4104
	Swelling			%	-	28,0549 ± 0,1054
	Biodegradability			%	-	16,1879 ± 0,1362
	Yield			%	77,5786 ± 0,1351	
Appearance	Form			-	Powder	Powder
	Color			-	White-Yellow	White
	Texture			-	Crude	Soft

The measurements of P-PO_4 are also threatened 2 times using 2 different methods and instruments, UV-Vis spectrophotometer and HACH Photometer. This measurements also aims to describe the detail of potential of P-PO_4 contains. The Ca and PO_4 contains need to describe in great detail because those minerals were the main minerals build the structure of HAp crystals. In the synthetic process from shells to HAp those minerals contains get decreasing, Ca get contains decreasing about ±57-58%, and P-PO_4 about ±9-10% from initial conditions. Another minerals measured with detail were K contains because several type of apatit also containing K [**45**] but the results of K measurements show the little amount then difficult to predicted the existence of K in the apatit formations.

The Ca and PO4 contains measurements also needed as base to describe the ratio of Ca/P in the HAp [**1,49,46**]. The ratio of Ca/P was the parameter needed to describe because with the ratio value able to approach the size of HAp crystals. The references report that the HAp with ratio of Ca/P 0,7-0,9% has the diameters range belongs to 250-125nm [1]. The ratio value is also able to predict the other ions bindings to the HAp structures. HAp with a ratio of Ca/P 0,7-0,9% predicted bond with Silicone (Si) ions [1,49,46]. In the cells, the Ca and PO4 contains caused by the feeding process using the foods which rich with proteins contains, the proteins can increase the estrogen produce then trigger the (biochemical) synthesis of Ca and P [3,20,37,47] in large amount to produce the good and thick eggshells [3,4,16,17,19,31,32,34,48].

The Porosity, Biodegradability, and Swelling ability measurements aim to describe the physical strength of the HAp when adapting the biochemical factors when applied (in the living bodies) [12,13,14]. The porosity of HAp was the vessels to produce the new bone matrix and the mineral link with the tissues when the HAp applied as bioceramics to the bones and interacting with the damage bone cells (and tissues) [41,46,47], but the large porosity will failing the physical structure of HAp [12,13,14] in macro and micro size [1,10], but in nano size the large porosity doesn't affected any fragile to the physical structure of HAp but just be better [1,10]. The biodegradability measurements aim to show the (approaching) mechanism that the HAp can dagradating (or not) by the biochemical factors. The chemical interaction with the living bodies tissues can cause the HAp structure will also get the degradation effect which can reducing the minerals, the Biodegradability measurements also can be proved the biocompatibility and biodegradability of the HAp but related with porosity, if the biodegradability too high can be fragiling the structure of HAp (in macro, micro and nano size). The Swelling ability measurements aim to describe the adaptation mechanism of the HAp when reacting with biochemical factors in the living body other than from hard tissues, like water, salt, and glucose solutions contained in the blood. If the HAp bioceramics absorb those solutions too intensely, the physical structure of HAp can be swelling and in swelling conditions the HAp structure tends to be fragile. From the measurements of Porosity, Biodegradability, and Swelling ability predicted that the HAp potentially to apply as remineralization and reparation agents to the hard tissues [12,13,14]. The Characterization of the HAp also threatened in solution form, 10g HAp dissolved until 100ml using aquadest and the solution were characterized. The result of HAp solution characterization showed at Table 2.

Table 2. The Characterization of HAp Solution (10%w/v)

Parameters	Density (g/ml)	Viscosity			MW (g/Mol)	Homogeneity			Z-Potential		
		Absolute (P.a)	Kinetic (CTs)	Dinamic (CPs)		Brix (%)	Solids (% w/v)	Solubility (%)	V (mV)	I (mA)	R (O)
HAp Solution 10% w/v	0,9979 ± 0,0005	0,97 ± 0,00	1,0281 ± 0,0000	0,1028 ± 0,0000	0,0007 ± 0,0000	0,22 ± 0,00	0,2885 ± 0,0286	99,7112 ± 0,0286	1,5 ± 0,00	15 ± 0,00	65 ± 0,00

The characterization of HAp solutions aims to complete the characterization of the HAp crystals (solid form). The density and Molecular weight (MW) measurements aims to describe the penetrating and interacting ability of HAp, small density and MW of HAp will able to cause the intense chemical interaction [10] when applied as bioceramics to manage the damage hard tissues, then the intense interaction will accelerating the healing process from the damage. The measurements of density and MW showed the small value mean the HAp synthesized from laying eggshells potentially cause the intense chemical interaction when it applied, the density measurements also aims to show the initial conditions of HAp before get the swelling effect when it applied in the body and reach with the biochemical factors [12]. Density measurements related with the Swelling ability measurements because swelling ability measurements are based on the increation of density [12]. The viscosity measurements are also related with the penetration and interaction but more focused on penetration ability. Small value of viscosity potentially cause the HAp can deeply penetrating into the damage tissues to manage it, the results of viscosity measurements to the HAp solutions showed the small value of viscosity means the HAp potentially able to penetrate to the damage tissues and triggering the produce of new (cels) bone matrix and accelerating the recovery of the tissues from crack or damage. The homogeneity measurements based on Brix scale aims to discover the solubility of HAp. The solubility related with the porosity and biodegradability parameter, the high solubility will produce the large value of porosity and biodegradability, also can proved that the HAp was biodegradable compounds.

The Z-Potential (electricity) measurements aim to provide the predictions about interaction of the HAp and also the absorption (to the tissues). The chemical interactions are mainly caused by the electricity factors called potential differences. Potential differences caused by the difference of ionic charge between two (or more) compounds cause the tug interactions. Potential differences are sometimes symbolized with Voltage parameters. The mechanism of electricity describe as; the HAp measured has the voltage so potentially interacted with the hard tissues causing the tug between the HAp and the tissues then the HAp will attracted towards the tissues, the low density, MW and viscosity predicted the HAp particles will easy to absorbed or bind to the tissues. The Z-Potential measurements proved that the HAp has the potential to cause chemical interactions when it is applied as the remineralization agent.

The next steps of characterization was the FTIR scanning to the eggshells and the HAp, the chemical structure change can be described by the FTIR sketch. The FTIR sketch of the HAp and the shells showed the differences in mineral positions mainly PO_4 and CO_3. The Results of FTIR scanning showed at Figure 2.

Based on equation 1 and 2 can conclude that the Ca contains in the substrates (raw materials / eggshells) [35-41] necessary in the HAp synthesis process to build the HAp structure [24,26,30,34]. Along the synthetic process of HAp, the main minerals from eggshells reduced [12,42-46], the Ca get 66,8% reduction and leaving ±0,73-1,82% and PO_4 get 2,41% reduction then leaving ±1,09-2,42% containing in HAp. The reduction of minerals is mainly caused by the incineration process using the temperatures 900-1100 ^oC along 3 hours. The PO_4 didn't get the high reduction levels because the Phosphate was not affected with high temperatures in apatite form. Chemical compounds are called as "Apatite'' because of the existence of PO_4 binding in the compounds, and mainly these compounds were crystallized at high temperature levels, because of that the PO_4 was not affected by the high temperatures in the incineration of brushite to produce the HAp.

The next steps of characterization was the FTIR scanning to the eggshells and the HAp, the chemical structure change can be described by the FTIR sketch [42,43,46,47]. The FTIR sketch of the HAp and the shells showed the differences in mineral positions mainly PO_4 and CO_3. The Results of FTIR scanning showed at Figure 2.

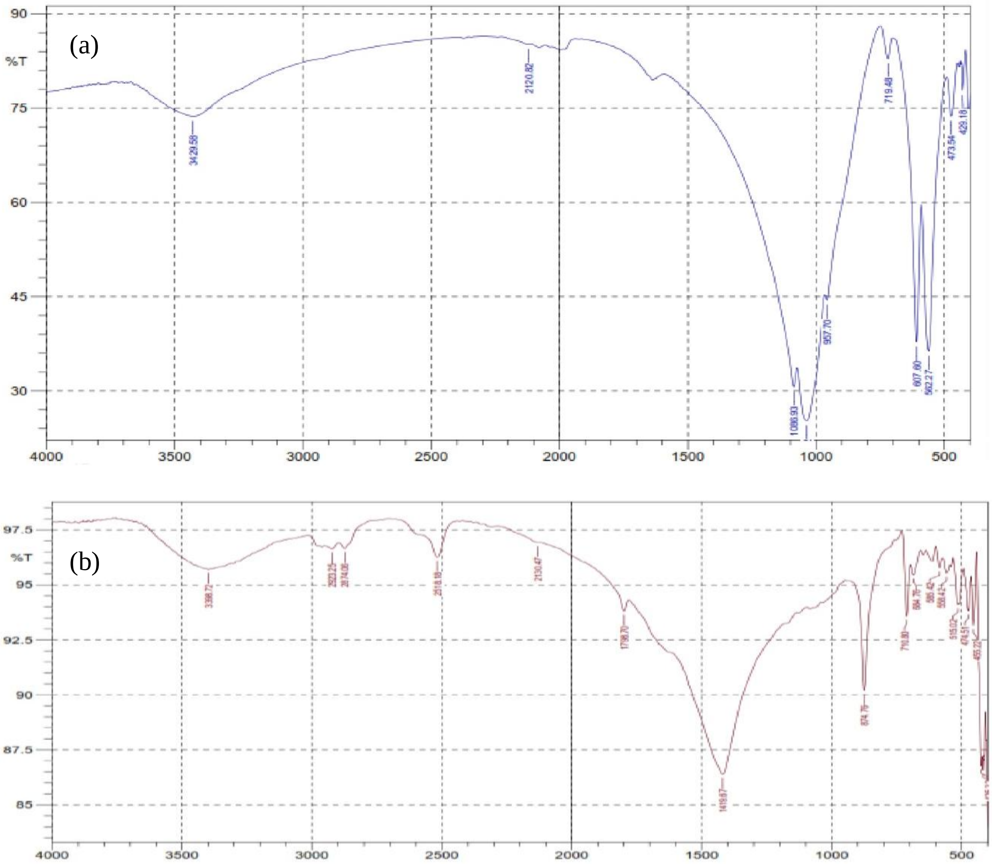

Figure 2. FTIR Image of The (a) Eggshells (b) HAp From The Eggshell

The Characterization by FTIR scanning aims to prove the structure change from the shells to the HAp after the synthesis process. The image from FTIR will show the differences, then prove the main structure after synthesis is not shells anymore but [$Ca_{10}(PO_4)_6(OH)_2$) also called HAp.

The characterization results need to be compared with the references to prove the Hypothesis about HAp synthesized from eggshells. The Ca and P measurements compared with the references then the comparison showed at Table 3.

Table 3. The Comparison of Minerals Contains (Ca and P-PO_4) from Eggshells Against The References

Mineral	Laying Hen Eggshells	Mineral Contains of Eggshells Based on References							
		[3]	[9]	[18]	[21]	[11]	[40]	[37]	[50]
Ca	0,7609 ± 0,0118 g/g	25,73 % (Local Hen)	70,088 ± 0,061 g/Kg (Laying	37,30%	94%	0,0188 ± 0,0007 g/g (Local Hen)	26,3972 ± 8,63mg.g (Hen)	1,19-1,21%	5,22% (Local Hen)

		23,67% (Duck)	Hen)			0,0195 ± 0,00 g/g (Laying Hen)	30,3334 ± 8,57mg/g (Duck)		6,41% (Laying Hen)
		20,67% (Laying Hen)				0,0195 ± 0,00 g/g (Duck)	31,7288 ± 13,14mg/g (Quail)		10,11 (Duck)
		21,70% (Quail)							9,69% (Quail)
P	0,0118 ± 0,0005 g/g	-	-	0,35%	1%	0,001349 g/g (Local Hen)	-	0,49-0,53	-
						0,001355 g/g (Laying Hen)			
						0,001294 g/g (Duck)			

Based on the comparisons, showed that the local chicken eggshells have the Ca and P [48-52] contains potentially to be applied in HAp synthesis as one of HAp sources [46,47]. The shell's potentiality based on synthetic yield was also compared with the other research, and the comparison showed at Table 4.

Table 4. The Synthetic Yield Compared With The References

Results	HAp yield from Laying Hen Eggshells	The Reference from Researches Before				
		[13]	[14]	[33]	[25]	[45]
% of yield (w/w)	77,5786 ± 0,1351	95,4320 ± 0,7085	85,2891 ± 4,2496	73,75	53	64
					60	
					71	

Based on rendement comparison showed that the rendement synthesis of HAp from laying chicken eggshells belongs to high range, the high rendements supported by the Ca and P-PO_4 containing in the shells, those two minerals role the main part of HAp formations, because the minerals were the main mineral build the HAp crystal structure. Along the synthetic process from shells to HAp, the mineral content gets reduced but still exists in the HAp. The measured mineral contains in HAp compared with the references and showed Table 5.

Table 5. The Results of Main Mineral Contains Measurements in HAp Against The References

Mineral	HAp from Laying Hen Eggshells	The Reference from Researches Before			
		[13]	[14]	[45]	[41]
Ca	0,1522 ± 0,0047 g/g	0,0095 ± 0,001g/g	0,0121 ± 0,0031g/g	9,78; 10,50% (atom)	88,88
PO_4	0,0135 ± 0,0019 g/g	0,0528 ± 0,008g/g	0,0167 ± 0,0013g/g	-	-
P	0,0068 ± 0,0010	0,0264 ± 0,004g/g	0,0084 ± 0,0007g/g	9,35; 8,97% (Atom)	8,78

The comparison showed that the Ca and P contains get reduced but still in the range referenced. The decrease (reduced) of the mineral contains mainly caused by the incineration process of brushite to produce the HAp. The Ca and P contains a need to describe the ratio of Ca/P. The ratio of Ca/P of the HAp also compared with the references in Table 6.

Table 6. The Results of Ratio Ca/P from The HAp Synthesized from Local Chicken Eggshells

HAp from Laying Hen Eggshells		The Reference from Researches Before							
		[13]	[14]	[42]	[30]	[10]	[46]	[1]	[45]
Ratio Ca/P (%)	0,9162 ± 0,0745 (B)	1,0307 ± 0,0932 (B)	1,3724 ± 0,2736 (B)	1,07	1,66	1,67	1,6760	1,60	0,67
				1,06	1,74		1,8209	1,67	1,67
					1,88		0,0433	1,74	2,67
	0,792 ± 0,331 (O)	0,96 ± 0,02 (O)	1,33 ± 1,32 (O)		1,78		0,0599		
							0,0423		

The biochemical parameters of HAp also compared with the references and showed at Table 7.

Table 7. The Biochemical Parameters against the References

Parameters	HAp from Laying Hen Eggshells	The Reference from Researches Before			
		[13]	[14]	[30]	[12]
Porosity (%)	70,5940 ± 0,4104	73,9598 ± 1,1320	9,9929 ± 0,7626	88,442	63,7476 ± 5,79145
				84,890	
				74,830	
				63,906	
Biodegradability (%)	16,1879 ± 0,1362	8,8206 ± 0,2176	10,8958 ± 1,1781	-	27,8833 ± 0,46448
Swelling (%)	28,0549 ± 0,1054	9,6067 ± 2,5365	24,8416 ± 1.4989	-	10,9983 ± 7,36003

CONCLUSION

The characterization of the eggshells showed that they contain Calcium 0,9809 ± 0,0118g/g; Phosphate 0,0236 ± 0,0009g/g; and ratio of Ca/P 2,0840%. The synthetic process by base precipitation method showed the yield 77,5786 ± 0,3509%; and from the synthetic obtained the HAp with contains of Calcium 0,1522 ± 0,0047g/g; Phosphate 0,01354 ± 0,0019g/g; and ratio of Ca/P 0,9162%. The Biochemical characterization of the HAp showed that the Porosity 70,5940 ± 0,4104%; Biodegradability 16,1879 ± 0,1362%; and Swelling Ability 28,0549 ± 0,1054%. The entire result of the research concluded that the sample eggshells potentially became one of the sources of HAp, and the HAp synthesized from the eggshells potentially to apply as remineralization agents.

MATERIAL AND METHOD

Preparation and Sterilization of The Sample Eggshells

The eggshells obtained from the market were washed and boiled to sterilize then continue with peeling of the organic fiber layers inside the shells. After peeling the shell, the shells dried for 24 hours using the oven and grinded into powder (eggshells powder).

The Shells Characterization

Moisture Measurements

The water contains (moisture) in the shells measuring using automatic moisture balance [11,13,14].

Ash Content

1g eggshells powder were ignited by a furnace in 650°C for 5 hours, after 5 hours the white ash was taken and weighed. The organic and carbons contains can be obtained from conversion calculation of the ash contains, and the silicone contains obtained by washing the ash using Hydrochloric Acid 1% (v/v) [11,13,14].

Eggshell solution

10g eggshells powder were dissolved in 100ml concentrated HCl (p.a) and filtering the solutions. 10ml eggshell solution diluted until 100mL by aquadest named as Stock solutions [3,11,13,14].

Calcium Contains

The Calcium contains measured using 10ml stock solutions added with the Murexide reagent then measuring the Ca contains using UV-Vis spectrophotometer at 515 nm. The Ca contains confirmed using the Flame photometer measurements, and titrimetric measurements using EDTA and EBT indicator [11,13,14].

Phosphorus (P/PO_4) Contains

The P/PO_4 contains 10 ml stock solutions added with the Blue Molybdate reagent and Ascorbic Acid 1% (w/v) solutions and measured using UV-Vis spectrophotometer at 800 nm. Ca contains confirmed using the HACH photometer measurements with Molybdenum-Vanadate reagent at 880 nm and setting number 490 [11,13,14].

FTIR Scanning

1g of shells powder scanning using FTIR spectrometer to describe the minerals composition.

The Synthesis of HAp Process

10g of Eggshells powder were dissolved in 100ml concentrated HCl (p.a) and filtering the solutions. 10ml eggshell solution diluted until 100mL by aquadest and added by 30ml Citric acid solutions and incubating it. After incubating, the solutions added with NH_4 solution until pH 9,5 then continued with the addition of KH_2PO_4 (8M) solutions with the drop speed 1 drop per second until the precipitation appeared. Separating the precipitated compounds (from the solutions) and dissolving it using concentrated HCl until pH 1. The solution with pH 1 was sintering in 70 °C until the condense, the solid phase called brushite. The brushite was ignited by a furnace at 900-1000 °C for 5 hours and produced the white HAp compounds [11,13,14,23].

HAp Characterization

HAp Solutions

10g HAp powder were dissolved in 100ml aquades (P.A) and vibrated the solutions using sonicator until the solution homogeny then filtering the solutions. The solution is by measuring the refraction index, density (in g/ml), viscosity (by rotary viscometer) and Z-Potential (by multimeter). This solutions called HAp stock solution.

Calcium Contains
The Calcium contains measured using 10ml HAp stock solutions added with the Murexide reagent then measuring the Ca contains using UV-Vis spectrophotometer at 515 nm. The Ca contains confirmed using the Flame photometer measurements, and titrimetric measurements using EDTA and EBT indicator [11,13,14].

Phosporus (P/PO_4) Contains
The P/PO_4 contains 10ml HAp stock solutions added with the Blue Molybdate reagent and Ascorbic Acid 1% (w/v) solutions and measured using UV-Vis spectrophotometer at 800 nm. Ca contains confirmed using the HACH photometer measurements with Molybdenum-Vanadate reagent at 880 nm and setting number 490 [11,13,14].

FTIR Scaning
1g of shell powder scanning using FTIR spectrometer to describe the minerals composition also to describe the structure change of the HAp [1,2,5,7].
Porosity Measurements
Porosity is measured gravimetrically by macerating the HAp in aquades along 24 hours in 37°C. After incubating, the HAp were dried and weighed [12,13,14].

Biodegradability Measurements
Biodegradability measured gravimetrically by maserating the HAp in Buffer PO_4 pH 7 along 24 hours in 37°C. After incubating, the HAp were dried and weighed [12,13,14].

Swelling Ability Measurements
Swelling ability measured gravimetrically by macerating the HAp in etanol 70% along 24 hours in 37°C. after incubating, the HAp were dried and weighed [12,13,14].

DECLARATION

There is no conflict of interest from authors for this research.

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