

## Characterization and Elemental Quantification of Natural Hydroxyapatite Produced from Cow Bone

*J. O. Akindoyo\**, *S. Ghazali\**, *M. D. H. Beg*, *N. Jeyaratnam*

*Faculty of Chemical & Natural Resources Engineering, Universiti Malaysia Pahang, 26300 Gambang, Pahang, Malaysia.*

*\*Corresponding author: [blessedbode@ymail.com](mailto:blessedbode@ymail.com); [suriati.ghazali@gmail.com](mailto:suriati.ghazali@gmail.com)*

### EXTENDED ABSTRACT

Demand for hydroxyapatite (HA) is increasing annually, due to the growing number of orthopedic patients. Therefore, there is a challenge of meeting this growing demand without interfering with the integrity of the environment. The aim of this study is to produce HA from natural sources. In this study, natural HA was extracted from cow bone through ultrasound treatment of the raw bone followed by calcination at different temperatures from 650-950 °C [1, 2]. Ultrasound treatment in ordinary water medium is found to be suitable for cleaning the bone and the HA produced after calcination at 950 °C was found to possess the desirable properties needed for pure HA.

Characterization of the produced HA was conducted through several other techniques including Field emission scanning electron microscopy (FESEM), X-ray diffraction analysis (XRD) and Fourier Transforms Infrared Spectroscopy (FTIR) among others. The HA revealed a nearly spherical morphology as confirmed through FESEM observation. The XRD diffractograms of the raw cow bone powder and the different calcined samples are illustrated in Figure 1. A standard XRD diffractogram for HA (ICCD standard HA, DB card number 01-074-9761) is also included in Figure 1 for validation of the prepared HA samples. Generally, the important peaks associated with standard HA can be seen in the diffractograms of the calcined samples which is an indication that ultrasound treatment followed by calcination process was able to remove collagen and other organic materials from the raw cow bone without observable disruption to the molecular skeletal of the HA produced [3]. In addition, the diffracted characteristic peaks of the calcined samples can be seen to be consistent with those of standard HA.

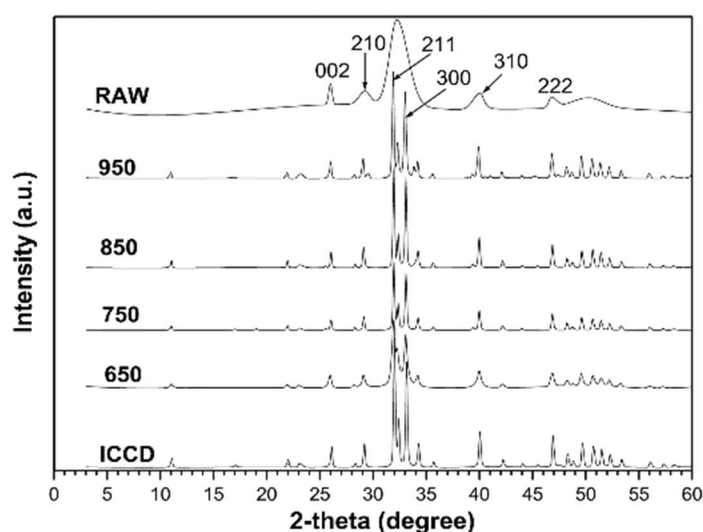


Fig 1: XRD diffractograms of samples calcined at different temperature and a standard HA

The FTIR spectrum of the HA is illustrated in Figure 2. Chemical functionality of the produced HA was investigated based on the functional groups represented in its spectrum. The FTIR spectrum for the raw cow bone powder (RAW) is also included in Figure 2. The band at higher wavelength from 3200-3500  $\text{cm}^{-1}$  is attributed to vibrational stretching of bonded hydroxyl groups [4, 5]. This representative functional group which also appeared at 1637  $\text{cm}^{-1}$  in the RAW spectrum can be associated with the presence of adsorbed surface water on the raw bone powder [6]. This was supported by the TGA result of this study. Manifestation of this peak may also be attributed to the hydroxyl groups from the HA structure [7]. The low intense band at 2840-2930  $\text{cm}^{-1}$  is an attribute of stretching vibrations of C-H bonds [4]. Presence of this band in the RAW spectrum indicates the presence of organic components in the raw bone powder as supported by TGA results. Absence of this peak from the HA spectrum indicates that there was full removal of organic components from the starting material as reported elsewhere [8]. The double split peaks which appeared at 1457  $\text{cm}^{-1}$  and 1411  $\text{cm}^{-1}$  are attributes of  $\text{CO}_3^{2-}$  asymmetric stretching which indicates that  $\text{CO}_3^{2-}$  actually entered the lattice of the obtained HA, which is in agreement with TGA and XRD results. At 1015  $\text{cm}^{-1}$  and 960  $\text{cm}^{-1}$ , vibrational stretching of phosphate ( $\text{PO}_4^{3-}$ ) ions was represented whereas its deformational vibration appeared around 550-650  $\text{cm}^{-1}$  [6]. The peak at 872  $\text{cm}^{-1}$  further confirms the ionic substitution of  $\text{CO}_3^{2-}$  into the HA.

In addition, elemental composition of the major and minor elements present in the produced HA was quantified through X-ray fluorescence (XRF) analysis. The results revealed large amount of calcium, and phosphorus whereas other minor elements were present in lesser amounts. Aside from calcium, phosphorus and oxygen, eleven other elements were detected in the produced HA. Most reports on natural HA often state that it actually contains other minor elements aside from calcium and phosphorus and despite sometimes very low amount of these elements they are actively involved in biochemical reactions during bone metabolism [9].

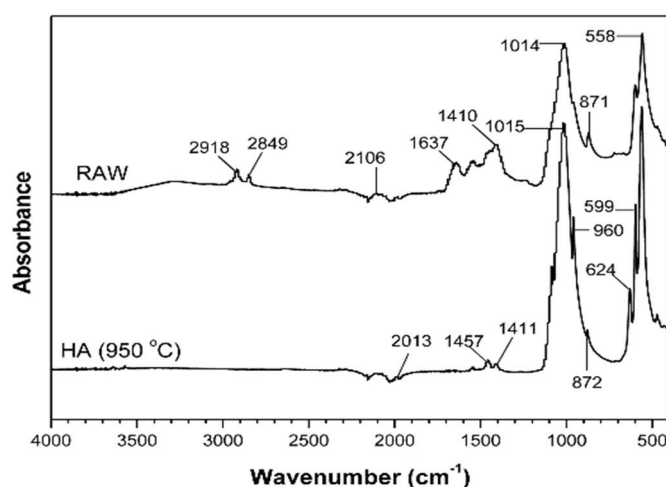


Fig 2: FTIR spectra of the HA produced after calcination at 950 °C, and raw cow bone powder

Incorporation of ultrasound into HA production from natural sources can be a more environmental friendly way of HA production as it offers an organic solvent free treatment method of the raw materials. It can also enhance large scale production as it can be employed at an industrial scale. Generally, the results indicated that the HA exhibit characteristic biomineral similarity with calcified tissues and it would be of immense benefit if it is employed for application in bone tissue engineering. Production of HA from natural sources is difficult to be totally replaced especially due to the low cost and environmental friendly characteristics of this approach.

Keywords: Hydroxyapatite; ultrasound; calcination; characterization; elemental analysis.

## Acknowledgement

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## References

- [1] Akindoyo J. O., Beg M. D .H., Ghazali S., Akindoyo E. O., Jeyaratnam N., (2017) Synthesis of Hydroxyapatite through Ultrasound and Calcination Techniques. IOP Conf. Series: Mater Sci & Eng 203:012003
- [2] Akindoyo J. O., Beg M. D. H., Ghazali S., Heim H. P., Feldmann M (2017) Effects of surface modification on dispersion, mechanical, thermal and dynamic mechanical properties of injection molded PLA-hydroxyapatite composites. Composites Part A: Applied Sci & Manufacturing 103:96-105.
- [3] Liu F, Wang R, Cheng Y, Jiang X, Zhang Q, Zhu M., (2013) Polymer grafted hydroxyapatite whisker as a filler for dental composite resin with enhanced physical and mechanical properties. Materials Science and Engineering: C, 33(8):4994-5000.
- [4] Akindoyo J. O., Beg M. D. H., Ghazali S., Islam M. R., (2015) Effects of poly (dimethyl siloxane) on the water absorption and natural degradation of poly (lactic acid)/oil-palm empty-fruit-bunch fiber biocomposites. J. App Polym. Sci 132:45.
- [5] Akindoyo J. O., Beg M. D. H., Ghazali S., Islam M. R, Mamun A. A., (2015) Preparation and Characterization of Poly (lactic acid)-Based Composites Reinforced with Poly Dimethyl Siloxane/Ultrasound-Treated Oil Palm Empty Fruit Bunch. Polymer-Plastics Technol. & Eng 54(13):1321-1333.
- [6] Liu Q, Matinlinna J. P, Chen Z, Ning C, Ni G, Pan H, Darvel B. W., (2015) Effect of thermal treatment on carbonated hydroxyapatite: Morphology, composition, crystal characteristics and solubility. Ceramics International 5:6149-6157
- [7] Jang D. W., Franco R. A., Sarkar S. K. Lee B. T., (2014) Fabrication of porous hydroxyapatite scaffolds as artificial bone preform and its biocompatibility evaluation. Asaio Journal **60** (2): 216.
- [8] Haberko K., Bucko M. M., Brzezinska-Miecznik J., Haberko M., Mosgawa W., Panz T, Pyda A., Zarebski J., (2006) Natural hydroxyapatite—its behaviour during heat treatment. Journal of the European Ceramic Society, 2006. 26 (4):537-542.
- [9] Murugan, R., Ramakrishna S., Rao K. P., (2006) Nanoporous hydroxy-carbonate apatite scaffold made of natural bone. Materials Letters 60(23):2844-2847.