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Characterization of Hydroxyapatite Extracted from Goat Bone

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ABSTRACT

This work presents the extraction of hydroxyapatite (HA) material from goat bone by thermal treatment. The raw goat-bone was heated at 750°C for 6 hours to achieve the fine powder. The obtained powder was characterized by using several physical-chemical methods such as X-ray diffraction (XRD), Fourier transmitted infra-red (FTIR), X-ray fluorescence (XRF), and Brunauer emmett teller (BET) methods. In addition, the synthetic powder was also tested for microbiological property. The obtained results confirmed the purity and crystallinity of HA material. The microbiological test confirmed the safety and hygiene of extracted HA without harmful-bacteria presence. The thermal treatment used in this work, is simply and efficiency method for HA extraction.

Keywords: *Goat bone, Hydroxyapatite (HA), Thermal extraction, Heat treatment.*

1. Introduction

Hydroxyapatite (HA) is a naturally occurring mineral with the formula $Ca_5(PO_4)_3OH$ or $Ca_{10}(PO_4)_6(OH)_2$. HA is a major component of bones and teeth of humans and animals, accounting for 65-70% of bone mass and 70-80% of teeth. HA materials are usually white, ivory, pale yellow or blue in color, depending on the formation conditions, particle size and aggregation state. HA has some physical properties such as melting point at 1760°C; boiling point at 2850°C; solubility in water is 0.7 g/l; molecular mass is 1004.60; density of 3.156 g/cm^3; hardness value on the Mohs scale is 5 [1]-[2]. Because HA is the main inorganic component in human bone, it has high bio-compatibility, and often used as bone material in orthopedic surgery, bone grafting, dental filling [3]-[4]. Through reference to the literature, HA material can be synthesized through physical-chemical methods such as precipitation, sol-gel, solid phase reaction, plasma methods; or isolated from animal bones. In which, HA material isolated from animal bones showed better biocompatibility due to the same natural properties [5]-[8]. To separate HA material from animal bones, the thermal decomposition method is commonly used. In the studies [7]-[10], HA material was separated from bovine, caprine, and galline bones by heat treatment of the original bone samples at temperatures ranging from 600°C to 1000°C. Research shows that HA was shown to have the best purity at about 750°C for a calcination time of 6 h. Besides, the purity, crystallinity, and particle sizes depend on the temperatures and the heating times of the initial samples. In this work, we separate HA material from goat bone.

Separation material is evaluated for physical and chemical properties such as phase composition, functional groups, elemental composition, porous structure, as well as microbiological criteria of the material.

2. Experiment and Methods

2.1. Experiment

Unprocessed natural goat bone was removed the flesh, fat and membrane that clings to the outside of the bone, and then cut the bone into long pieces (Fig.1a). Bones after being cut, were washed with distilled water to clean,

removed mechanical impurities and organic compounds, and then boiled for several hours. The goat bone samples were removed, washed, dried (Fig.1b), and then ground into bone meal (Fig.1c).

The bone meal was heated at 750°C for 6 hours based on previous research [7]-[8], then ground into a fine ivory-white powder (Fig.1d).

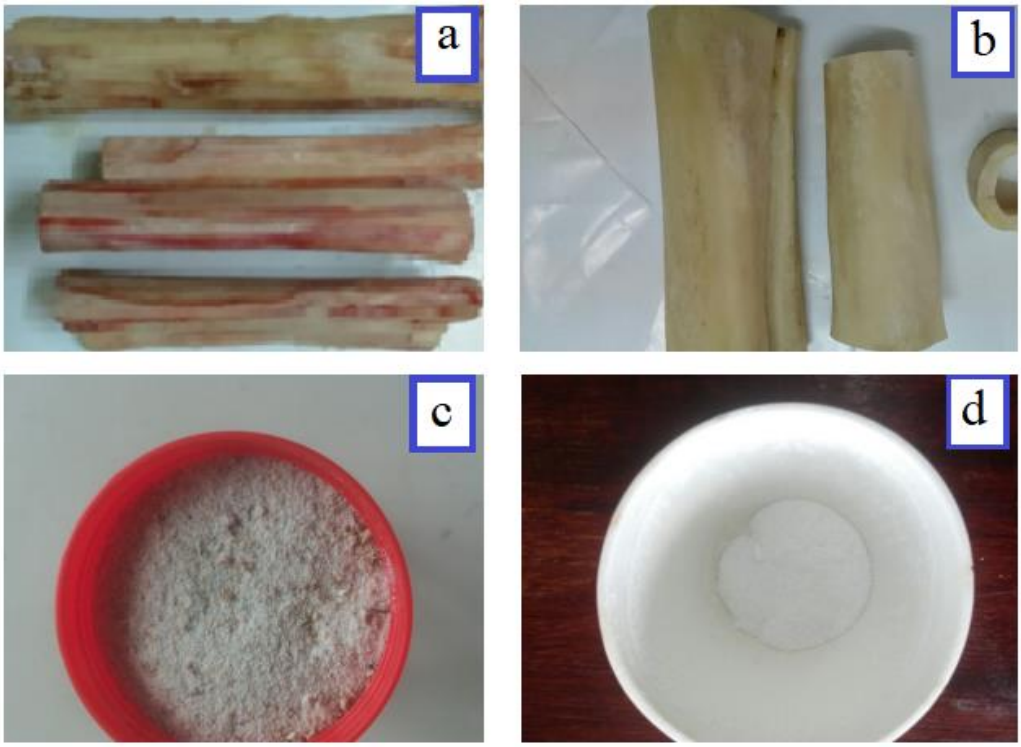

Fig.1. Protocols of HA synthesis by thermal treatment: (a) unprocessed bone, (b) pieces of cleaned bone, (c) raw bone-powder, (d) synthetic bone powder

2.2. Method for characterization

The phase composition of bone powder was characterized by using XRD. The functional groups were analyzed by FTIR technique. The elemental composition was identified by XRF. The texture of bone powder was analyzed by using BET method. Microbiological examination is performed with some causative bacteria such as Coliforms, Escherichia coli, Staphylococcus aureus.

3. Results and Discussion

3.1. XRD Investigation

Fig.2 presents the XRD diagram of bone powder synthesized from goat bone by the thermal treatment. XRD diagram of bone powder separated at 750°C showed the appearance of sharp and narrow peaks, confirming the crystalline state of the bone powder. All observed peaks correspond perfectly to those of the standard XRD diagram of pure hydroxyapatite [11]. The peaks of the bone meal sample when compared with the standard sample include main peaks at 26°, 32° and 50° (2θ) regions as well as other minor peaks. In addition, no strange peaks was observed. This result shows that the bone meal sample was heat treated at 750°C for 6 hours for completely pure HA material.

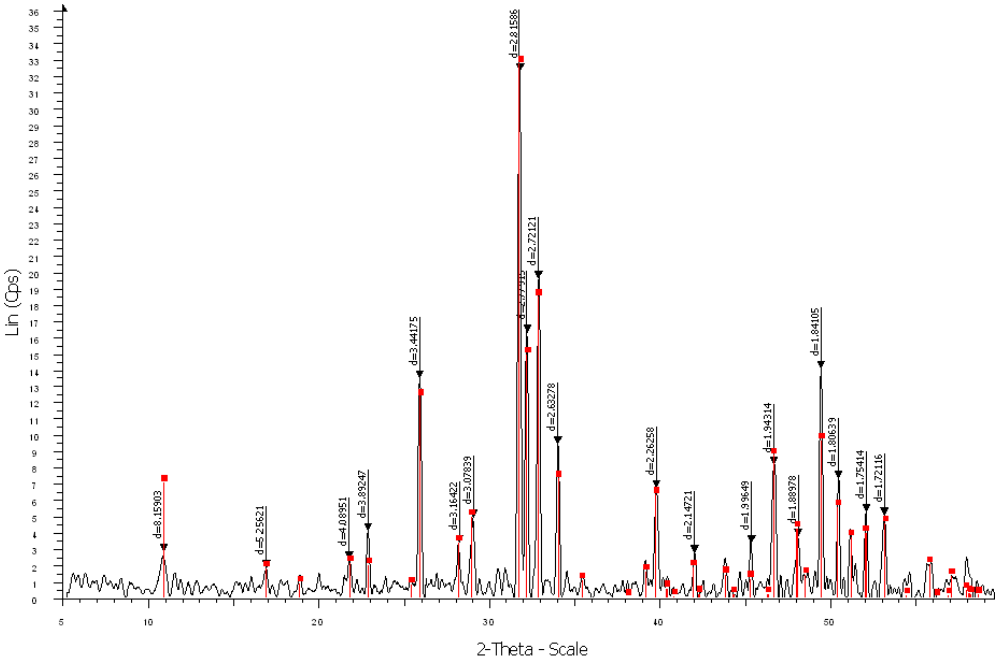

Fig.2. XRD diagram of bone powder synthesized at 750°C during 6 hours

3.2. FTIR Analysis

Fig.3 presents the FTIR spectrum of HA separated from goat bone by heat treatment. Comparison with the standard HA sample [12]-[14], the result shows that the bone powder has all the characteristic spectral bands of HA. Specifically, the spectral bands at 474.71; 570.97; 603.11; 632.91; 963.67; 1047.08; 1089.89 cm^{-1} are typical for PO_4^{3-} group oscillations in the HA crystal structure. In addition, spectral bands of CO_3^{2-} and OH^- were also observed, which could be explained by CO_2 and water vapor absorption during sample storage as well as sample transfer to infrared spectroscopy.

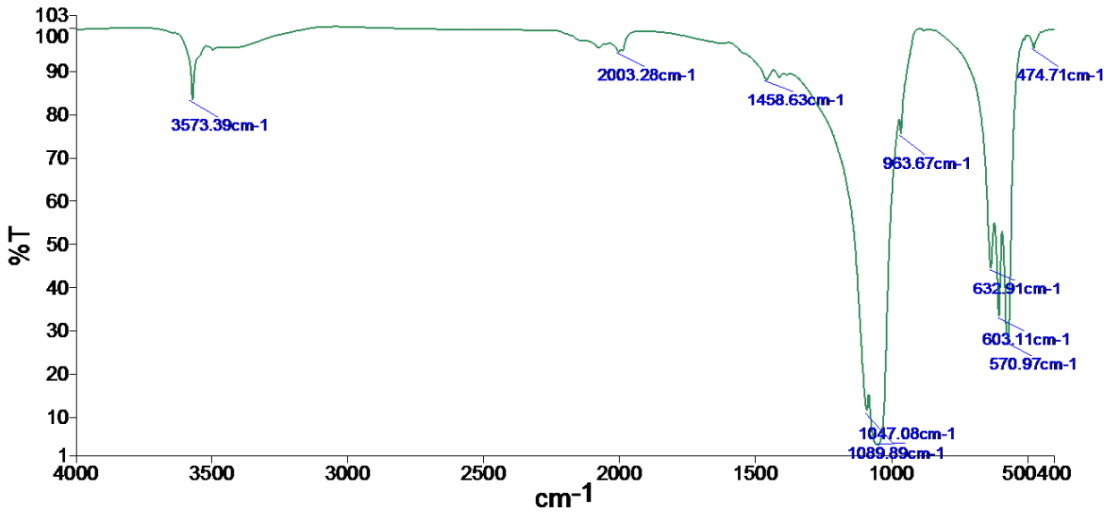

Fig.3. FTIR Spectrum of bone powder synthesized at 750°C during 6 hours

3.3. XRF Analysis

The elemental composition of bone powder was tested by using XRF technique (Table 1). From the obtained data, calcium and phosphorous are the principal elements, were found. Besides, the minor amounts of other elements

such as magnesium, potassium, sodium, strontium were detected. This analysis result is similar to that in the previous study [15], confirmed the success of HA extraction.

Table 1. XRF analysis for elemental composition of bone powder

Elemental names	Wt.%	Error.%
Ca	70.6	0.08
P	27.15	0.1
Mg	1.03	0.03
Na	1.12	0.02
K	0.07	0.002
Sr	0.03	0.001

3.4. BET Characterization

Fig.4 presents the nitrogen adsorption/desorption isotherm of bone powder. The feature mentions that the isotherm can be attributed for type IV according to the International Union of Pure and Applied Chemistry (IUPAC) classification. The bone powder is identified as meso-porous material [16]. From the Barrett-Joyner-Halenda (BJH) calculation, the pore size of bone powder is ranged from 1 to 100 nm, with the pore diameters centered at 3 nm. This result shows that the obtained HA powder is quite fine with small pore size in nanoscale (Fig.5). The BET multi-points measurement is showed in the Fig.6. The calculated value of specific surface area is 4.910 m^2/g.

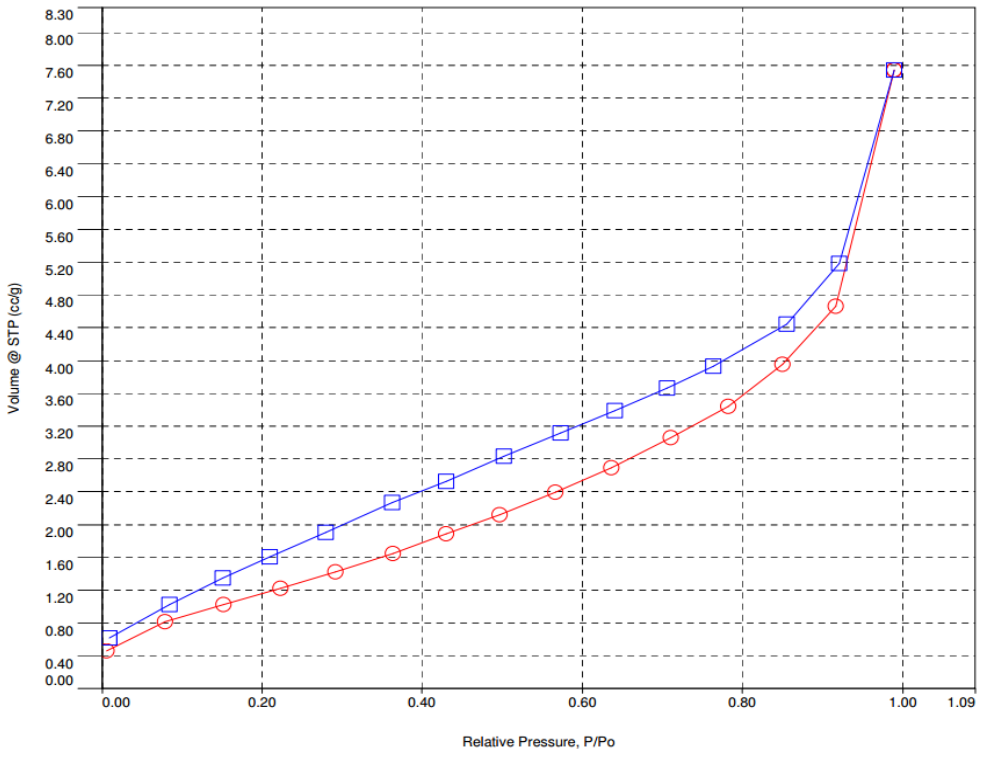

Fig.4. BET isotherm study of bone powder

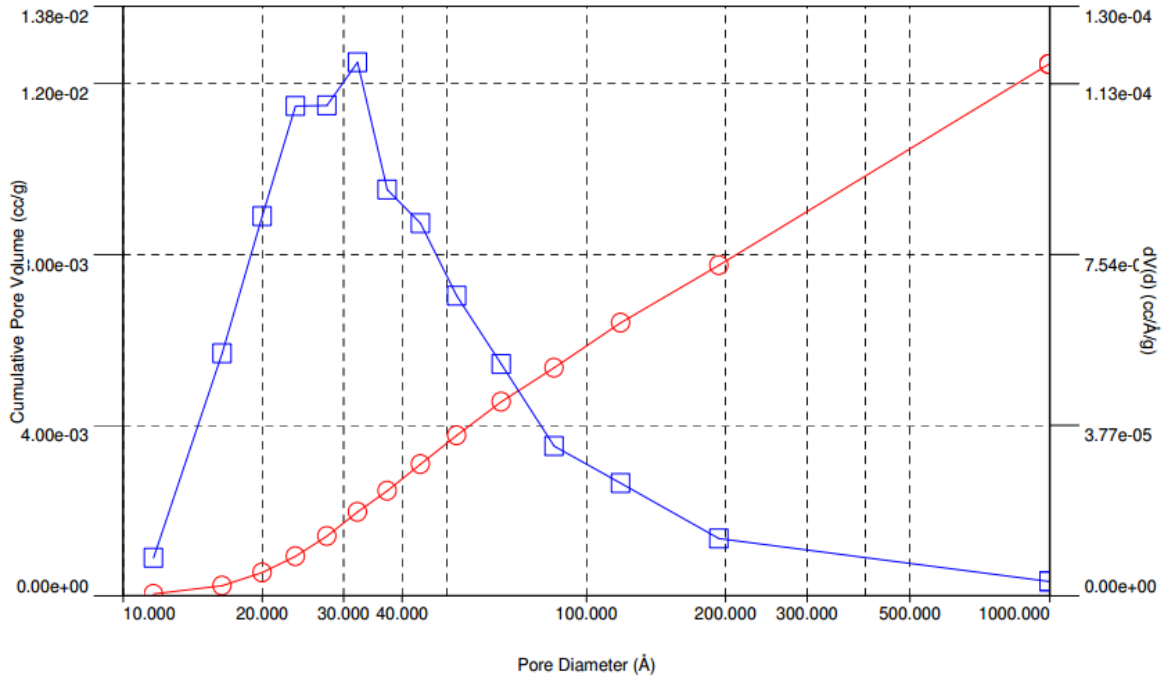

Fig.5. BJH pore size distribution of bone powder

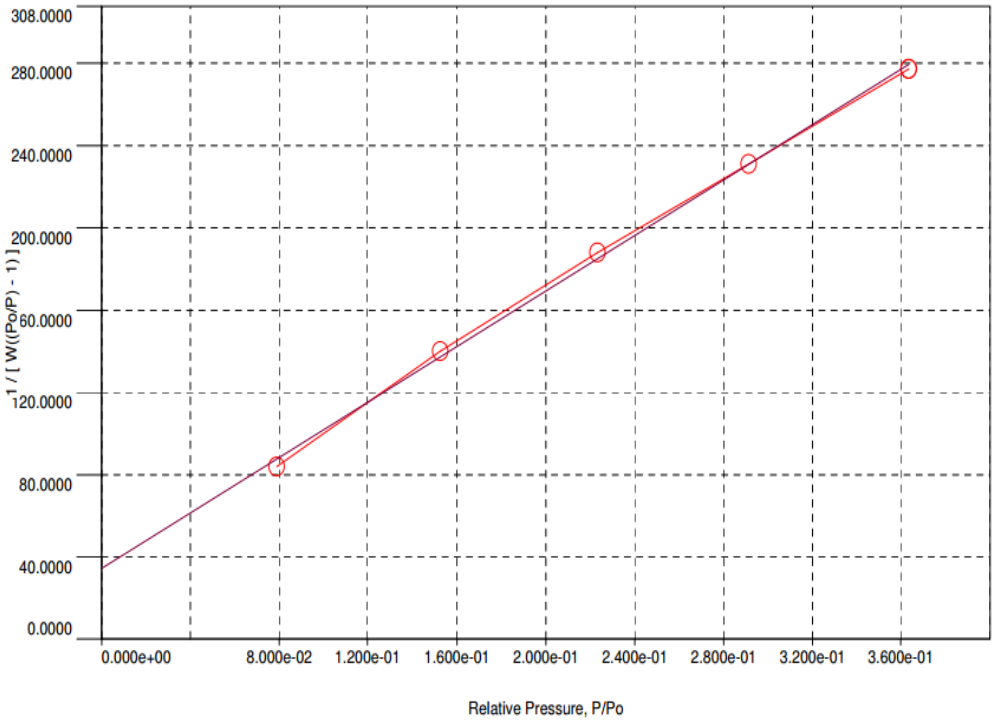

Fig.6. BET multi-point measurement for bone powder

3.5. Microbiological Evaluation

Microbiological testing was effectuated with some harmful bacteria, including Coliforms, Escherichia coli, Staphylococcus aureus. The experiments were performed at the Center of Analytical Services Experimentation HCMC. The collected data is shown in Table 2. The obtained results show that HA material powder separated from goat bone is completely clean with harmful bacteria. HA material can bring practical application values as a safe and hygienic product.

Table 2. Microbiological testing for bacteria

Bacterial Names	Calculated Unit	Result	Testing Method
Coliforms	CFU/g	<10	ISO 4832:2006
Escherichia coli	CFU/g	No detection	ISO 16649-3:2015
Staphylococcus aureus	CFU/g	No detection	ISO 6888-3:2003

4. Conclusion

We have successfully extracted HA powder from goat bone using the thermal technique. The obtained results from XRD and FTIR confirmed the high purity and crystallinity of the synthetic HA material. The BET measurement confirmed the mesoporous structure of HA powder with the pore size centered at 3 nm. The microbiological assay confirmed the safety and hygiene of extracted HA powder. Thus, the thermal treatment proved to be a potential process for extraction of HA powder from natural bone. This processing is quite simple and effective, can produce a large amount of HA materials with good quality.

Declarations

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This research did not receive any grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing Interests Statement

The author declares no competing financial, professional and personal interests.

Consent for publication

Author declares that he/she consented for the publication of this research work.

Availability of data and material

Author is willing to share the data and material according to relevant needs.

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