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CHARACTERISATION OF NATURAL HYDROXYAPATITE (HAP) DERIVED FROM DIFFERENT TYPES OF TILAPIA FISH BONES AND SCALES

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Hydroxyapatite (HAp), $Ca_{10}(PO4)_6(OH)_2$, is recognised as a biomaterial that is widely used for bone implant due to its chemical and structural similarity to the mineral components in human bone and enamel. The elements of HAp are primarily composed of calcium and phosphorous with molar ratio of calcium to phosphorous is 1.67 capable to promote bone in-growth into prosthetic implant. HAp can be produced from synthesis process and from natural resources such as coral and fish. Enormous amounts of by-product waste produced from fish factories generated an undesirable environmental impact. Thus, this study was conducted to obtain natural biological HAp from fish scales and different types of bones of tilapia fish from fishery waste. Therefore, fish bones and scales are cheap source to produce biological HAp for medical applications. For this purpose, fish bones and scales of tilapia fish were boiled at 100°C to remove adhere meat and other impurities. Later, fish bones and scales were separated into several groups and subjected to different calcination temperatures (800° C and 900° C) for 3 hrs. All calcined samples were crushed to fine powder. The XRD result revealed the presence of derived HAp from the samples powder and were identical with standard HAp. Thermo Gravimetric Analysis was carried out to show thermal stability of the HAp powder from different types of fish bones and scales. SEM results shows porous structure appeared in calcined samples compared to raw samples. The findings is the promising alternative to produce calcium phosphate from fishery wastes that beneficial to medical applications.

INTRODUCTION

Fish are caught in Malaysia around 1.42 Million tonnes in 2010 [1] which is much less compared to the total of the whole world fish production estimated more than 91 Million tonnes catch every year. From this catch estimates barely about 40-50% are considered as by-product wastes [2]. It had been reported that waste by-product from fish is considered as worthless, impracticable and dismissed as a waste [3]. Most of the fish by products are currently employed to produce fish oil, fishmeal, fertilizer, pet food and fish silage. Recent studies have recognized a number of compounds such as hydroxyapatite occurred in remaining fish muscle proteins, collagen and gelatin, fish oil, fish bone, internal organs, fish scales and shellfish and crustacean shells. Fish bones mainly composed 30% of collagen as organic component [4] and the rest is inorganic component such as sodium, magnesium and hydroxyapatite which is obtained after calcinations [5]. HAp material have been extensively used as implant materials due to

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their close similarity in composition with natural bone. It is composed primarily of calcium phosphate and exit in two phases, α and β. The β phase is change to α with heat treatment at higher temperature from 1250°C to 1300°C [6]. HAp has all the characteristic features of biomaterials, such as biocompatible, bioactive, osteoconductive, non-toxic, non-inflammatory and non-immunogenic properties [5]. It is the most widely established biomaterial for the repairand reconstruction of bone tissue defects due to compositional similarities to human bones and teeth with stoichiometric ratio of Ca/P is 1.667 [7]. Their compositions make them an excellent choice for most medical applications. There are several techniques used to produce HAp which are from synthetic process or obtained from natural sources.

However, HAp extracted from natural sources has better metabolic activity and more environmental friendly compared to the synthetic one [7]. HAp extracted from natural source are biologically safe from foreign chemical and it is an economically desirable process.

Beside, synthetic HAp mostly possesses similar stoichiometric to pure HAp. However, it does not contain any minerals such as Na, Mg, K, F and Cl which can be found in natural sources. These minerals are able to stimulate better osteoconductive in reconstruction of bone fracture [8]. Moreover, the process of producing synthetic HAp are normally expensive, complicated, and time consuming. Therefore, the chemical synthesis method is limited only to synthesis in small quantities [9]. Thus, in this paper, a simple method of derived HAp from natural sources (fish bones and scale) had been introduced. HAp is derived via calcination process from fish scales and different parts of fish bones. The obtained HAp were then characterised in terms of its microstructure and chemical compositions.

MATERIALS AND METHODS

Sample Preparation

Tilapia fish bones and skins with scales were collected from fishery factory located in Perak, Malaysia as by-product wastes in frozen condition. Fish scales were immediately removed from skin using fish scaler. Both bones and scales were directly boiled in hot water at temperature 100°C for 1 h. Later, they were cleaned with tab water to remove adhere fish meat and other impurities. This procedure is repeated twice to make sure the samples were well cleaned. The cleaned samples were separated into several groups as shown in Table 1. Thereafter, bones and scales were dried in oven at temperature 60ºC for 3 hrs and placed into desiccator.

TABLE 1. *Parts of Black Tilapia fish wastes used for HAp extraction.*

Type of Sample	Details
Head bone	Opercular, skull, upper jaw, lower jaw, clavicle,
Body bone	Vertebra, neural spine, hemal spine, rib
Fins	Dorsal fin, caudal fins ray, anal fins ray, pectoral fin ray, pelvic girdle, pelvic fin ray, hypural
Scales	All fish scales

Calcination of Samples

The dried samples were then calcined in furnace (Protherm) at 800°C and 900°C with heating rate of 10°C/min. The temperatures were maintained for 3 hrs to remove the organic matrix and cooled. Completing calcination process, samples were milled

separately using Planetary Pulveristte (Fritsch, Germany) in order to form fine powder.

Characterization

Scanning electron microscope (JEOL JSM-6380L, Japan) with an accelerating voltage of 20kV equipped with an energy dispersive X-Ray spectroscope (EDS) was used to determine the microstructure of the samples. Each sample was sputtered with platinum prior to the analysis EDS analysis was performed to identify the elemental composition of materials. Meanwhile, the crystalline structure and phase composition of the calcined samples powder were identified by using a high resolution Bruker Advance D8 XRD diffractometer. X-ray diffraction operating in the Bragg-Brentano configuration with Cu-Kα (λ = 1.5406 Å) radiation at a current of 40 mA and an accelerating voltage of 40 KV. Intensity data were collected by step counting method where step was set at 0.02° and time 0.5 s in the range 2θ = 20° – 80°. Identification of phases were compared with the diffraction patterns of HAp with HAp standards (JCPDS, Card No. 9-432). Thermo gravimetric analysis was performed using a Mettler Toledo TGA machine. All powder samples were subjected to heat at a rate of 10°C/min from 25°C to 950°C under nitrogen atmosphere.

RESULTS AND DISCUSSIONS

Mineralogy

Figure 1, Figure 2 and Figure 3 show the XRD spectra of the raw and calcined powders at 800°C and 900°C for comparison. The spectra have been validated with the standard HAp from JCPDS No. 9-432 [10,11]. As shown in Figure 2 and Figure 3, all the peaks corresponding to the standard hydroxyapatite are clearly identified in the spectra of the calcined samples. Therefore, it can be concluded that the calcination process has eliminated the present of collagen and organic compounds in the raw samples. For the raw samples, the spectra are observed to be broad and not crystalline due to the presence of fibrous collagen which disperses the X-Ray radiations as shown in Figure 1. Meanwhile, increasing the temperatures to 800°C and 900°C have created more intense and sharp peaks, related to an increase in the mineral crystallinity for all samples [12]. The increase of mineral crystallinity is compatible with crystallite growth and the elimination of carbonate from the lattice. The narrowing of the diffraction peaks is related

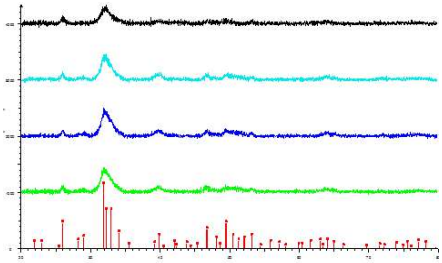

Fig. 1: X-ray diffraction patterns of separation parts of raw samples with compared to XRD data of standard Hap.

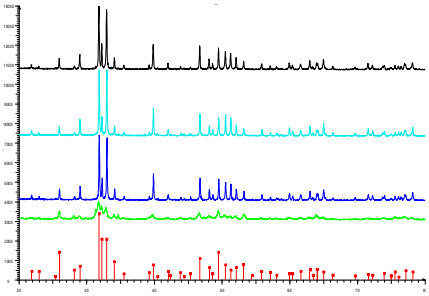

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Fig. 2: *X-ray diffraction patterns of separation parts of caclined samples at temperature 800ºC with compared to XRD*

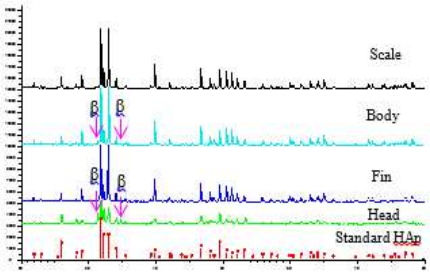


Fig. 3: X-ray diffraction patterns of separation parts of caclined samples at temperature 900ºC with compared to XRD

changes in the crystallite size that increases with calcination temperature. In head sample calcined at 800ºC, β-tricalcium phosphate (β-TCP; $Ca_3(PO_4)_2$) was detected. This scenario happened again when the calcination temperature was increased to 900ºC. Meanwhile, β-TCP could not been trace in the scales and fins bone. The presence of this compound is depended on the heat treatment temperature that indicates a change in the relative content of the two phase during calcination process.

Microstructure

Figure 4 shows the surface morphology of raw samples. The samples were found to be in compacted form due to the presence of water and organic compounds such as collagen, protein, and lipids. In addition, the size of porosity for each samples were different. The body sample had larger porous size compared to the rest. Meanwhile, small voids are depicted all over the surface of head sample. Moreover, a single mineralized collagen bundle observed in fin sample. Usually, the morphology of the scale can be divided into three region: posterior, lateral and anterior as shows in scale micrograph in Figure 4. The surface of the scale is separated into several interradial areas by radial grooves. The scale ridges shown in scale morphology are considered as grows rings and it growth on the surface of lateral and anterior regions forming concentric arcs [13]. Furthermore, the posterior area structures of rounded particles and tiny holes.

SEM were also used to characterize the calcined samples surface morphology. Table 2 shows SEM micrograph images as comparison between raw samples and calcined samples at 900ºC. The microstructures of raw samples appeared to be dense and less porous compared to the calcined samples. The SEM micrograph shows those samples consist of very small regular grains and slightly elongated grains after calcination. The increasing of porous structure are obviously seen at the surface morphology of each sample after calcination at 900ºC.

Ca/P Ratio

Table 3 shows a comparison of molar Ca/P ratios obtained in each of calcined samples. All Ca/p ratios of those samples are identified higher as compared to the stoichiometric HA (1.67). Other authors have also observed higher Ca/P ratio in hydroxyapatite from sword fish and tuna bones [7].

Furthermore, the variation of Ca/P molar ratio could be due to the presence of trace element found in the natural organic source [8] as shown in EDS spectra. The presence of the Na and Mg ions on calcined samples can be observed in Figure 5. The presence of allof these minor elements can be beneficial for properties such as biocompatibility and bioactivity of HAp [6].

Thermogravimetric Analysis

The thermogravimetric curve of different samples are shown in Figure 6 and Figure 7, with the corresponding heat flow range from 25°C to 950°C. The thermal plots of the samples show typically three successive steps of weight loss [15,16]. At the first stage (100-300ºC) release of water molecules or the dehydration of bone from samples was observed, and corresponding mass loss was ~10%. The second step corresponds (300-600ºC) to the greatest weight loss nearly 20% is due to the decomposition and burning of the organic component of bone (such as collagen, protein, and lipids) [17,18]. The later step of weight loss (10%), observed at temperatures above 600°C, is mainly caused by the release of CO_2 from the apatite lattice, due to carbonate decomposition (decarbonation) [5] for Fin, Body, and Head

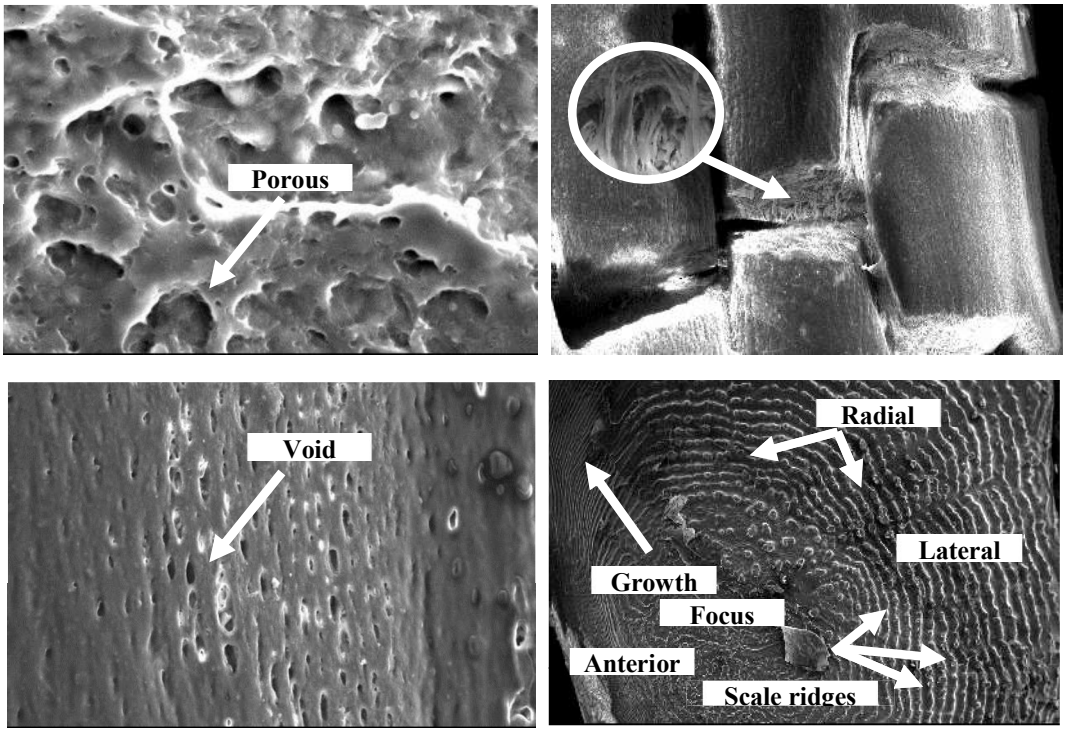
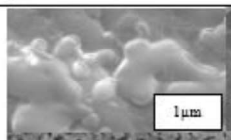
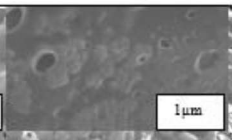
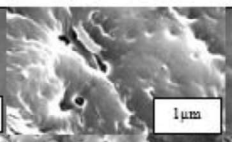
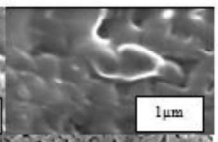
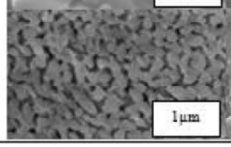
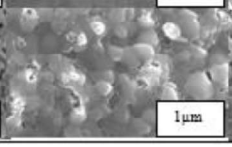
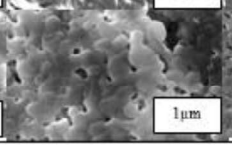
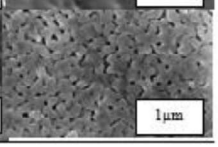


Fig. 4: SEM micrograph of different types of raw tilapia bones and scale.

TABLE 2. *Comparison of SEM micrograph picture between different types of calcined (900ºC) samples with raw samples.*

Sample	Micrograph			
	Body	Fin	Head	Scale
Raw				
Calcined (900°C)				

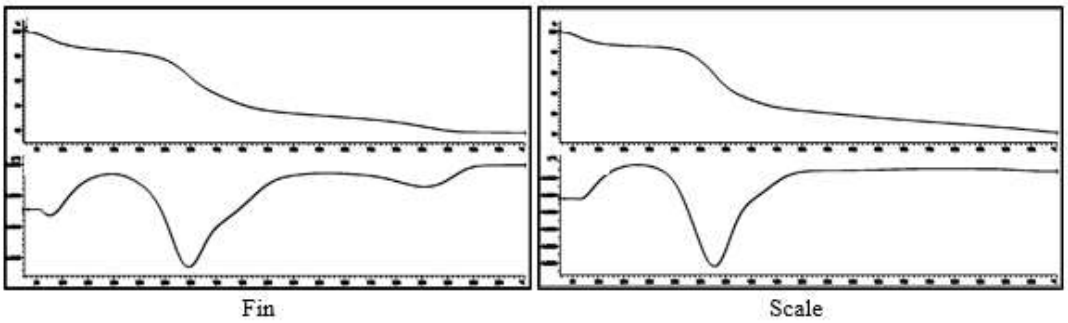


FIG. 7: *Thermal plots of fin and scale samples.*

TABLE 3. *Comparison of Ca/P ratio between different types of fish bone and scale after calcination.*

Calcined Temperature, °C	Sample	Average Ca/P ratio
900	Body	1.90
	Head	1.83
	Fin	1.52
	Scale	1.95

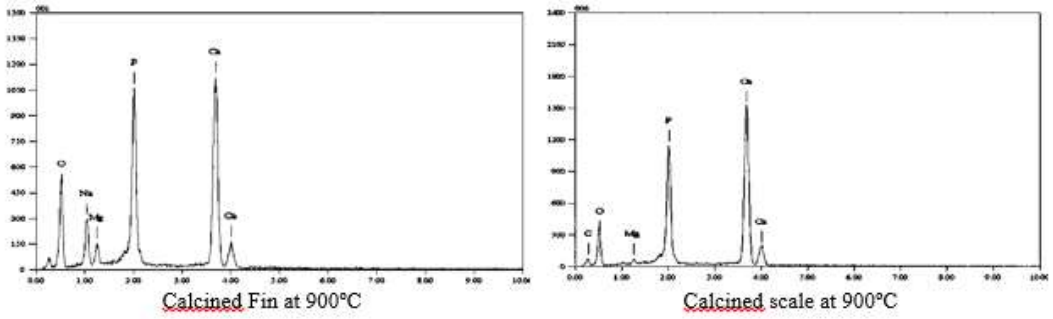


FIG. 5: *EDS spectra of chemical composition of samples obtained after calcination process.*

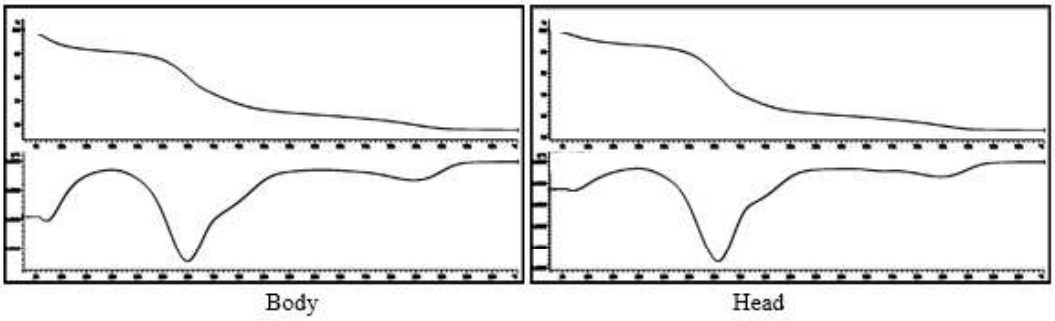


FIG. 6: *Thermal plots of body and head samples*

CONCLUSION

Black tilapia fish wastes (bone, fin, head, and scale) were found to have great potential in extraction of HAp. Sample of fin from tilapia fish shows good properties and has close Ca/P molar ratio to the stoichiometric HAp compared to other samples. It can be concluded that β-TCP phase occurred in calcined samples when calcination temperature are increased. The collagen and organic compounds in the raw samples clearly had been eliminate during calcination process. The method of extraction used in this study is a simple and low cost method for HAp production. As a conclusion, this work shows that studied at fin, body and scale samples calcined at 800ºC exhibit a promising chemical composition and structure of pure HAp form.

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REFERENCES

[1] Anon. Annual Fisheries Statistic, Dep of Fish (DOF), Malaysia (2010).
[2] T. Rustad, Agri & Food Chem. 2(4), 458-463 (2003).
[3] Y. C. Huang, P. C. Hsiao and H. J. Chai, Ceram Int. 37:1825–1831 (2011).
[4] T. Nagai, M. Izumi and M. Ishii, Int J. of Food Sci & Tech. 39, 239–244 (2004).
[5] M. Boutinguiza, J. Pou, R. Comesaña, F. Lusquiños, , A. de Carlos and B. León, Mat Sci & Eng: C, 32(3), 478-486 (2012).
[6] J. Pena, and M. Vallet-Regı, J. of Euro Ceram Society, *23*(10), 1687-1696 (2003).
[7] V. P. Orlovskii, V. S. Komlev, and S. M. Barinov, Inorgc Mat 38, no. 10: 973-984 (2002).
[8] M. Akram, A. Rashid, S. Imran, A. W. I. Wan, and H. Rafaqat, J. of Mat Sci 49, no. 4: 1461-1475 (2014).
[9] M. K. Herliansyah, C. Muzafar, and A. E. Tontowi, Proc of the Asia Pac Indust Eng & Man Syst Conf: 1137–1146 (2012).
[10] C. M. Lopatin, V. Pizziconi, T. L. Alford and T. Laursen, :326, 227 (1998).
[11] JCPDS Card No. 9-432, (1994).
[12] F. Zhou, Y. Wang, Q. Feng, A. Kienzle, and W. E. Müller, Mat Sci and Eng: C 43: 145-152 (2014).
[13] M. Figueiredo, A. Fernando, G. Martins, J. Freitas, F. Judas, and H. Figueiredo, Ceram Int 36, no. 8: 2383-2393 (2010).
[14] Antonakos, Anastasios, E. Liarokapis, and T. Leventouri, Biomaterials 28, no. 19: 3043-3054 (2007).
[15] A. Mortier, J. Lemaitre, and P. G. Rouxhet, Therm Act 143: 265-282 (1989).
[16] M. Ozawa and S. Suzuki, J. of the Amrca Ceram Socie 85, no. 5: 1315-1317 (2002).
[17] K. Haberko, M. M. Buc´ko, J. Brzezin´ska-Miecznik, M. Haberko, W. Mozgawa, T. Panz, A. Pyda and J. Zare¸bski, J. Eur. Ceram. Soc. 26 537–542 (2006).
[18] S. Mondal, M. Biswanath, D. Apurba, and S. M. Sudit, J. of Mins & Mats Charac & Eng 11: 55 (2012).