Sample Preparation of the Natural Source Hydroxyapatite (HAp) derived from Black Tilapia Fish Scales

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Abstract. Hydroxyapatite (HAp) is a biomaterial with the chemical formula Ca\textsubscript{10}(PO\textsubscript{4})\textsubscript{6}(OH)\textsubscript{2}. Normally, it was used and applied in biomedical applications, cooking recipes and healthy food products. Due to this, it has attracted the researcher to work on HAp synthesis and extraction. In this study, the hydroxyapatite was extracted from fresh water Black Tilapia Fish Scales (BTFS) was analysed. The crystallinity of the HAp was characterized by using an X-ray diffractometer (XRD) whereas the Fourier transform infrared (FTIR) analysis was used to characterize the presence of HAp from the samples. Scanning electron microscopy (SEM) provided with an Energy Dispersive Spectroscopy, (EDS) was used to investigate the morphology and element of the hydroxyapatite powders. The BTFS samples were heated at 100 °C to composed and eliminated the remaining meat and other impurities. A mechanical crusher was applied to make a miniscule and fine hydroxyapatite powder using a thermal calcination technique using a furnace. The BTFS samples powder were calcined at a temperature of 900 °C for 3 hours correspondingly. The XRD spectra findings, uncovered the existence of the obtained BTFS HAp are articulate with Joint Committee on Powder Diffraction Powder Standard data (JCPDS) from the library. From the SEM results, HAp powder presence in hexagonal shape. The findings show the potential of producing high-value products from fishing waste, such as HAp with Ca/P ratio 1.74, for biomedical applications.

1 Introduction

Calcium phosphates (CP) were categorized as biomaterials that made up of calcium and phosphorus at different molar ratios ranging from 0.5 to 2.0 \cite{1}. Between these, hydroxyapatite, HAp [(Ca)\textsubscript{10} (PO\textsubscript{4})\textsubscript{6} (OH)\textsubscript{2}] is a common CaP-created biomaterial that closely resembles the mineral elements found in natural teeth and bones \cite{2}. Furthermore, in biomedical applications, HAp with calcium to phosphorus molar ratio of 1.67 demonstrates exceptional bioactivity, osteoconductive, biodegradability and biocompatibility \cite{3}. Because of its non-provocative, and non-noxious qualities, it has been employed as a alveolar consonant and bone replacement as an alternative material \cite{4}. Electrodeposition, chemical sleet, sol-gel method, flare, microwave, solvothermal, hydrothermal, and neutralisation method, amalgamation at low pressure, disintegration molecular, method of condensation,
and initial response in solid state process have all been developed so far for the groundwork and dealing out of HAp [5]. However, obtaining the natural HAp from bio-wastes or material from fish that have no direct use such as fish scales [6], fish bones [7], egg shells [8], coral/sea shells [9], and bovine cortical bones [10] is even now difficult. The human bones mechanism, which consists of underlying connective tissues, is extremely essential in the human body. This system's abnormalities are especially unsafe to one's health. A congenital condition or accidental tissue/organ injury can cause a bone deformity[11]. Tissue engineering employing innovative biomaterials can be used to overcome the healing and regeneration tissues. The goal of tissue engineering is to offer structural support to help wounded body parts heal faster [12].

Generally, there is more than fifty percent of fish industries capture or product is not manufactured as a worthwhile food item for consumption and contains of million tons of undesirable trash from fish [13]. Huge volumes produce from by-products of the fishing industry are thrown away, having a negative influence on the ecosystem. Fish scale waste has recently been used as a economical and new source of preparing HAp [14]. The quality and properties of HAp synthesised from fish waste and typical commercial stoichiometric HAp, on the other hand, are naturally dissimilar from one another and in their characteristics [15]. The fact that exists imperative negative ion such as Cl- and F-, as well as positive ion such as Mg2+, Al3+, Sr2+, Zn2+, K+, and Na+, in HAp made from the junks (fish waste) exhibited increased biological activities, and the availability of both ions significantly improves biomedical uses, particularly regarding fast bone regeneration [16]. The accessibility of Mg2+ and Na+ ions is important for bone and tooth growtht and stability, whereas their removal or insufficient quantity might cause bone loss or make it more vulnerable.

In this study, simple heat treatment process via calcination method for extracting the natural source hydroxyapatite from the waste of BTFS. The crystallinity, surface physicochemical characteristics and element of the extract HAp sample powder were characterize using several techniques including XRD evaluation, SEM, FTIR elemental graph assessment, and EDS calcium to phosphorus ratio. The result shows that, the natural source hydroxyapatite showed outstanding biocompatibility which demonstrated that black tilapia fish scale biowaste produced HAp could be a prospective replacement biomaterial and biomedical for implantation purposes.

2 Methodology

2.1 Samples preparation

The raw material of the BTFS was gain and harvested from G3 lake inside Universiti Tun Hussein Onn Malaysia (UTHM), Batu Pahat (Johor, Malaysia) and the process fish scale were separated as a by-product trash in raw form. Fig. 1 shows the harvested Black Tilapia fish. Prior to the procedure, the BTFS were repeatedly boiled in boiling water for 1 hour to eradicate any skin, raw flesh, and any other undesired material from the scale. After that, it was washed and dried to remove any residual fish meat or skins. Sequentially, BTFS was dried out in a dry up stove (Termo-line, China) at temperature 95 °C for 3 hours and crushed using a mechanical grinder to a pleasant powder and have been palleted using a hydraulic presser.
2.2 Calcination process

The BTFS powder samples was then calcined in a furnace (Protherm) at 900 °C with 10 °C / min of heating rate and natural cooling. The calcination duration was well-maintained for 3 hours and cooled precisely to eliminate all layers of contaminants and entire undesirable biological characteristics. After the calcination treatment was performed, the hydroxyapatite samples were carefully placed in a vacuum space desiccator to avoid contamination and reduce water surface captivation.

2.3 Characterization of HAp

The constitution of the phases and crystallinity of the BTFS pallet HAp were studied using a high-resolution Bruker Advance D8 X-ray diffractometer (XRD), X-ray diffraction functioning in the Bragg-Brentano arrangement through Cu-Kα (λ = 1.5405 Å) emission at a specific current (40 mA) and applied voltage (40 kV), while the Fourier Transform Infrared (FTIR), Perkin Elmer Spectrum 100 Optica was used to characterize the present of HAp from the BTFS samples. Scanning electron microscope (SEM) (SU1510 Hitachi, Japan) with voltage that accelerates at 20kV equipped with an Energy Dispersive Spectroscopy, (EDS) (Oxford, United Kingdom) with a speed up voltage of 15 kV was used to investigate the morphology, microstructure and elements of the hydroxyapatite powders and pallet samples.
3 Results and discussion

3.1 Minerology

The size of the BTFS sample powder had been determined after being sieved was ~50 μm. Fig. 2 shows the pattern from XRD analysis of the fish scale (raw) and BTFS HAp sample peaks that has been calcined at 900 °C. Based on the XRD result (Figure 2), the most prominent or sharpest peaks were found to relate to high crystalline standard hydroxyapatite materials [17]. All peaks for calcined BTFS are similar to the standard HAp in X-pert high score library JCPDS No. 9-432. There are some missing peaks for the raw BTFS powder. It is due to the hydroxyapatite in amorphous phase. Based on library collection, the spectrum also approximated the usual XRD pattern of hydroxyapatite. Consequently, it is possible to establish that the recommended pre-treatment with heat via calcination development has removed or comprised the collagen and natural mixtures from the scale of Black Tilapia also didn't change the behaviour and molecular form or chemical formulation of the BTFS hydroxyapatite.

Fig. 2. XRD pattern for raw scale sample and calcined BTFS at 900°C.

3.2 Functional group

Fig. 3 shows the graph spectra obtain from FTIR analysis of the raw solid fish scale uncalcined samples and calcined BTFS HAp at 900 °C. The spectra point to the occurrence of phosphate (PO₄³⁻), carbonate (CO₃²⁻) and hydroxyl (OH⁻) groups. The stretching and
bending vibrations of OH were seen in the absorption bands of uncalcined raw BTFS at 3571 and 1644 cm\(^{-1}\), respectively. These spectra are additional and obviously noticed from the samples calcine BTFS HAp, because of the calcination method has demolished the cross-correlated formation for the raw BTFS HAp powder. The existence of major hydroxyl (O-H) stretching vibrations of cellulose molecules is related to the broad peak that formed between 3500 cm\(^{-1}\) and 3300 cm\(^{-1}\), according to prior investigations [18].

![Fig. 3. The FTIR spectra of raw BTFS samples and calcine at 900°C.](image)

### 3.3 Microstructure

Fig. 4 shows the raw material's surface morphology of solid fish scale before crushed which is the raw BTFS sample and calcined at temperature 900°C. Both samples were analysed using the SEM images. The BTFS were observed to be a cycloid scale. Comparing the exterior morphology of the BTFS raw solid uncalcined samples to boiled and heat-treated fish scales samples, raw BTFS samples contain collagen fibre. When the black tilapia fish scales were heated in boiling water, the collagen fibre was seldom discovered on the exterior, implying that the collagen solubilized into galantine for the duration of central heating [19]. The existence of minerals, biological substances (collagen and lipids) and water are expected to increase the amount of compressed microstructure. Furthermore, when raw fish scales were compared to calcined hydroxyapatite sample powders at 900 °C, different morphologies were detected, the final experimental result was observed had a thin particles form. This result was caused by the disappearance of all natural substances from the BTFS raw powder throughout the calcination technique, leaving only non-living substances. Therefore, the proposed calcination method to extract the BTFS HAp was the cheap and suitable ways to obtain the natural sources HAp.
3.4 Ca/P molar ratio

Chemical compositions of the BTFS HAp raw samples beforehand and after calcination method at temperatures 900 °C were calculated by using EDX analysis. Table 1 shows the calcium to phosphorus ratio for raw BTFS and sample calcined at 900 °C. The Ca/ P ratio represented from BTFS HAp samples for raw and calcined was calculated and the ratios are 1.11 and 1.74 respectively. Which is closed to stoichiometric hydroxyapatite. The average Ca/P molar ratio for raw scale was lower than the stoichiometric hydroxyapatite. Fig. 5 shows the SEM image and EDX spectra for calcine samples at 900 °C. Fig. 6 shows the SEM and EDX for the raw samples. It was identified by exchanging the phosphate because of the occurrence of carbonate ions, implying the occurrence of B-type carbonate hydroxyapatite. It's typical of this type of biological apatite's mineral phase. The present of trace element in small amounts of ions in the scale could explain the inconsistency in Ca/P ratio. Those ions, on the other hand, are precisely controlling several physiological responses linked to bone metabolism. The ratios are equivalent with previous study [20].

| Temperature   | Elemental Average | Ca         | P         | Ca/P      |
|---------------|-------------------|------------|-----------|-----------|
| Raw scale     |                   | 47.68      | 42.63     | 1.11      |
| 900 °C        |                   | 62.51      | 35.75     | 1.74      |

Table 1. Ca/P molar ratio between BTFS raw sample and after calcined at 900 °C

Fig. 4. The SEM image of the (a) raw BTFS and (b) samples calcine at 900 °C.

Fig. 5. The SEM image and EDX spectra of the samples calcined at 900°C.
Fig. 6. The SEM image and EDX spectra of the raw samples.

4 Conclusion

According to the findings of this study, the utilisation of BTFS to produce natural source hydroxyapatite is important in biomedical applications or bone restoration defects in the human body. It was feasible to produce hydroxyapatite and calcium phosphate using a simple heat treatment via calcination approach. Consequently, observation from the suggested heat treatment via calcination process have destroyed and removed the collagen and natural element combinations from the fish scale and didn’t alter the molecular shape or chemical formula of the hydroxyapatite. The XRD pattern of the fish scale powder that has been calcined at 900°C HAp, which is similar to the standard HAp (JCPDS No. 9-432). The Ca/P ratios of the extracted BTFS HAp pallet samples were evaluated. The ratios were 1.74, which is closed to stoichiometric hydroxyapatite. Hence, the BTFS HAp was identified as the potential sustainable sources available to produce the HAp.

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