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### **Nanohydroxiapatite Using Chicken Eggshell Waste And Its Characterization**

Journal:	<i>Malaysian Journal of Medicine &amp; Health Sciences</i>
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Keywords:	Nanohydroxyapatite, Chicken Eggshell Waste, Characterization

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## Abstract

Hydroxiapatite (HAp) is a multiused biomaterial and it can stimulates hard tissue repair. HAp is biocompatible, nontoxic and similar with bone and teeth structure. It can be synthesized from natural sources, such as eggshell waste. Eggshells waste contain almost 94% calcium carbonate, which preferable for producing CaO as calcium resource for synthesizing pure HAp powder with nanocrystalline form. Compared to other poultry eggshells, chicken eggshell has higher HAp composition. This study aims to review nano hydroxyapatite (nanoHAp) from chicken eggshell waste and its characterization using Scanning Electron Microscopy (SEM), Energy Dispersive X-ray spectroscopy (EDX), X-ray Diffraction Analysis (XRD), and Fourier Transform (FTIR) Spectroscopy.

**Key Words** : Nanohydroxyapatite, Chicken Eggshell Waste, Characterization

## Introduction

Hydroxyapatite is biomaterial that widely used. Its chemical properties has an excellent biocompatibility, bioactivity, stimulates growth of bone tissue and economically cost. There were several experiment concerning to produce best quality HAp because of its advantages. It was synthesized through various methods.(1-2) There are many natural sources of HAp, such as bovin bone, aquatic source, and eggshells.(3-4) Among them, Chicken eggshell is interesting to be reviewed because it contains 94% calcium carbonate and low cost biomaterials. It consists of higher hydroxyapatite than other poultry eggshells.(2,5)

## Function Of Hydroxiapatite In Dentistry

In Implantology, calcium source from nanoHAp creates osseogenesis, inhibits the growth of bacteria, reduced inflammatory, and reconstruct bone defects.(6) In periodontology, HAp is used to fill bone loss in surgical procedure and binds chemically in osseointegration process.(7) Nowadays, HAp is also used in tissue engineering, It is known as excellent material approach for hard tissue reconstruction and repair. It support alginate or other polymers as reinforcement

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3 and osteoconductive material to promote a successful tissue regeneration.(8-9) Another study  
4 performed gelatin,magnesium doped hydroxyapatite mixed with alginate thus they concluded  
5 that it is possible to achieve scaffolds with fine microscopic pore.(10) Earlier clinical tested the  
6 hypersensitivity effect of nanohydroxyapatite compared with Pro-Argin and fluoride varnish,  
7 and it showed nanohydroxyapatite effective as dentin desensitizing.(11)  
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### 14 **Source of Hydroxyapatite**

16 HAp can be synthesized from natural and synthetic source. Synthetic HAp was common  
17 material used in tissue engineering, bone regeneration and replacement. It was suitable to  
18 human hard tissue with 1,67 stoichiometry. The main of its composition is calcium, so similar  
19 to HAp from living source. Natural HAp carries advantages such as reduces the impurity and  
20 production cost, also biological origin and overcomes environment pollutant.(12-13) It can be  
21 synthesized from mammalian bone, clam shell, coral, poultry eggshell.(14-15) Chicken  
22 eggshell is one of common derived HAp with economically cost, and consumed tons yearly.(2)  
23 It also contains higher HAp (0,0950 g/g) than other poultry eggshells (0,3315 g/g - 0,0559  
24 g/g).(5) Synthetic nanoHAp can achieved 534 nm particle size and natural HAp can be  
25 synthesized to 250–550 nm with appropriate milling process, so natural HAp is acceptable for  
26 bone replacement application.(16)  
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### 41 **Review of HAp Characterization**

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44 Characterization of HAp derived from eggshell usually uses several tools , such as Scanning  
45 Electron Microscopy (SEM)-Energy Diffraction Spectroscopy (EDX) which can observes the  
46 morphological and elemental study. Heating process with appropriate method resulted in well  
47 shaped nano HAp particle with agglomerates shape creates pore in between.(2) Calcination at  
48 1100 °C can performed smooth surface of agglomeration in spherical shape.(16) Figure 1  
49 (Fig.1) showed SEM analysis of HAp at 2 hours aging time performed fluffy agglomerates  
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3 rounded edge morphology, and highly agglomerated HAp figure at 1000 °C calcination  
4 (Fig.2).(3,17)  
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7 Crystalline structure and composition phase can be determined by XRD analysis. XRD uses  
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9 CuK $\alpha$  radiation with 40 - 45 kV voltage and data collects from  $10^{\circ} < 2\theta < 80^{\circ}$ .(12,17) The  
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11 diffraction pattern performed compatible HAp characterization phase with crystal size range  
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13 at 20.12 and 19.93 nm in eggshell HAp which calcinated in 1000°C and there were no  
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15 secondary phase (Fig.3). It suitable with bone tissue HAp which has 15 nm crystallite size.(3)  
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17 FTIR used to observe the type of chemical bonds and functional group in samples.(12) Usually  
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19 samples uses with KBr to make a pellet and it is tested with 4000-400  $\text{cm}^{-1}$  wavenumber  
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21 range.(18) Figure 4 showed adsorbance of H $_2$ O in graph broad band at 1643.48  $\text{cm}^{-1}$  and  
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23 3449.01  $\text{cm}^{-1}$ . Vibration mode was known by observed peak at 878.26  $\text{cm}^{-1}$  and 1460.87  $\text{cm}^{-1}$   
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25 which indicates elimination CO $_3^{2-}$  ion because of calcination process of HAp. Band at 633,14  
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27 confirmed the OH structure in HAp. Peak at 926.81 showed starching mode of PO $_3^{3-}$ . From this  
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29 graph it was confirmed crystalline phase.(2)  
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37 Several study concerned in HAp characterization are performed by Horta et al who synthesized  
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39 nanohydroxyapatite by precipitation method using hen eggshell with 2 different aging time  
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41 (Table I), Khandelwal and Prakash synthesized HAp powder from eggshell with wet chemical  
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43 method also characterized it by SEM-EDX, XRD, FTIR, TGA-DTA (Thermogravimetric/  
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45 Differential Thermal Analysis) (Table II). Another study performed due to develop tissue  
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47 engineering biomaterial by Agbabiaka et al who also characterized HAp from eggshell using  
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49 three calcination temperature (Table III).(17) Hamidi et al characterized derived HAp from  
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51 eggshell using calcination and ball milling method with different rotational speed and heat  
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53 treatment temperature using SEM,XRD and FTIR. (Table IV).(16)  
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## 57 **Conclusion**

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Hydroxiapatite is a biomaterial used due to its biocompatibility and the similarity with bone and teeth structure, whether it was synthetic or natural source. Eggshell waste is one of popular natural source which can be synthesized into nanohydroxiapatite. According to several research before, calcination using high temperature and appropriate ball milling process has considered to give several effect in achieved proper HAp. Selection of suitable HAp characterization tools can performed appropriate interpretation result.

#### Conflict of Interest

Author declared no potential conflicts of interest with respect to the authorship and/or publication of this article.

#### References

1. Sossa PAF, Giraldo BS, Garcia BCG, Parra ER, Arango PJA. Comparative study between natural and synthetic Hydroxyapatite: structural, morphological and bioactivity properties. *Matéria (Rio J)* [Internet]. 2018 Dec 6 [cited 2021 Mar 4];23(4). Available from: [http://www.scielo.br/scielo.php?script=sci\\_arttext&pid=S1517-70762018000400408&lng=en&tlng=en](http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1517-70762018000400408&lng=en&tlng=en)
2. Khandelwal H, Prakash S. Synthesis and Characterization of Hydroxyapatite Powder by Eggshell. *JMMCE*. 2016;04(02):119–26.
3. Horta M, Aguilar M, Moura F, Campos J, Ramos V, Quizunda A. Synthesis and characterization of green nanohydroxyapatite from hen eggshell by precipitation method. *Materials Today: Proceedings*. 2019;14:716–21.
4. Mohd Pu'ad NAS, Koshy P, Abdullah HZ, Idris MI, Lee TC. Syntheses of hydroxyapatite from natural sources. *Heliyon*. 2019 May;5(5):e01588.
5. Gintu AR, Salenusu MW, Wadu I, Hartini S. Sintesis Biokeramik Hidroksiapatit (Hap) Dari Kerabang Telur Ayam Kampung, Ayam Broiler, Dan Bebek Menggunakan Metode Pengendapan Basa Dan Hidrolisis. *Bioma*. 2017;6(2):11.
6. Bordea IR, Candrea S, Alexescu GT, Bran S, Băciuț M, Băciuț G, et al. Nano-hydroxyapatite use in dentistry: a systematic review. *Drug Metabolism Reviews*. 2020 Apr 2;52(2):319–32.
7. Pepla E, Besharat LK, Palaia G, Tenore G, Guido Migliau. Nano-hydroxyapatite and its applications in preventive, restorative and regenerative dentistry: a review of literature. *ADS* [Internet]. 2014 [cited 2021 Mar 4]; Available from: <http://www.annalidistomatologia.com/common/php/portiere.php?ID=152fe78586592c638a38bef5e4b82eb2>
8. Sancilio S, Gallorini M, Di Nisio C, Marsich E, Di Pietro R, Schweikl H, et al. Alginate/Hydroxyapatite-Based Nanocomposite Scaffolds for Bone Tissue Engineering Improve Dental Pulp Biomineralization and Differentiation. *Stem Cells Int*. 2018 Aug 2;2018:1–13.
9. Sathiyavimal S, Vasantharaj S, LewisOscar F, Selvaraj R, Brindhadevi K, Pugazhendhi A. Natural organic and inorganic–hydroxyapatite biopolymer composite for biomedical applications. *Progress in Organic Coatings*. 2020 Oct;147:105858.

10. Panseri S, Montesi M, Dozio SM, Savini E, Tampieri A, Sandri M. Biomimetic Scaffold with Aligned Microporosity Designed for Dentin Regeneration. *Front Bioeng Biotechnol* [Internet]. 2016 Jun 8 [cited 2020 Sep 27];4. Available from: <http://journal.frontiersin.org/Article/10.3389/fbioe.2016.00048/abstract>
11. Wang L, Magalhães A, Francisoni-dos-Rios L, Calabria M, Araújo D, Buzalaf M, et al. Treatment of Dentin Hypersensitivity Using Nano-Hydroxyapatite Pastes: A Randomized Three-Month Clinical Trial. *Oper*. 2016 Jul 1;41(4):E93–101.
12. Hammood AS, Hassan SS, Alkhafagy MT. Comparison of Natural and Nano-synthetically-Produced Hydroxyapatite Powder. *JOM*. 2019 Jan;71(1):272–8.
13. Ketta M, Tůmová E. Eggshell structure, measurements, and quality-affecting factors in laying hens: a review. *Czech J Anim Sci*. 2016 Jul 24;61(07):299–309.
14. Yusril Yusuf, Dyah Uswatun Khasanah Firda Yanuar Syafaat Ishak Pawarangan, Mona Sari, Vicky Julius Mawuntu Yazida Rizkayanti. *Hidroksiapatit Berbahan Dasar Biogenik*. Gadjah Mada University Press; 2019.
15. Mozartha M. *Hidroksiapatit Dan Aplikasinya Di Bidang Kedokteran Gigi*. *Cakradonya Dent J*. 2015;7(2):807–68.
16. Hamidi AA, Salimi MN, Yusoff AHM. Synthesis and characterization of eggshell-derived hydroxyapatite via mechanochemical method: A comparative study. In Kaohsiung City, Taiwan; 2017 [cited 2021 May 12]. p. 020045. Available from: <http://aip.scitation.org/doi/abs/10.1063/1.4981867>
17. Agbabiaka OG, Oladele IO, Akinwekomi AD, Adediran AA, Balogun AO, Olasunkanm OG, et al. Effect of calcination temperature on hydroxyapatite developed from waste poultry eggshell. *Scientific African*. 2020 Jul;8:e00452.
18. Hikmawati D, Maulida HN, Putra AP, Budiatin AS, Syahrom A. Synthesis and Characterization of Nanohydroxyapatite-Gelatin Composite with Streptomycin as Antituberculosis Injectable Bone Substitute. *International Journal of Biomaterials*. 2019 Jun 25;2019:1–8.

#### Figure Legend

Fig.1 SEM analysis of HAp performed fluffy agglomerates rounded edge morphology (Horta et al., 2019)

Fig.2 SEM analysis of HAp with highly agglomerated morphology (Agbabiaka et al., 2020)

Fig.3 The XRD pattern performed compatible characterization phase in eggshell HAp which calcinated in 1000°C (Horta et al., 2019)

Fig.4 FTIR graph confirmed crystalline phase (Khandelwal and Prakash., 2016)

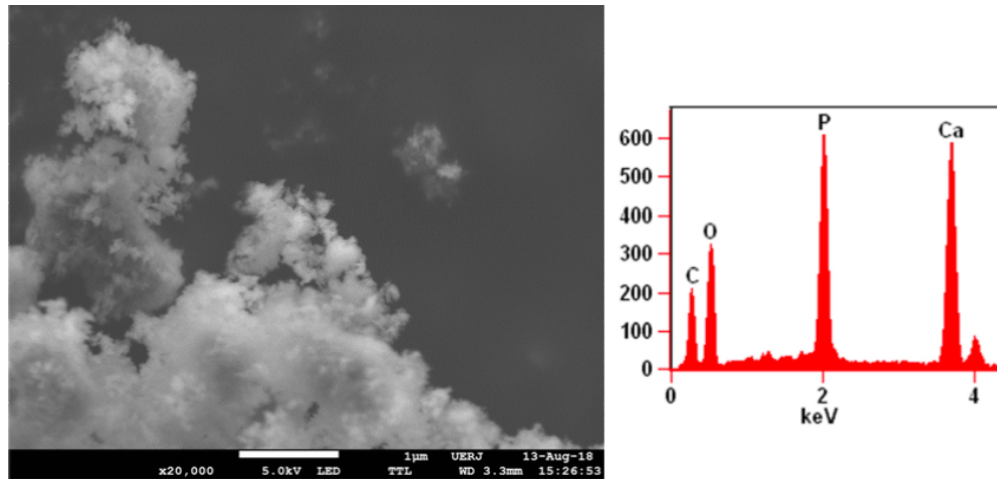


Fig.1 SEM analysis of HAp performed fluffy agglomerates rounded edge morphology (Horta et al., 2019)

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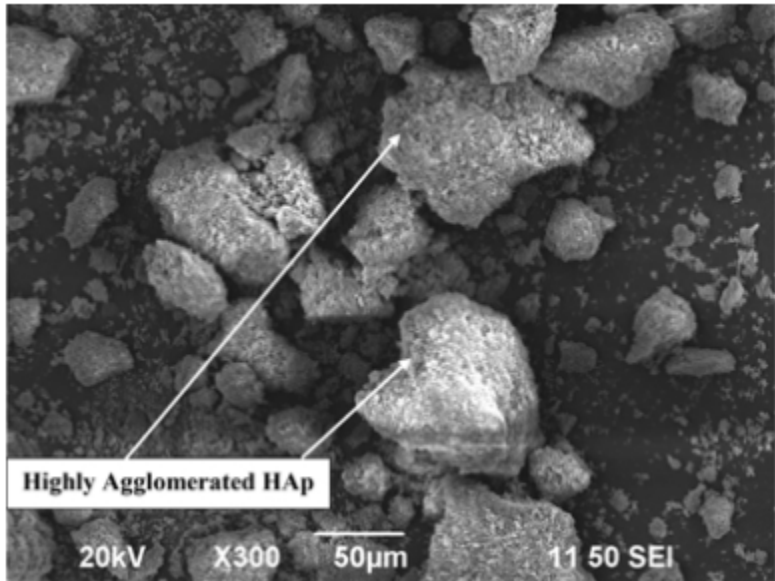


Fig.2 SEM analysis of HAp with highly agglomerated morphology (Agbabiaka et al., 2020)  
136x102mm (72 x 72 DPI)

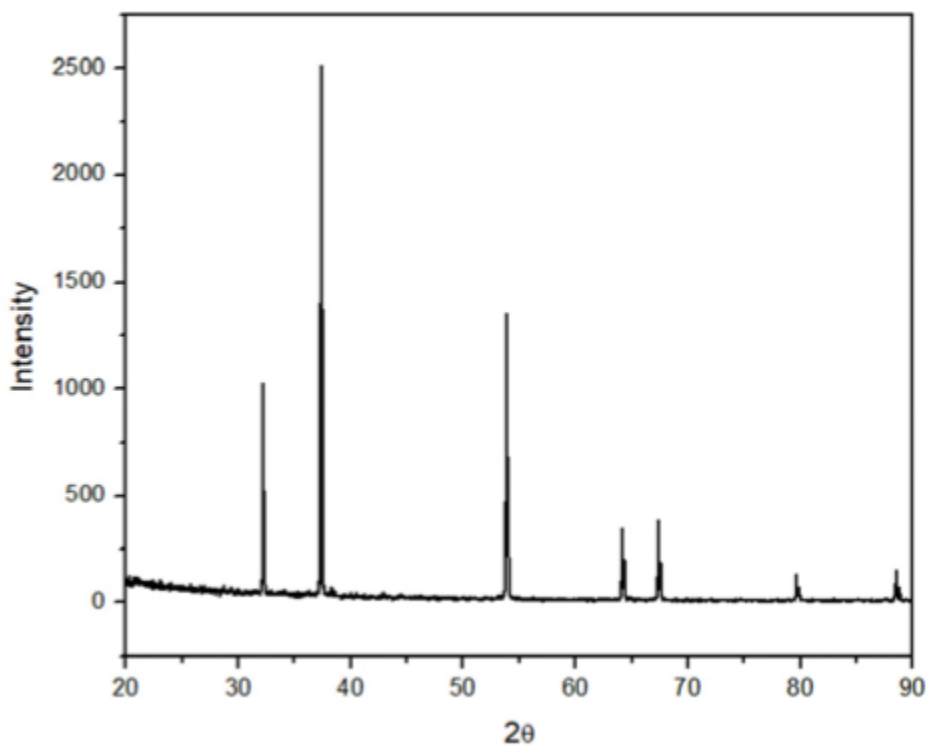


Fig.3 The XRD pattern performed compatible characterization phase in eggshell HAp which calcinated in 1000°C (Horta et al., 2019)

168x134mm (72 x 72 DPI)

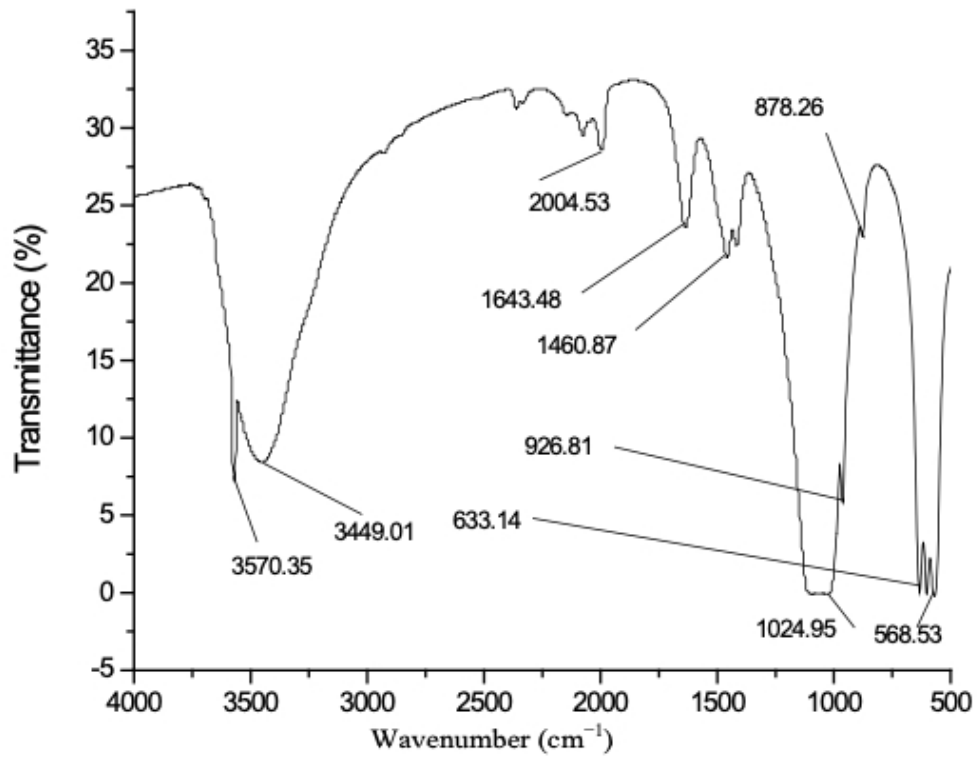


Fig.4 FTIR graph confirmed crystalline phase (Khandelwal and Prakash., 2016)

185x142mm (72 x 72 DPI)

**Table I. Horta et al HAp characterization result**

Characterization Method	Remarks	
	Precipitation method using 2 aging time	
	1 hour	2 hours
SEM	lamellar, such as plates like	Fluffy morphology (Fig.1)
EDX	Ca/P ratio : 1.59	Ca/P ratio : 1.49
XRD	complete decomposition of CaCO <sub>3</sub> to CaO monoclinic hydroxyapatite phase with no secondary phases average crystallite size: 20.12	complete decomposition of CaCO <sub>3</sub> to CaO monoclinic hydroxyapatite phase with no secondary phases average crystallite size: 19.93
Specific Surface BET	73.17 m <sup>2</sup> /g	68.15m <sup>2</sup> /g
Concluding remark : Aging time variation performed no significant differences in the characteristics of the materials, Chicken eggshell were very promising for biomedic applications		

For Review Only



**Table II. Khandelwal and Prakash HAp characterization result**

Characterization Method	Remarks
	Wet Chemical method with 900°C calcination for 2 hours
SEM	Microcrystalline molecule with irregular agglomerates shapes with pores
EDX	Ca/P ratio : 1.68 and it was acceptable.
XRD	2θ range 15 °C to 80 °C. Intense reflection peak between 31.8 - 32.5 of 2θ values, confirmed of the apatite phase, with 31,5 nm average particle size
FTIR	H <sub>2</sub> O adsorbance at 1643.48 cm <sup>-1</sup> and 3449.01 cm <sup>-1</sup> Vibration mode CO <sub>3</sub> <sup>2-</sup> ion at 878.26 cm <sup>-1</sup> and 1460.87 cm <sup>-1</sup> , confirmed elimination of CO <sub>3</sub> <sup>2-</sup> because of calcination process OH stretching bond at 3570.35 cm <sup>-1</sup> due to water adsorbance, at 633.14 confirmed OH in HAp. Peak at 926,81 confirmed HAp PO <sub>4</sub> <sup>3-</sup> stretching mode at 565.53 showed crystalline phase (Fig.4)
TGA-DTA	At 1400°C temperature obtained thermal stability without major loss of weight HAp samples
Concluding remark : HAp powder which Calcinate at 900°C can performed pure and single apatit phase, with Ca/P ratio 1,68	

For Review Only

**Table III. Agbabiaka et al characterization result**

Characterization Method	Remarks		
	800°C calcination	900°C calcination	1000°C calcination
SEM	crystallites flake /crystallites agglomerate like arbitrary flower structure	Agglomerates in spherical shape	Irregular agglomerated shape (Fig.2)
EDX	Ca/P:0.55, below stoichiometry ratio	Ca/P :1.26 with monetite and calcium hydrogen phosphate hydrate phase	Ca/P ratio : 1.65 , there were complete hydroxyapatite phase
XRD	The temperature wasn't suitable so there were no HAp phase identified	Main phase was monetite and hydroxyapatite. There were incomplete process of CaO during calcination, so $\text{Ca}_4\text{H}_2(\text{P}_3\text{O}_{10})_2$ phase wasn't available	There were strong peak HAp phase
Concluding remark: HAp 1000°C performed strong peak of HAp in XRD analysis, EDX result also similar to stoichiometry ratio,morphology were vary depends on the synthesis of HAp,			

**Table IV. Hamidi characterization result**

Characterization Method	Remarks			
	200 rpm (800°C and 1100°C)		400 rpm (800°C and 1100°C)	800 rpm (800°C and 1100°C)
SEM	800°C : irregular form with spherical shape, small and large particles clusters with mean particle size: 263 nm. 1100 °C : mean particles 513 nm (Fig.1)		Large agglomerates with fine particles , mean particles 257 nm	smooth surface of agglomeration in spherical shape.
FTIR	1 reaction process	2 reaction process	1 reaction process	1 reaction process
	H <sub>2</sub> O adsorbtion at 3600 and 2600 cm <sup>-1</sup> At temperature 800 and 1100 ° C: major peak at 3435 cm <sup>-1</sup>  PO <sub>4</sub> <sup>3-</sup> weak stretching peak at 963 cm <sup>-1</sup>	Lower H <sub>2</sub> O adsorbtion at 3448 cm <sup>-1</sup> (less water molecule)  Stretching OH group : peak at 3642cm <sup>-1</sup> (HAp is present in the sample)  PO <sub>4</sub> <sup>3-</sup> asymmetrical stretching vibration modes 602	H <sub>2</sub> O adsorbtion at 3436 cm <sup>-1</sup>  Stretching OH group : peak at 3567 (HAp is present in the sample)  PO <sub>4</sub> <sup>3-</sup> asymmetrical stretching vibration modes : 566cm <sup>-1</sup>	H <sub>2</sub> O adsorbtion at 3435cm <sup>-1</sup>  Stretching OH group : peak at 3569 cm <sup>-1</sup> (HAp is present in the sample)  PO <sub>4</sub> <sup>3-</sup> asymmetrical stretching vibration modes: 1040 cm <sup>-1</sup> , and and 471
XRD	800°C : Low crystallite size (19.00 nm) 1100 °C : high HA crystallite size (34.89 nm)	HAp crystal size 0 nm (no HAp phase)	Suitable crystal size	Suitable crystal size
Concluding remark: higher rotational speed forms chemically more complete HAp, heat treatment to 1100 °C results in stoichiometric HAp and promoted the particles to agglomerate and form bigger size due to increase of degree of crystallinity				