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Research Paper

Crystallinity of Hydroxyapatite Powders from Acid Washed and Thermally-Treated Tilapia Bones

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ABSTRACT

This paper presents the production of quality hydroxyapatite (HAp) powders from tilapia bones using thermal treatment. Hydroxyapatite was successfully synthesized by calcination of 0.5M HCl pre-treated tilapia bones. Powder X-ray diffraction (XRD) revealed the nano-crystalline hexagonal structure of HAp powders. Peaks associated with impurities such as hydrates and carbonates decreased and vanished along with the coalescence of particles as calcination temperature increased. XRD results also showed better crystal formation at 800 and 900°C. The synthesized HAp powder in this study conformed well to the properties of biological apatites.

Keywords: Bioceramic, Biomaterial, HAp, Nano-crystalline material

INTRODUCTION

Developing ceramic materials to replace broken bones, teeth, and hard tissues is one of the latest trends of research works by material scientists for the past two decades. There have been extensive studies on bioceramic materials (Park, Condrate, & Hoelzer 1998; Park et al., 1998), which reported that the most suitable material as a substitute for bones is the mineral hydroxyapatite (HAp) (Hench, 1998). It is chemically represented as $Ca_{10}(PO_4)_6(OH)_2$ and is the principal component of bones and teeth (Afshar, Ghorbani, Ehsani, Saeri, & Sorrel, 2003). This is mainly applied as a substitute material for hard tissue implantation due to its excellent biocompatibility, bioactivity, and osteoconductivity (Park et al., 1998; Park et al., 1998; Hench, 1998).

Hydroxyapatite has diverse applications in the following fields: chromatography (Morales, Burgues, Boix, Frails, & Clemente, 2000), solid state ionics (Aizawa, Hanazawa, Itatani, Howell, & Kishioka, 1999), catalyst (Sugiyama et al., 1999; Tanaka, Chikazawa, Kandori, & Ishikawa, 2000), drug delivery system (Panda, Ming-Fa, Chung, & Chin, 2001) and fuel cells (Gross, Berdt, Stephens, & Dinnebier, 1998; Verges et al., 2000) and has promising application as a chemical gas sensor (Gross et al., 1998; Verges et al., 2000; Nagar, Nishino, & Saeki, 1988; Mahabole, & Aiver, 2005). Because of these, various techniques have been developed to synthesize HAp bioceramic materials including hydrothermal processing (Yoshimura, Suda, Okamoto, & Ioku, 1994), microwave route (Vaidhayanathan & Rao, 1996), ultrasonic spray pyrolysis (Aizawa et al., 1999), precipitation routes (Ikoma & Yamazaki, (1999; Sugiyama et al., 1999), emulsion system (Furuzono, Walsh, Sato, Sonoda, & Tanaka, 2001), sonochemical synthesis (Kim & Saito, 2001) and sol-gel method (Jillavenkatesa & Condrate Sr, 1998). The main goal of these techniques is to produce HAp

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powders with high specific surface area and fine grain size with as little particle agglomeration as possible (Sung et al., 2004). Although promising, the use of these techniques in large-scale operations is still very costly (Vijayalaksmi & Rajeswari, 2006), so there is a need to develop new techniques to synthesize HAp powder at significantly lower costs. Fortunately, there are promising results of HAp powder synthesis from eggshells (Sasikumar & Vijayaraghavan, 2006) and fish bones (Prabakaran & Rajeswari, 2006; 2006; Butanas, 2010; Butanas & Vequizo, 2013; Fara & Abdullah, 2015) using the thermal treatment method. Eggshells and fish bones are ideal starting materials. Eggshells are rich in calcium while fishbones naturally contain appatite-like substances (Currey, 2001). Previous studies (Prabakaran & Rajeswari, 2006; Butanas, 2010; Butanas & Vequizo, 2013; Fara & Abdullah, 2015) have shown that formation of HAp typically occurred at 900^{o}C. Moreover, Butanas and Vequizo (2013) reported that pre-treatment by acid washing produced well-crystallized HAp as suggested by their scanning electron microscopy (SEM) photomicrographs. However, further characterization of the synthesized HAp powder is necessary for it to have any potential Osseo applications.

This paper presents the X-ray powder diffraction (XRD) characterization of HAp's synthesized by acid pre-treatment with 0.5 M HCl and thermal treatment of tilapia bones. Tilapia ranks third among the most important fish in the world, in inland fisheries after carps with production reaching 723,000 tons in 2006 (FAO, 2009). The Philippines is very fortunate since it is considered as a major producer of marine and inland fisheries in the world (FAO, 2009) having tilapia as the second most cultured fish next to milkfish (BFAR, 2007). Making use of tilapia bones as a raw material is an advantage given the fact that the Philippines, specifically Bukidnon, has an abundant supply of it. Typically, these bones are dumped as a waste product during fish processing and food consumption thus allowing the researchers to produce high-quality HAp powders from a relatively cheaper source.

METHODOLOGY

HAp Synthesis

The fish bones were collected during fish processing as a waste product at the central market of Iligan City, Lanao del Norte, Philippines and then boiled in distilled water for 1 hour to soften the attached meat. The fish bones were then washed using strong flowing water from the faucet to remove the remaining fish meat. These were then washed with reagent grade 0.5M

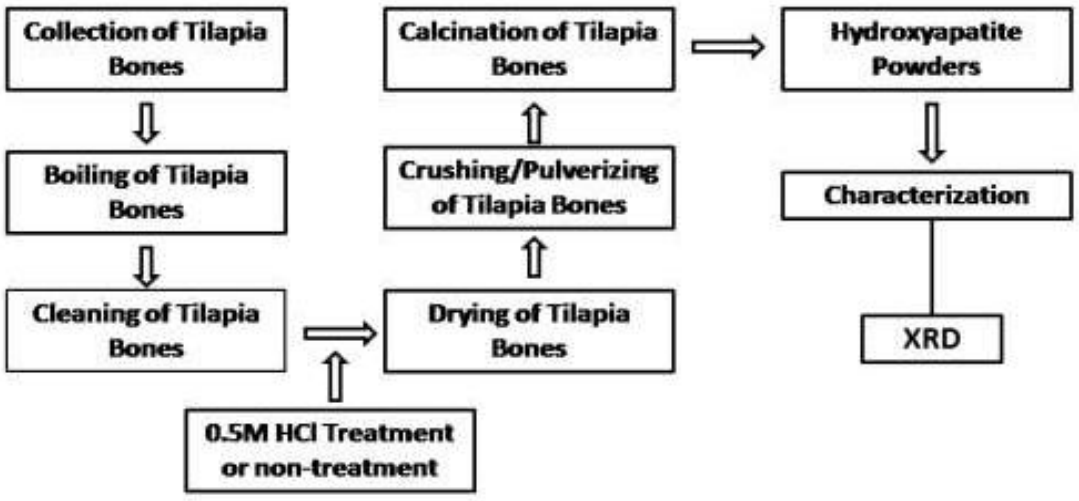

Figure 1. A Schematic Flow Chart of HAp Powder Synthesis and Characterization

hydrochloric acid (HCl) (Merck, Philippines) to eliminate organic residues further. The bones were then thoroughly washed with ethanol and deionized water to remove residual HCl and other contaminants. After drying at 150°C for three hours the bones were crushed in a blender and calcined at various temperatures (i.e., 700°C, 800°C, 900°C and 1000°C) for 5 hours using a furnace (ThermolyneTM Industrial Benchtop Muffle Furnace, Thermo Fisher Scientific Inc., USA). The calcined powders were then ground to <50 µm using an agate mortar and pestle prior to the XRD analysis.

XRD Characterization

The crystal structure and crystallinity of HAp powders were determined using XRD. The X-ray diffraction patterns of samples calcined at various temperatures were obtained in the range 2θ = 5°-90° at a scan speed of 0.02°/min. with an X-ray diffractometer (XRD-7000, Shimadzu Corporation, Japan) operating at 40kV and 30mA generating CuKα with a wavelength of 0.154 nm. For determination of diffraction line positions (2θ-values and $d_{2\theta}$ values), the prepared samples were mixed with pre-ground and annealed ZnO powders (100°C) that serve as an internal standard for corrections.

The obtained raw data were plotted. The plotted XRD patterns were corrected using the standards 2θ-values of ZnO then compared to Powders Diffraction Files of ZnO, PDF # 36-1451, and HAp (Marcovic, Fowler, & Tung, 2004).

RESULTS AND DISCUSSION

Calcination Mass Loss

Figure 2 shows that there was a higher loss of mass that occurred as the calcination temperature increases. This trend was to be expected because of the more extensive desorption of water molecules and decomposition of organic materials in the raw bone sample at a higher temperature. At 700°C, about 35% of mass loss was observed. This was attributed to the decomposition of organic compounds of the raw bone structure (Ozawa & Suzuki, 2002) such as collagens, fats, etc. There was also mass loss due to removal of physisorbed and chemisorbed water (Marcovic et al., 2004).

A slight mass loss increase of about 1% was observed as the samples were calcined at 800°-1000°C. This was probably due to the further removal of remaining traces of carbonates.

XRD ANALYSES

Figure 3 shows the XRD pattern of tilapia bone powder calcined at 700°C, 800°C, 900°C, and 1000°C. Notice that we only considered the

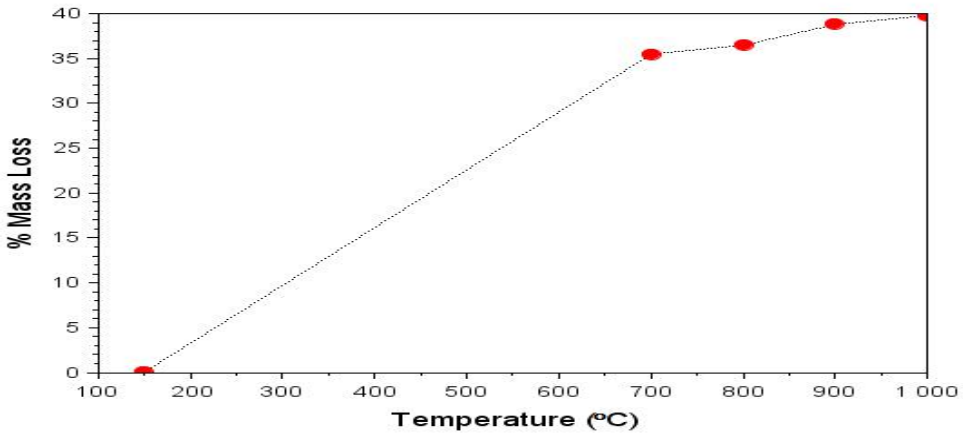

Figure 2. Average Mass Loss of Tilapia Bone Powders Calcined at 700°C-1000°C

peaks in 2θ = 5° - 60° since our standard reference material only uses this range. As shown in Figure 3, peaks that correspond to crystalline ZnO were easily identified through PDF # 36-1451. Around 2θ = 31.76°, overlapping of (211) plane associated with HAp and (100) plane of ZnO occurred. The (211) plane was the strongest peak for HAp as depicted by the broad peak around (100) ZnO plane.

Calcination had a strong effect on the tilapia bone powders at 700°C. More peaks associated with the crystal planes of HAp appeared (i.e., (100), (111), (102), (300), (310), (311), (222), (321), (410), (402), and (004)). The increasing crystallinity of HAp was apparent as the (002), (210), and (213) peaks became more intense and narrower. These results suggested that the powders are at a submicron level and consistent with the FTIR spectra and SEM photomicrographs obtained by Butanas and Vequizo (2013).

Tilapia powders calcined at 800°C exhibited a greater crystallinity of HAp crystals as suggested by the appearance of additional peaks (212), (302), (113), (312), (213), (501), and (420). The major peaks, i.e., (002), (211), (213), (222), (310), (004), (210), (321) and (312), (501), and (420). The major peaks, i.e., (002), (211), (213), (222), (310), (004), (210), (321), and (312), of HAp also became more distinct, narrower, and more intense compared with crystal formed at 700°C. Broadening becomes less, and this was likely due to decomposition of remaining traces of volatile material at 800°C. Peaks greater than 2θ = 60° were beyond the range of the HAp's standard 2θ range and did not belong to ZnO peaks.

More distinct peaks of HAp were observed at 900°C. The crystallinity of calcined product at this temperature was less ambiguous than those synthesized at 700, 800, and 1000°C, which indicates better HAp crystal formation at higher temperatures.

Calcination of tilapia bone powders at 1000°C also formed crystalline HAp with negligible amounts of tricalcium phosphates (TCP). This confirmed the absence of the TCP absorption bands in the FTIR results of Butanas and Vequizo (2013).

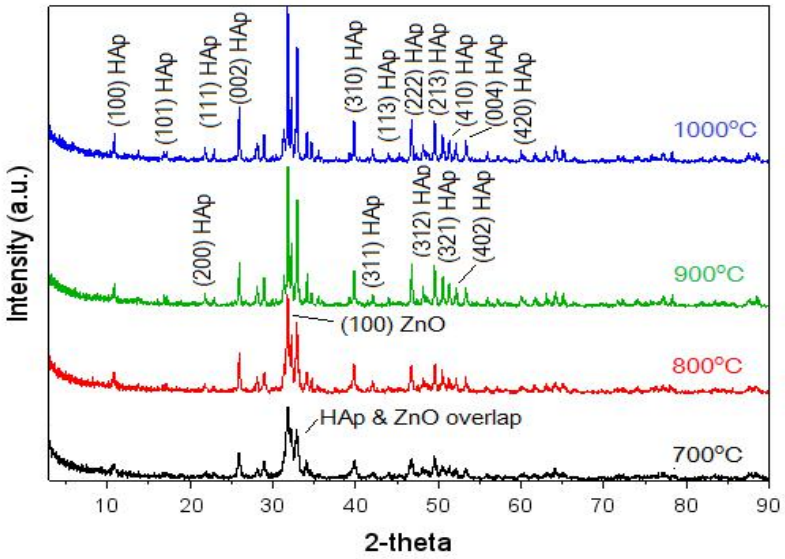

Figure 3. XRD Patterns of HAp Samples Calcined at Temperatures 700-1000°C

CONCLUSIONS

Crystalline HAp powders were successfully synthesized from Tilapia bones using acid washing and thermal treatment. XRD results revealed that the best calcination temperature for the formation of well-crystallized HAp was around 800°C. As calcination temperature increased, tilapia bone powder continued to transform into a well-crystallized and crystalline HAp. Finally, the characterization results conform to the hexagonal structure of HAp as well as to the characteristics and composition of biological apatites (LeGeros, 1991).

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