Effect of the Calcination Temperature on the Properties of Hydroxyapatite from Black Tilapia Fish Bone

Siti Khadijah Dermawan\textsuperscript{1}, Zamratul Maisarah Mohd Ismail\textsuperscript{1}, Muhamad Zaki Jaffri\textsuperscript{1}, and Hasan Zuhudi Abdullah\textsuperscript{1,1}

\textsuperscript{1}Department of Manufacturing Engineering, Faculty of Mechanical and Manufacturing Engineering, Universiti Tun Hussein Onn Malaysia, 86400 Parit Raja, Batu Pahat, Johor, Malaysia

Abstract. Hydroxyapatite, also known as HAp, \(\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2\) is a naturally present form of calcium phosphate, which make up a large portion of inorganic components in human bones. Because of its near resemblance in structure to natural bone, it has been commonly used in orthopaedic applications. The ecosystem is negatively impacted by large amounts of by-product waste from fisheries factories. Thus, the aim of this study is to extract the HAp from black tilapia fish bones (BTFB) from a fish fillet factory. Fish bone can serve as a low-cost source of HAp and contribute significantly to biomedical applications. The BTFB was calcined for 3 hours at 700 °C and 900 °C, respectively. The results of X-ray diffraction (XRD) revealed the presence of derived HAp, which matched data from the Joint Committee on Powder Diffraction Powder Standard (JCPDS). For functional group analysis, Fourier Transform Infrared Spectroscopy (FTIR) was used, and the organic compounds were removed throughout the calcination process according to the spectra. The chemical composition of Ca and P was revealed by Energy Dispersive Spectroscopy (EDX), with traces of magnesium, Mg, and sodium, Na present. In the BTFB samples, the Ca/P molar ratio was determined to be 1.67 which is the stoichiometries HAp. These findings have potential as a biomaterial for biomedical applications.

1 Introduction

According to the Food and Agriculture Organization's (FAO) publication The State of World Fisheries and Aquaculture 2020, global fish production in 2018 was anticipated to reach 179 million tonnes \cite{1}. In most parts of the world, between 30 and 35 percent of that amount is lost to fish and waste. The trash has negative environmental and over-exploitation implications. Fish by-products are often offered as frozen-at-sea fillets after the bones and scales have been removed. The bones were discarded as waste because they were declared impractical and useless. Those fragments, on the other hand, might be employed as a low-
cost supply of calcium phosphate or referred to as hydroxyapatite due to their similarity to human bone characteristics [2]. Hydroxyapatite is non-immunogenic, non-inflammatory, biocompatible, non-toxic, and bioactive material [3].

To extract natural HAp, calcination, alkaline hydrolysis, hydrothermal, or a combination of techniques are commonly utilised [4]. Because no chemicals are used in the extraction of HAp from bio-waste, it is regarded as a safe process. It is also an economically desirable process. This is because of the global demand for hydroxyapatite bioceramics is high [5]. Additionally, natural sources are easy to get, low in cost, simple to process, and limitless in supply, particularly for waste by-products. Other marine hydroxyapatite sources include fish bone [6, 7], fish scale [8], and cuttlefish [9]. Thus, this study was done to extract and analyse HAp from natural sources, specifically the bones of black tilapia fish (*Oreochromis niloticus*), for biomedical applications. Tilapia is a robust, prolific, and fast developing tropical fish, and black tilapia is the most widely produced tilapia species in the world [10]. The HAp is derived via a straightforward calcination procedure because the high temperature through this process will leave the organic part found in the fish bones that is HAp as desired [11].

2 Materials and experimental methods

2.1 Preparation of samples

In a frozen form, fish bones from black tilapia were received as by-product wastes from a fishery factory in Perak, Malaysia. BTFB were boiled for 1 hour at 100°C to eliminate any clinging fish meat or other contaminants, followed by rinsed with tap water. The process was repeated twice to confirm that the samples have been fully cleaned. The bones were then dried for 3 hours at 120°C in oven before being stored in a desiccator. For size reduction, the dried materials were milled using a rotor mill pulverisette 14 classic line (Fritsch, Germany) machine.

2.2 Calcination of samples

The powder samples approximately 10 g were calcined in a furnace (Protherm) at several temperatures which are 700 °C and 900 °C with heating rate at 10 °C / min in the air. To eliminate the organic matrix, the temperatures were retained for 3 hours, then cooled to room temperature.

2.3 Characterization of samples

The chemical composition of the BTFB samples was characterized by using a high-resolution Bruker Advance D8 XRD diffractometer, the phase composition and crystalline structure of the calcined BTFB samples powder were determined. X-ray diffraction in the Bragg-Brentano configuration using Cu-Kα (λ = 1.5405 Å) radiation at the specific current (40 mA) and voltage (40 kV). Step counting method was used to collect intensity data in the range 20 between 10° to 90°, with a step of 0.02° and a time of 0.5 s. Fourier-transform infrared spectroscopy (FTIR), Perkin Elmer Spectrum 100 Optica FTIR Spectrometer was used to identify the functional groups of HAp. The infrared transmission studies were done in the regions of 400 cm⁻¹ to 4000 cm⁻¹ using 32 scans with a spectral resolution of 4 cm⁻¹. The element of the samples was characterized by using Energy Dispersive Spectroscopy, EDX (Oxford, United Kingdom) equipped with a Scanning Electron Microscope, SEM SU1510 (Hitachi, Japan).
3 Results and discussion

3.1 XRD analysis

The XRD patterns of calcinated samples at 700 °C and 900 °C are shown in Figure 1. The standard HAp from JCPDS 00-009-0432 [12] was used for validation. The XRD peaks in the picture corresponded to the standard HAp (JCPDS 00-009-0432) and were indexed as (002), (210), (211), (112), (202), (301), (311), (222), (213), (321), (410), (402), (322), (502), (304), (511), (431), and (522), and crystallise in a hexagonal crystal structure with space group P63/m. At 25.88°, 28.97°, 31.77°, 32.20°, 32.90°, 34.05°, 39.82°, 42.03°, 46.71°, 49.47°, 50.49°, 51.29°, 52.10°, 55.88°, 63.01°, 64.08°, 65.03°, 71.65°, and 78.23° are the 2θ positions of the peaks, respectively.

The spectrum of HAp is more visible in the calcined samples at 900 °C than at 700 °C. The suggested heat treatment approach removed collagen and organic substances from fish bones without affecting the hydroxyapatite molecular structure. Furthermore, raising the temperature to 900 °C resulted in more intense and sharp peaks, indicating an increase in mineral crystallinity [13]. The samples grow more crystalline because of the crystal absorbing enough energy during heat treatment. Crystallite formation and carbonate removal from the lattice are consistent with enhanced mineral crystallinity. The sharpness of the diffraction peaks relates to transformation in crystallite size which as expected, increases with increasing temperature of calcination.

Fig. 1. The XRD pattern of the calcined BTFB at 700 °C and 900 °C.

3.2 FTIR analysis

Figure 2 depicts the FTIR spectra of the calcined BTFB at 700 °C and 900 °C. Organic molecules, such as amide I and II, as well as inorganic compounds, such as PO₄³⁻, CO₃²⁻, and
OH⁻ chemical groups, make up the characteristic absorption bands of HAp. The hydroxyl group was recognised by a broad peak at approximately 3500 cm⁻¹ for sample calcined at 700 °C. According to prior research, water molecules are assigned to the broad absorption band at 3000 cm⁻¹ to 3600 cm⁻¹ [14]. Moreover, the amide I and II spectra were observed at 1638 and 1560 cm⁻¹, respectively for calcined BTFB at 700 °C. The C-H group, amide I, and amide II peaks for calcined BTFB at 700 °C are sharper than calcined BTFB at 900 °C, indicating that the amount of organic compound was higher in the calcined BTFB at 700 °C than calcined BTFB at 900 °C.

The mineral component, which corresponds to the carbonate ions, CO₃²⁻, is also visible within the range 880 – 870 cm⁻¹ and 1500 – 1400 cm⁻¹ wavenumbers. The intensity of the carbonate peak decreases as the temperature increases due to decarbonated hydroxyapatite [15]. The spectra depict the PO₄³⁻ group's distinctive bands, which are divided into two major sections. The first zone of phosphate ions is represented by the peak range of 1200 - 960 cm⁻¹, and the second region is denoted by the peak range of 650 - 570 cm⁻¹. The crystallinity of the HAp has risen in terms of intensity due to apatite that has settled in the lattice during calcination with corresponded to the temperature. All calcined BTFB peaks have been ascribed to a similar HAp spectrum.

![Fig. 2. The FTIR spectra of the calcined BTFB at 700 °C and 900 °C.](image)

### 3.3 Ca/P ratio

Table 1 shows that the average of Ca/P molar ratio of the BTFB which is closed to stoichiometric hydroxyapatite. Figure 3 shows the most abundant elements in the black tilapia fish bone were discovered to be calcium and phosphorus, with sodium and magnesium showing in much lesser proportions. When carbonate ions substitute for phosphate following heat treatment, the Ca/P value rises, suggesting the existence of carbonate hydroxyapatite of the B-type [16]. This form of HAp is found in natural bone's mineral phase of apatite. Aside from that, the existence of trace ions in the natural organic source could explain the variance
in Ca/P molar ratio. These ions, on the other hand, have a direct impact on several metabolic events connected to bone metabolism. Mg and Na, for example, can induce bone growth in in vitro and in vivo experiments that mimic human bone development [17, 18]. From the result, the weight percent of traces element increase regarding the temperature.

| Sample | Elemental Composition (wt.%) | Ca/P ratio |
|--------|----------------------------|------------|
|        | Ca            | P          | Mg    | Na    |          |
| 700 °C | 60.05         | 36.31      | 1.85  | 1.78  | 1.65     |
| 900 °C | 51.83         | 33.17      | 4.26  | 10.74 | 1.56     |

**Fig. 3.** The SEM images and EDX spectra for calcined BTFB at (a) 700 °C and (b) 900 °C.

**4 Conclusion**

The extraction of hydroxyapatite from BTFB waste by-products was shown the potential, particularly in the biomedical field. According to the findings, the calcination temperatures of the bone powders were influenced their properties. At higher temperatures, resulting more pure of hydroxyapatite and higher crystallinity degrees. It was discovered that a sample that had been calcined at 900 °C had greater intensity than BTFB calcined at 700 °C. The FTIR spectra indicate that the organic components in the raw samples are eliminated throughout the calcination process at 900 °C. Meanwhile, because of the existence of trace amounts of ions that aid in bone development, the Ca/P molar ratio found from samples is near to HAp stoichiometric, 1.67. As a result, BTFB have been discovered as a low-cost source of HAp and the extraction process utilised in this work is a simple and low-cost method of HAp synthesis.
The authors would like to thank the Ministry of Higher Education Malaysia for supporting this research under Fundamental Research Grant Scheme Vot No. FRGS/1/2018/STG07/UTHM/02/2 and partially sponsored by Universiti Tun Hussein Onn Malaysia.

References

1. FAO, (Rome, Italy, 2020)
2. M. Boutinguiza, J. Pou, R. Comesañ, F. Lusquinos, A. De Carlos, and B. León, Mater. Sci. Eng. C, \textbf{32}, 478–486 (2012)
3. K. Prabakaran and S. Rajeswari, \textit{Journal of Physics: Conference Series} \textbf{2169}, (2022) 012034
4. N. A. S. Mohd Pu'ad, P. Koshy, H. Z. Abdullah, M. I. Idris, and T. C. Lee, Heliyon, \textbf{5}, e01588 (2019)
5. N. A. M. Barakat, K.A. Khalil, F.A. Sheikh, A.M. Omran, B. Gaihre, S.M. Khil, H.Y. Kim, Mater. Sci. Eng. C, \textbf{28}, 1381–1387 (2008)
6. A. S. Hammood, S. S. Hassan, M. T. Alkhafagy, and H. L. Jaber, SN Appl. Sci., \textbf{1}, (2019)
7. T. M. Coelho, E.S. Nogueira, W.R. Weinand, W.M. Lima, A. Steinacher, A.N. Medina, M.L. Baesso, A.C. Bento, \textbf{084701}, (2013)
8. B. Mondal, S. Mondal, A. Mondal, and N. Mandal, Mater. Charact., \textbf{121}, 112–124 (2016)
9. J. Venkatesan, P. D. Rekha, S. Anil, I. Bhatnagar, P. N. Sudha, C. Dechsakulwatana, K. Se-Kwon, and M.S. Shim, Biotechnol. Bioprocess Eng., \textbf{23}, 383–393 (2018)
10. G. Yue, H. Lin, and J. Li, Int. J. Mar. Sci. Ocean Technol. (IJMO), \textbf{31}(1), 11–13, (2016)
11. N. A. S. Mohd Pu’ad, R. H. Abdul Haq, H. Mohd Noh, H. Z. Abdullah, M. I. Idris, and T. C. Lee, Mater. Today Proc., (2020)
12. JCPDS Card No. 9-432, (1994)
13. M. Figueiredo, A. Fernando, G. Martins, J. Freitas, F. Judas, and H. Figueiredo, Ceram. Int., \textbf{36}, 2383–2393 (2010)
14. T. Goto, I. Y. Kim, K. Kikuta, and C. Ohtsuki, Mater. Sci. Eng. C, \textbf{32}, 397–403 (2012)
15. G. Muralithran and S. Ramesh, Ceram. Int., \textbf{26}, 221–230 (2000)
16. A. N. K. A. Fara, M. A. bin Yahya, and H. Z. Abdullah, Adv. Mater. Res., \textbf{1087}, 152–156 (2015)
17. H. Begam, B. Kundu, A. Chanda, and S. K. Nandi, Ceram. Int., \textbf{43}, 3752–3760 (2017)
18. I. Cacciotti, A. Bianco, M. Lombardi, and L. Montanaro, J. Eur. Ceram. Soc., \textbf{29}, 2969–2978 (2009)