PROPERTIES OF NATURAL HYDROXYAPATITE (HAp) EXTRACTED FROM BLACK TILAPIA FISH (BONE AND SCALE) USING CALCINATION APPROACH

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Abstract. Natural hydroxyapatite (HAp) was used from the bone and scales of black Tilapia *(Oreochromis Niloticus)* fish, because it is less expensive than HAp made synthetically and safer than the mammalians bone. In this research, the calcination method at 1000 ºC was used to prepare and examine the characteristics of natural HAp from the black tilapia fish's bones and scales. Prior to heat treatment, grinding process was done in order to produce the natural HAp fine powder. The sample was characterized using thermal gravimetric analysis (TGA) for thermal stability, field emission scanning electron microscopy (FESEM) for morphological analysis, X-rays diffraction (XRD) for phase identification, and energy dispersive X-rays spectroscopy (EDX) for chemical composition. From the result, TGA shows the extracted HAp had a relatively high thermal degradation temperature and the peaks of the HAp were visible on XRD. Additionally, the weight loss was tracked and used to validate that the sample's organic components had been eliminated during the thermal calcination process. The morphological analysis from FESEM shows the particles have rod-like shape HAp crystals and EDX result showed a Ca/P molar ratio of both samples is close to stoichiometric HAp (1.67) which is 1.69 for fish bone and 1.73 for fish scale. Therefore, the calcination approach is an effective way to extract the natural HAp from fish waste.

Keywords: Hydroxyapatite, biomaterial, black tilapia, fish bone, fish scale

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Introduction

A well-known biomaterial is hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), that was utilized generally in therapeutic diagnostics for instance dental paste, bone implants, and cements [1], owing to its chemical characteristics resembling the inorganic part of mammalian bones and hard tissues. Numerous synthetic methods, including aqueous precipitation reaction, sol-gel crystallization, sintering [2], electrodeposition, hydrothermal technique [3], spray pyrolysis, spray-drying method, have been developed in the literature to manufacture HAp. Utilizing a chemical reaction between calcium and phosphate ions, synthetic methods are applied. These procedures make it simple to monitor the shape, stoichiometric elemental composition (1.67), and crystallinity of HAp preparation [4]. These techniques, however, are difficult, time-consuming, expensive, and delivered HAp with a low activity [5]. Due to the high expense of producing synthetic HAp, several studies have concentrated on using natural resources to make affordable HAp.

The main natural resources for HAp include biological sources like animal bones from bovine and pigs [6]. However, because of pathogens, natural resources like fish scales have emerged as the best HAp substitutes. According to reports, the majority of fish scales contain about 50% weight of collagen and HAp, two organic and inorganic substances [7]. Fish scales are safer and more cost-effective than animal bones for the extraction of HAp. There are a several techniques, including alkaline treatment [8], enzymatic treatment [9], and calcination, that can be used to obtain HAp from the fish scales. One of the simplest and most affordable techniques for extracting natural HAp from fish scales is calcination [10]. The samples of fish bone and scale were calcined at 1000 °C in a furnace to eliminate the organic components and the result HAp is the only compound material left behind in ashes form.

Food waste, wastewater treatment, and by-product management are problems that have an impact on both the aquatic and terrestrial environments in addition to the economy and food security. The researcher sought out by-product waste products with high added value out of a great concern for reducing the environmental impact caused by overfishing [11]. It has been reported that waste products from the fish industry, such as fish scales and bones are a useful combination of organic and inorganic elements, primarily collagen and hydroxyapatite [12]. Numerous studies have shown that HAp be able to extract from fish bone and scale [13]. Fish waste can therefore be used to produce inexpensive HAp for use in implant materials. In order to extract natural HAp from a Tilapia fish bone and scale, a straightforward approach based on calcination or heat treatment is proposed in this study.

Materials and Methods

Samples and Preparation

At Universiti Tun Hussein Onn Malaysia (UTHM) in Batu Pahat, Johor Malaysia, the Black Tilapia fish were caught and taken from the G3 lake and they were leftover waste that had not been treated. Figure 1 reveals the black Tilapia fish collected. The fish bones and scale were isolated from the fish waste. Before starting the calcination process, both samples (bone and scale) were boiled several times, for one hour, in order to eliminate the leftovers of any raw meat and foreign matter that might be contaminated. The samples were dried out at room temperature. After that, by using an oven (Termoline, China), the dried-out process continues at 95 °C for 1 hour. Both samples will become brittle, and easy to grind and crush using the mortar and pestle into small fine powder.

Figure 1: The black Tilapia fish harvested in UTHM

Calcinations Process

Both samples (in powder form) which are the scale and bone were calcined in a Protherm furnace at 1000 °C and 10 °C/min heating rate. The calcination technique applied at holding time was 3 hours and natural cooled to get rid of the biologically detrimental components and layers of contaminant. To minimize water capture and avoid contamination, both HAp samples were carefully located inside a vacuum area desiccator after the calcination procedure.

Samples Pellet

After the calcination procedure, both HAp samples in ash form were crushed into an even finer powder and sieved once more to ensure that their sizes were consistent and would not interfere with the powder's ability to solidify into pellets. The samples were prepared in pellet form in order to obtain constant size and weight for every experiment applied. The weight of the powder will be measured using a digital scale at 1.1 gram for improved accuracy. The sample was then put into the hydraulic jack pressure pellet mold. The ideal pressure to ensure that the pellet wouldn’t destroy and return to ashes was 9 tons of pressure.

Characterization of the Samples

A Simultaneous thermogravimetry and differential scanning calorimetry (TG –DSC) (Linseis, STA PT 1600 Germany) was performed to investigate the thermal stability, and phase identification of the decomposition stage for both HAp samples. Alumina crucible (cup) containing 15.1 mg of powdered sample was heated under nitrogen at a rate of 10 °C/min from 25 °C to 1000 °C. In the interim, a higher-resolution Bruker Advance D8 X-RD diffractometer validated the transparent arrangement and stage composition of the conducted experiment. X-ray diffraction functioning in the Bragg-Brentano arrangement through Cu-Kα (λ = 1.5405 Å) emission at a current of 40 mA and an increased speed voltage of 40 kV. The samples' microstructure was verified, using field emission scanning electron microscopy (FESEM) JEOL JSM – 7600F from Japan with an accelerating voltage 5.0 kV, 20 000 magnification and imaging at nano scale equip with and an energy dispersive X-ray spectroscope (EDS) (Oxford, United Kingdom) with a speed up voltage of 15 kV. EDS was applied to validate specimen elemental composition.

Results and Discussion

Thermal Gravimetric Analysis (TGA)

The thermogravimetric curve for the sample of fish scales and bones, with an associated heat flow range of 25 °C to 1000 °C, is demonstrated in Figure 2. The samples' thermal graphs typically show weight loss in three different stages. There are three steps: below 300 °C, between 300 °C and 650 °C, and between 650 °C and 1000 °C. The weight fluctuation is not much altered above this temperature. The former is known to be dehydration (removal of water content) [14]. The degradation of the organic matters, which occurs in the second stage, is what causes the biggest weight loss (for example protein, collagen, and lipids) [15]. The fish scale sample has greater weight loss than fish bone at this stage indicated the amount of organic compounds is higher in fish scale than fish bone. The release of carbon dioxide from the apatite lattice expected to carbonate breakdown is primarily what causes the later stage of weight loss, which is visible at temperatures above 650 °C [16].

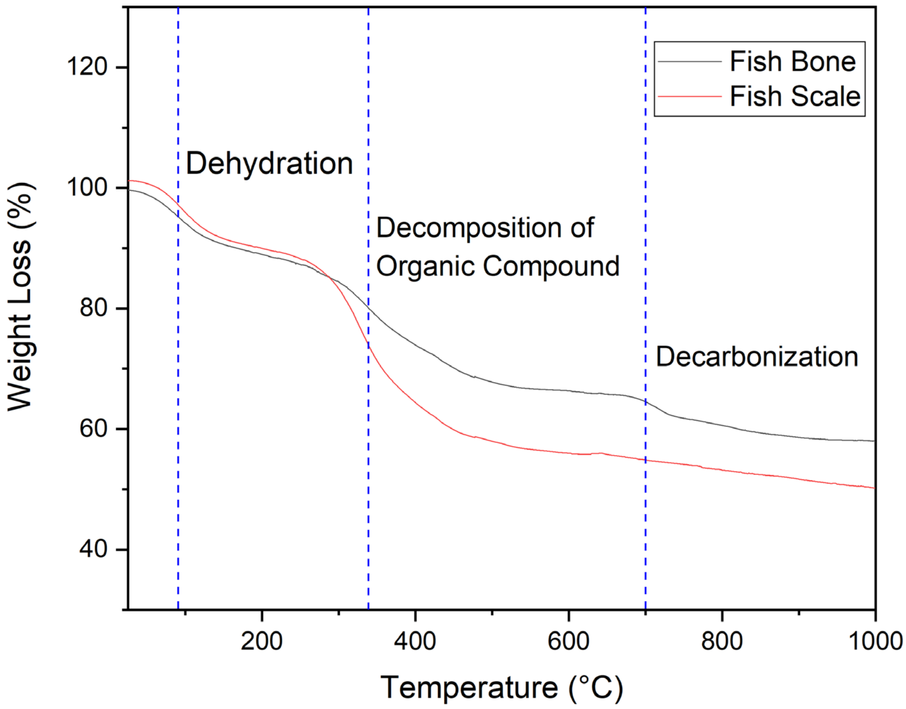

Figure 2: The thermal behaviour of the black tilapia fish bone and scale

X- rays Diffraction (XRD)

Figure 3 shows the XRD spectra of calcined fish bone and scale. Standard HAp from JCPDS No. 00-009-0432 was used for confirmation. From the graph, the spectra of both samples just matched the standard HAp with high intensity due to high temperature applied on the samples. The peak intensity is at its maximum, at a 2θ value of 32.01° for fish scale, and 33.51° for fish bone. The acute peaks reflect that the mineral crystallinity is high due to the removal of the organic molecule and sufficient energy absorption by the crystal via heat treatment. Other than that, secondary material for both samples was discovered to be β-TCP which has a good resorbability [17]. For the purpose of overcoming HAp's poor biodegradability, biphasic calcium phosphate (HAp + β-TCP) composite has recently been explored [18].

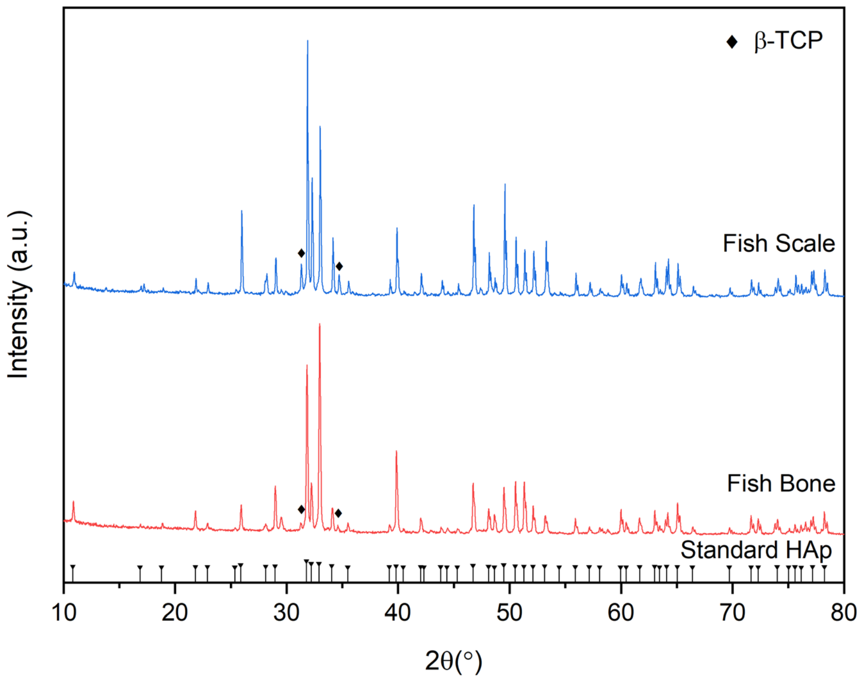

Figure 3: XRD pattern of black tilapia fish bone and scale calcined at 1000 °C

Field Emission Scanning Electron Microscopy (FESEM)

Figure 4 illustrates the surface morphology of the calcined fish bone and scales after FESEM analysis. Calcined samples have shaped in equiaxed patterns and morphologically homogeneous rod-like shape [19]. For the calcined fish bone, the hexagonal crystal shape is more angular and longer than calcined fish scale that has shorter sub-rounded shape. This is due to the organic content in fish scale is higher than in fish bone. It proves the finding in TGA analysis, which the fish scale has a higher organic compound decomposition rate than fish bone. So that, the only materials left in the inorganic compound, HAp crystals.

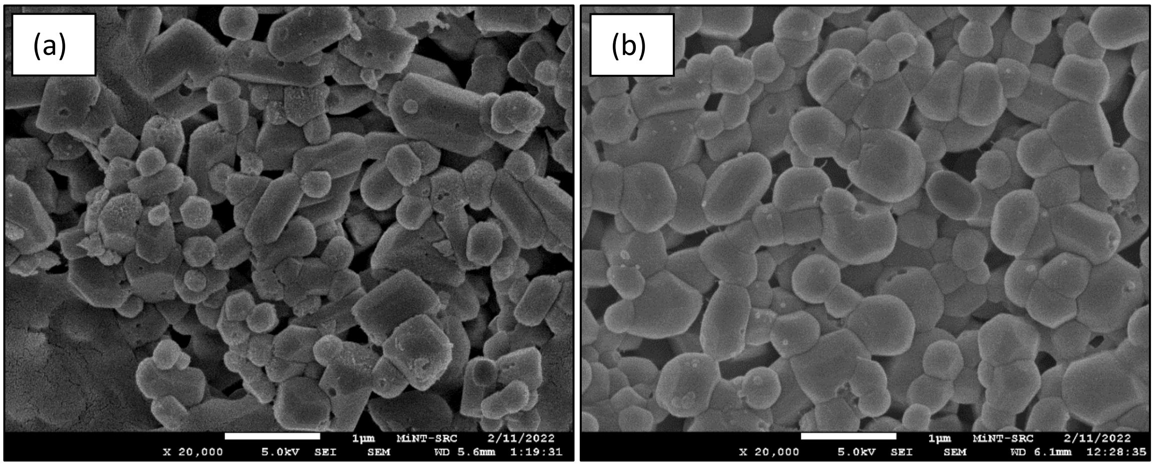

Figure 4: The surface morphology of the calcined (a) fish bone and (b) scales

Energy Dispersive X-rays spectroscopy (EDX).

As shown in Table 1, the molar ratio of Ca/P for fish scale and bone was comparable to stoichiometric HAp, 1.67. Calcined fish scales had a Ca/P ratio of 1.73, whereas the ratio of calcined fish bones was 1.69. Phosphorus (P) and Calcium (Ca) make up the primary of the composition with trace elements of Sodium (Na) and Magnesium (Mg) also present. The presence of trace elements from this natural source could be the cause of the fluctuation of the Ca/P ratio. Mg and Na perform an significant character in tests (in-vitro and in-vivo) that imitate the natural bone growth and stimulate the development of new bone which directly enhanced osteoblast formation [20].

Table 1: The Ca/P molar ratio for calcined black tilapia fish bone and scale

Sample	**Elemental Composition (at%)**				**Ca/P**
	Ca	**P**	**Mg**	**Na**	
Fish Bone	61.49	36.48	0.49	1.54	1.69
Fish Scale	31.00	17.94	0.40	0.35	1.73

Conclusions

The ability to extract hydroxyapatite from the bones and scales of black tilapia fish has been found to have many applications, particularly in biomedical production. The discoveries imply that the samples are thermally stable and highly crystalline with a temperature applied which is 1000 °C. Both samples are just in line with standard HAp with the presentation of β-TCP as biphasic material help in better resorbability. Furthermore, the morphology of both samples was in agreement with the hexagonal crystal system of HAp. This natural HAp had a close similarity of Ca/P molar ratio with real bone and the trace elements were also offered to encourage the creation of new bone. Fish bones and scales have been discovered to be a cheap source of HAp as a result, and the extraction process utilized in this study offers a straightforward and secure way to produce HAp.

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Author Contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure of Conflict of Interest

The authors have no disclosures to declare

Compliance with Ethical Standards

The work is compliant with ethical standards

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