International Journal of Academic Multidisciplinary Research (IJAMR)
ISSN: 2643-9670
Vol. 8 Issue 4 April - 2024, Pages: 21-27

Crystallization and Characterization of Hydroxyapatite from Billy and Nanny Femur bones through Calcination process

Orlando Ketebu, Favour Ossai Uche

Department of Chemical Engineering
Niger Delta University
Wilberforce Island Amassoma, Nigeria

Abstract: Hydroxyapatite (HAp) is a ceramic material that contains calcium-phosphate compound that is applicable in chemical engineering, biomedical and medical fields. It can be used as an adsorbent for dyes and heavy metals from oil and waste water. In medical and tissue engineering, it is applied in bleaching and teeth repair, toothpastes formation, coating of dental implant and orthopedic applications. This research project synthesizes HAp through calcination process from waste Billy and Nanny femur bones left as waste after removal of the meat from the bones. The experimental results showed that the colour of the HAp changes as heat is applied from butter cream colour (raw grounded powder) to cream colour (200°C), black (400°C), light grey (600°C), milky white (800°C) and pure white (1000°C). This changes in colour are attributed to organic materials in bones being removed and the crystallization of pure HAp at 1000°C. SEM analysis showed that the HAps synthesized from Billy and Nanny femur bones had rough, dense aggregated morphology at 2000 magnification with no clear differences in surface morphology. The XRD diffractogram for Billy bone synthesized HAp showed peaks at 2θ angles of 25.99º, 29.09º, 31.89º, 33.01º, 34.17º, 39.91º, 46.82º, 49.60º, and 64º corresponding to the 002, 210, 211, 300, 202, 310, 222, 213, and 304 planes of crystalline HAp. Similar peaks were observed for Nanny bone HAp with slight variations in the 2θ angles. This indicates that the crystallized HAp for Billy and Nanny femur bones has a hexagonal structure. And FTIR spectra showed that the HAp synthesized from both bones had similar functional groups of phosphate at wavelength 1088.4cm-1 and 1021.3 cm-1, hydroxyl groups at wavelength 3574.5 cm-1 and carbonate groups at wavelength 1546.8 cm-1, 1457.4 cm-1, and 1408.9 cm-1 respectively. The characterized results indicates that there is no significant difference in the HAps synthesized from Billy and Nanny femur bones**.**

Keywords—Thermal calcination; Billy and Nanny bones; Hydroxyapatite; XRD; SEM; FTIR

1. INTRODUCTION

Hydroxyapatite (HAp) is an important material applicable in chemical engineering, biomedical and medical fields. In chemical engineering it can be used as an adsorbent or used to form adsorbent compound with polymers for dyes, pigments and heavy metals removal from waste effluents. In medical and biomedical applications, it is used in wound healing, tissue repair, orthopedic, implant coatings etc. The important role hydroxyapatite plays have made researchers to think of easy and cheap sources for its production. One easy and cheap sources of hydroxyapatite are from waste bones of animals, fishes and other mammalian. Animal bones are bio-waste and if not properly disposed causes environmental pollution that affects human health and the environment. Thus, converting these waste animal bones to hydroxyapatite is one way of recycling the bones and reducing environmental pollution.

Hydroxyapatite (HAp) is biological ceramic material that contains calcium and phosphate compounds ($[Ca_{10}(PO_4)_6(OH)_2]$). It is similar to hard tissues found in humans in terms of its morphology and composition [1]. It is a biocompatibility material that functions like the human natural apatite in bone enhancement during bone and tissue repairs [2-3]. It has also found useful application in chemical engineering as adsorbents for removal of pollutants from soil and water ways [4-7].

HAp can be obtained from organic and inorganic compounds. The natural organic sources for HAp are waste biological materials like bovine, fish, poultry bones, cattle etc. The HAp obtained from inorganic compounds are called synthetic HAp. Different methods such as micro-emulsion, sol-gel, solvothermal, hydrothermal, homogeneous precipitation, chemical precipitation and calcination have been used in HAp synthesis [8]. Some of these methods are also applicable in the synthesis of the organic HAp. The major problem facing the inorganic synthesized HAp from inorganic sources, is the expensive nature of the synthetic processes involved in the use of calcium and phosphorus.

HAp has been synthesized from different biological materials by researchers for various medical and engineering applications such as chicken bone [8-9], bovine bone [10], bovine and catfish bones [11], marine, mammalian and plants [12].

This project looks at the synthesis of hydroxyapatite from Billy and Nanny femur bones through calcination process and their characterization and comparison. Calcination is a process where substances such as solid materials are heated at high temperature in a regulated system to form ceramic powder. To the best of our knowledge no report has been written on synthesis, characterization and comparison of hydroxyapatite from Billy and Nanny femur bones.

Billy is the name for male goat and Nanny is for female goat. Their femur bones are bones obtained from killed male

and female goat in an abattoir that are left as waste materials after the meat has been removed.

2. MATERIALS AND EQUIPMENT

The following are equipment for the experiment; 250 ml beakers, drying oven, grinder, weighing balance, magnetic stirrer, 200 ml measuring cylinders, crucibles and the materials and chemical are Billy and Nanny femur bones, distilled water, Acetone, ethanol and ether.

2.1 Synthesis of hydroxyapatite through thermal calcination of the Billy and Nanny femur bones

The synthesis procedure follows similar method reported by Challoob and co-researchers [13] with modifications. The femur bones of Billy and Nanny were sourced from local abattoir in Yenagoa, Bayelsa State in Nigeria. The bones were washed with water and little soap to remove dirt on the bones and heated at 100ºC for 4 hours in a covered crucible to remove impurities.

After heating and allowed to cool, the bones were washed severally with distilled water and soaked in acetone/ether mixture with a ratio of 3:1 respectively for 3 hours to eliminate unseen fats in the bones. The bones were further dried at 120ºC for 17 hours in an oven to eliminate shoots development when grinding the bones.

The bones were grounded mechanically and the grounded powder was calcinated in a furnace at temperature range 900-1000ºC for 2 hours at a pace of 5ºC per minute. The temperature 900-1000ºC was chosen because researchers have shown that HAp is formed at this temperature range [13].

2.2 Characterization of the synthesized hydroxyapatite

Synthesized HAp from Billy and Nanny femur bones were characterized using XRD to determine the crystalline nature of the HAp. SEM analysis was carried out to determine the HAp surface features and morphology and FTIR to identify functional groups and bonds present in the HAp.

3. RESULTS AND DISCUSSION

Fig. 1 (a) shows the Billy and Nanny bones after washing and heating at 100ºC for 4 hours to eliminate impurities. Fig. 1 (b) shows the bones soaked in acetone/ether mixture to eliminate unseen fat from the bones. Fig. 1 (c) shows the bones rinsed severally with distilled water to remove acetone, ether and unseen fats from the bones, before being drying at 120ºC for 17 hours as shown in Fig. 2 to eliminate shoots development during grinding of the bones.

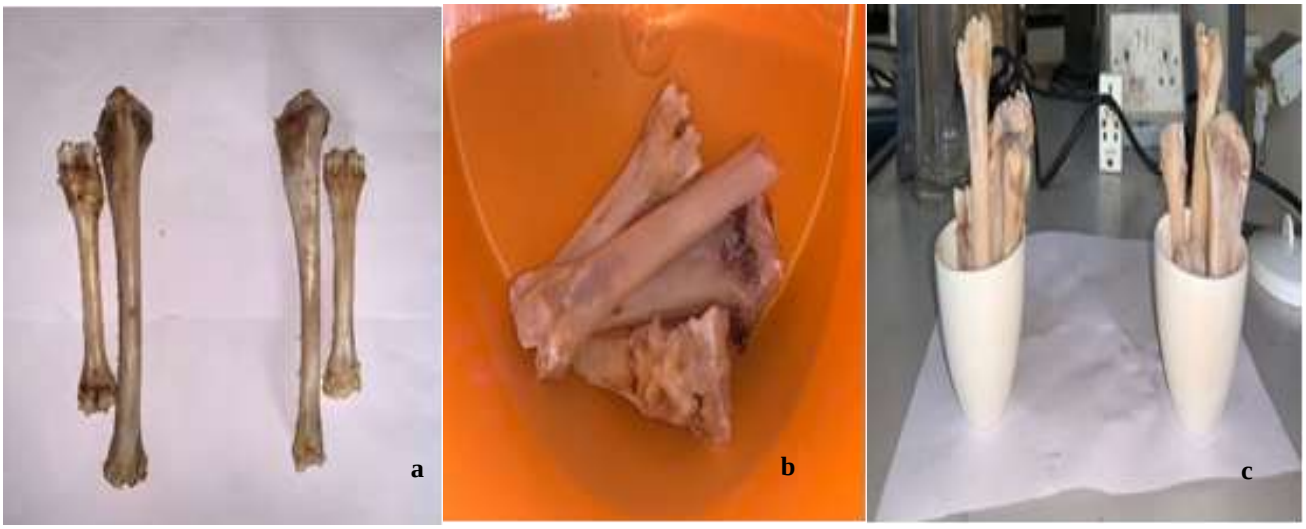

Fig. 1 Treated bones (a) dried at 100ºC for 4 hours, (b) Bones soaked in acetone/ether mixture (c) Bones washed with distilled water to remove acetone/ether mixture

In Fig. 2, you can see that the bones are well dried with Fig. 2 (a) showing the Nanny dried femur bones and Fig. 2 (b), the Billy dried femur bones. The bones were broken so as to fit in the crucible and furnace for heating.

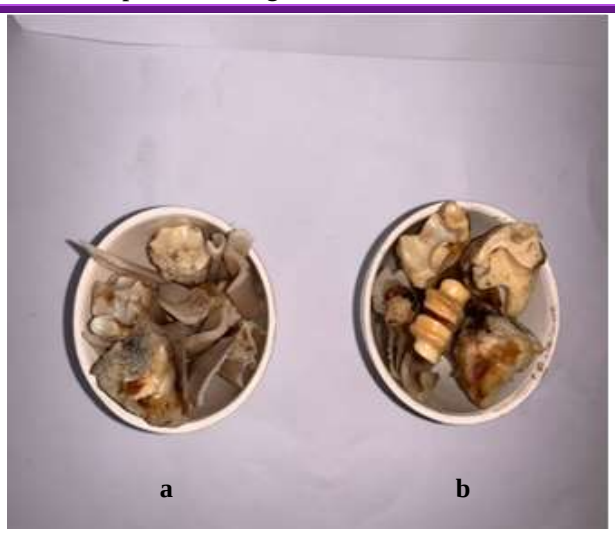

Fig. 2 Dried bones at 120ºC for 17 hours (a) Nanny femur bones (b) Billy femur bones

Fig. 3 (a) shows the grounded bone using a blender and Fig. 3 (b) shows the grounded bones after filtering to fine powder with butter cream colour using a filter mesh to remove larger ungrounded bones particles before calcinating.

Fig. 3 Grounded bones (a) inside grinder cup (b) fine powder bones after filtering.

Fig. 4 shows the fine powdered bones in crucible placed inside furnace heating to 1000ºC for the formation of Hydroxyapatite. Crucible Fig. 4 (a) contains the Nanny bone powder while crucible Fig. 4 (b) contains the Billy femur bone powder.

The calcination process was repeated for the bone powders at different temperatures (200ºC, 400ºC, 600ºC, 800ºC, and 1000ºC) for 2 hours respectively. The result showed that the goat bones powder from 200 to 1000ºC had varying colours during calcination process. The colour ranged from cream colour (200 ºC), black (400ºC), light gray (600ºC) to white colour (800 and 1000ºC) as shown in Fig. 5. Fig. 5 (a) shows the grounded bone before calcination with butter cream colour, Fig. 5 (b) shows the change in colour of the bone heated for 2 hours at 200ºC to cream colour, Fig. 5 (c) at 400ºC had black colour, Fig. 5 (d) at 600ºC, the colour turned light grey. Fig. 5 (e) at 800ºC and Fig. 5 (f) at 1000ºC, the colour changed from white milky to pure white. This change in colours at different temperatures indicates that the organic materials in the bones are highly carbonated and amorphous. But heating at 800 to 1000ºC, the colour changed from white milky to pure white at 1000ºC. This showed that the organic materials in the bones are eliminated and pure HAp particles crystallized.

Fig. 4 Calcination process (a) Nanny femur bone powder in crucible (b) Billy femur bone powder in crucible

Fig. 5 Calcinated bone powder at different temperatures (a) raw bone powder (b) bone powder at 200ºC (c) at 400ºC, (d) at 600ºC, (e) at 800ºC and (f) at 1000ºC.

Fig. 6 shows the SEM image of the synthesized Haps (a) Billy femur bone HAp (b) Nanny femur bone HAp synthesized at 1000ºC. The SEM images shows a rough, dense and

aggregated surface morphologies of the Haps. The SEM image for Billy and Nanny HAps showed no clear difference in surface morphology at 2000 magnification. This result was further corroborated with the XRD analysis in Fig. 7 for Billy femur bone HAp and Fig. 8 for Nanny femur Haps.

The XRD showed that the crystallized HAp from Billy bone powder had peaks at 2θ angles of 25.99º, 29.09º, 31.89º, 33.01º, 34.17º, 39.91º, 46.82º, 49.60º, and 64º corresponding to the 002, 210, 211, 300, 202, 310, 222, 213, and 304 planes of crystalline HAp. Indicating that the crystallized HAp has a hexagonal structure based on standard phase of HAp as recorded in JCDPS 09-432 card.

Similar diffraction peaks at 2θ angles were observed for the crystallized Nanny bone HAp as shown in Fig. 8. This indicates that the crystallized HAp for both Billy and Nanny femur bones had hexagonal structure.

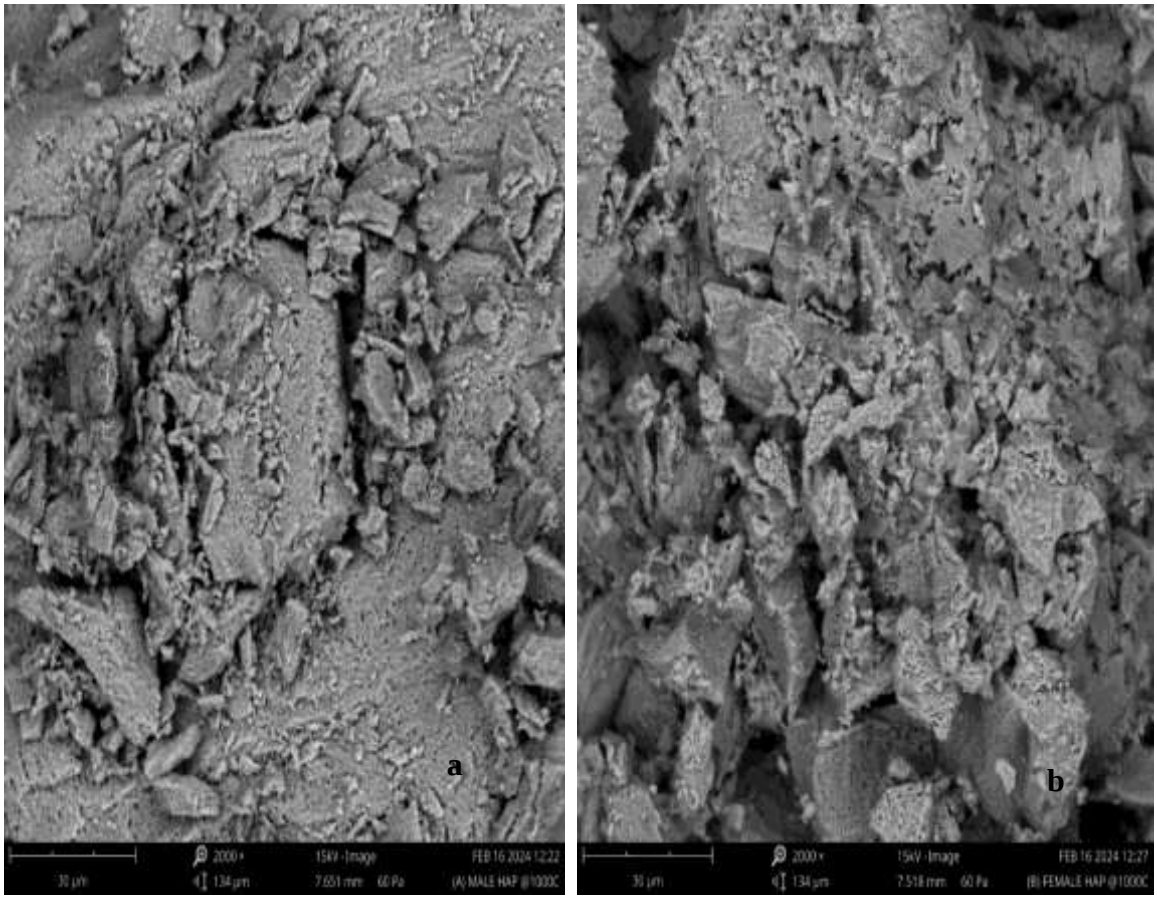

Fig. 6 SEM images (a) Billy femur bone HAp (b) Nanny femur bone HAp

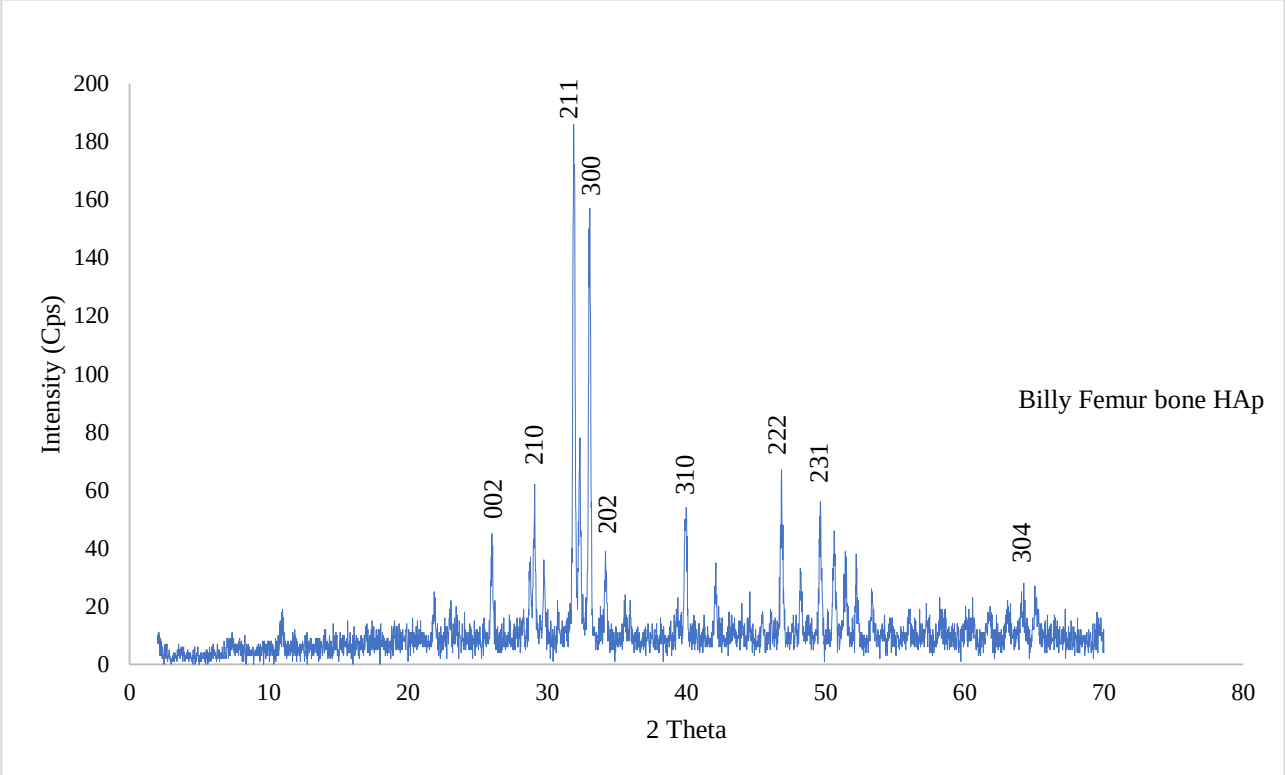

Fig. 7 XRD diffractogram of Billy femur bone synthesized HAp

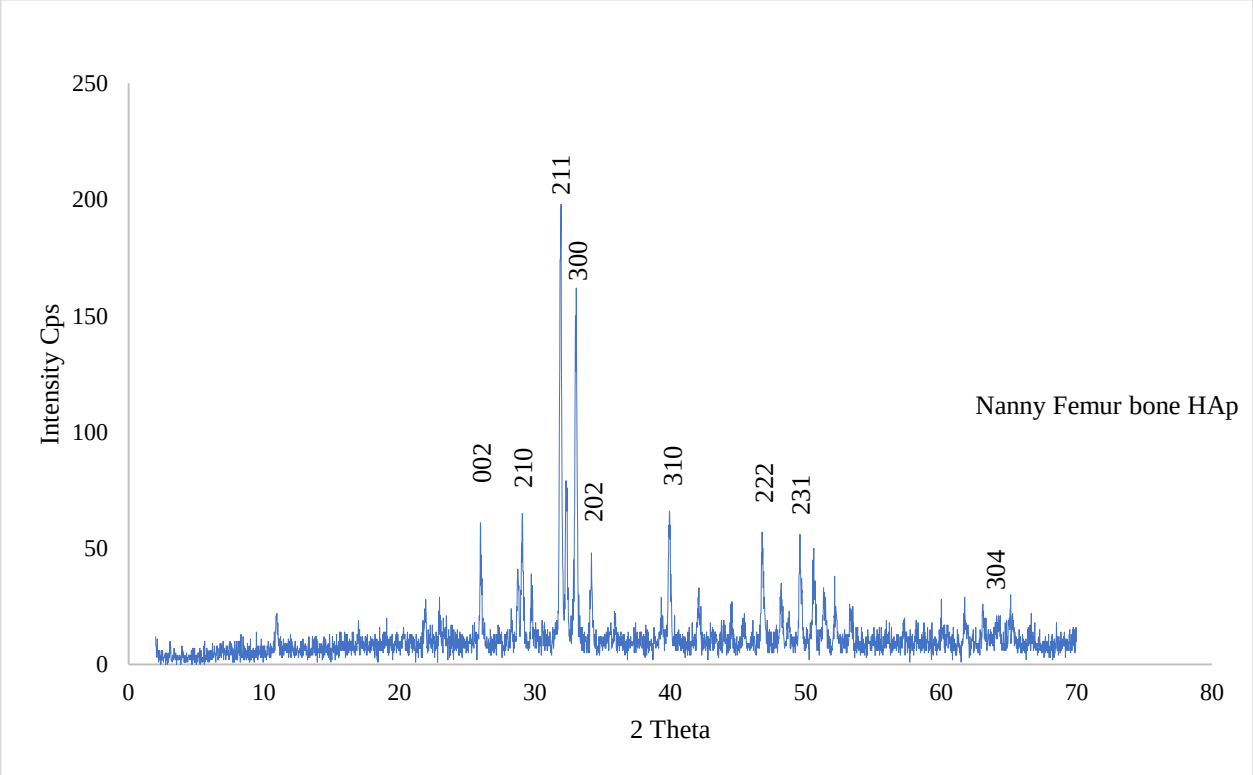

Fig. 8 XRD diffractogram of Nanny femur bone synthesized HAp

Fig. 9 and Fig. 10 shows the FTIR spectra for the Billy femur bone and Nanny femur bone synthesized HAp respectively. The FTIR spectra showed the functional groups in the HAps. Fig. 9 showed the presence of phosphate functional groups (PO_4^{3-}) at wavelength at 1088.4cm^{-1} and 1021.3 cm^{-1}. It also showed the presence of stretched OH functional group at wavelength 3574.5 cm^{-1}. The sharpness of the bands for the phosphate and hydroxyl groups in the figures indicated the crystallization HAp. Thus, higher calcination temperature (1000°C) further increased the phosphate group peak sharpness, which indicates higher crystallinity. Also carbonate (CO_3^{2-}) functional groups were observed in the FTIR spectra in Fig. 9 at wavelength 1546.8 cm^{-1}, 1457.4 cm^{-1}, and 1408.9 cm^{-1} respectively. This showed that carbonate groups are present in the formed HAp. Similar functional groups with slight difference in wavelengths were observed for the for the HAp crystallized from Nanny bone as shown in Fig. 10.

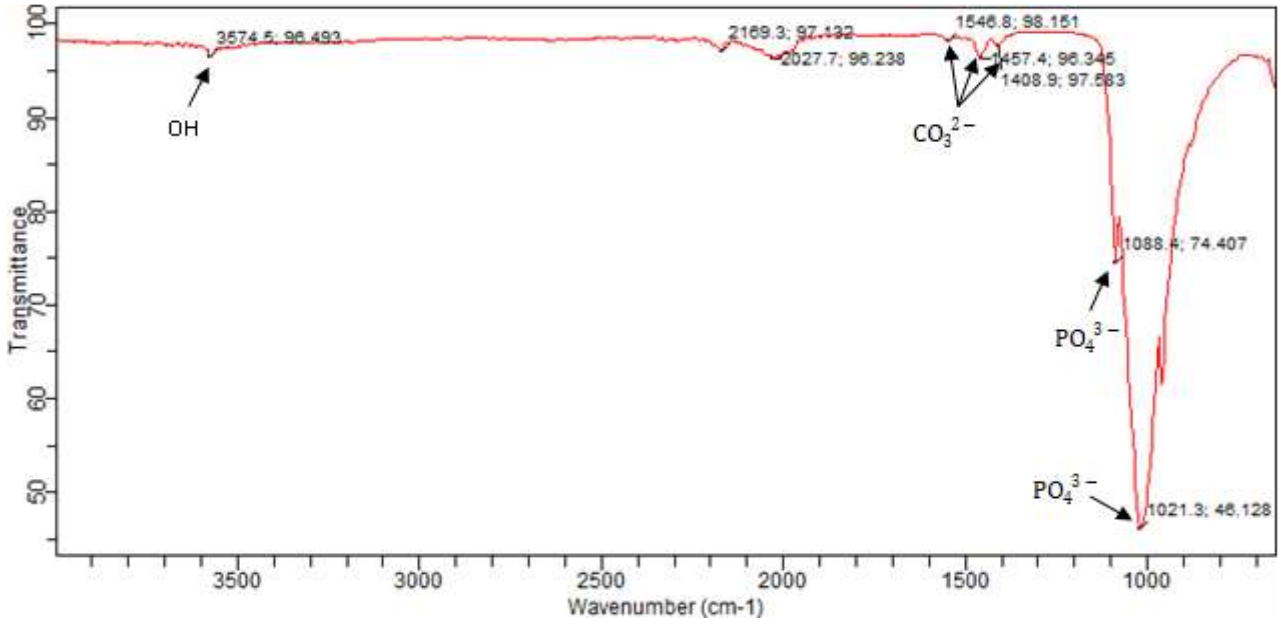

Fig. 9 FTIR spectra for synthesized HAp from Billy femur bone powder

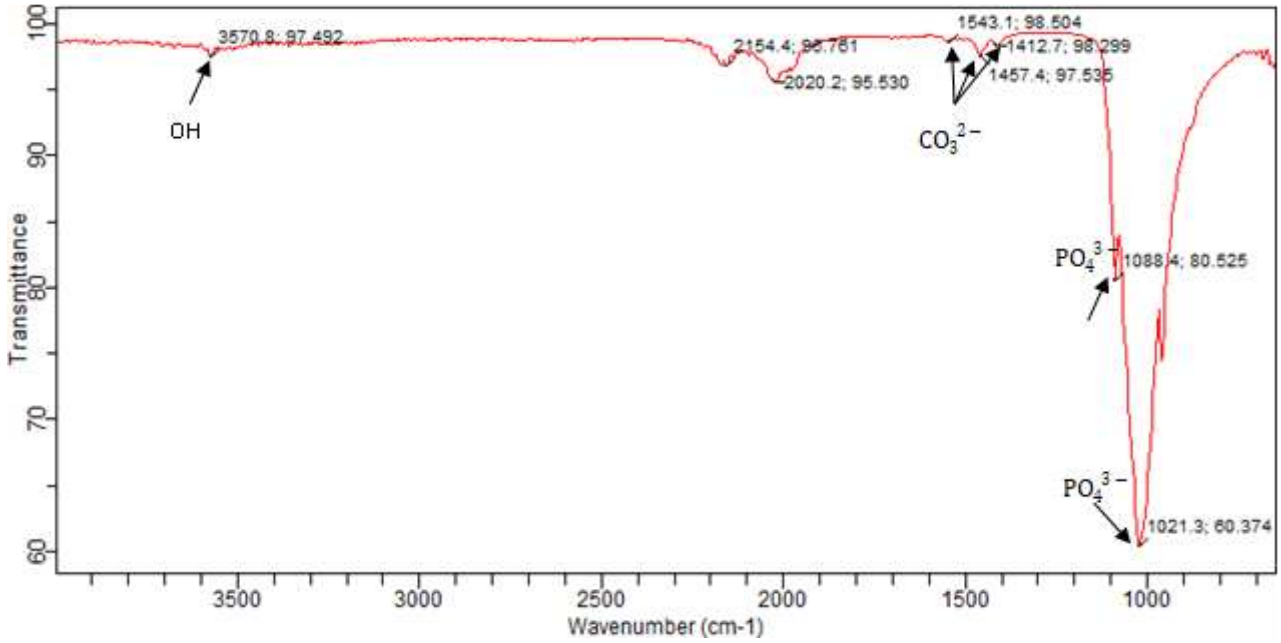

Fig. 10 FTIR spectra for synthesized HAp from Nanny femur bone powder.

4. CONCLUSION

In conclusion, hydroxyapatite was successfully synthesized from Billy and Nanny femur bones through calcination process at 1000°C. The result showed that the colour of the HAp changes as heat is applied from butter cream colour (raw grounded powder) to cream colour (200°C), black (400°C), light grey (600°C), milky white (800°C) and pure white (1000°C). The change in colour is attributed to the removal of organic materials and the crystallization of pure. Characterization of the HAps using SEM showed a rough, dense aggregated morphology of the HAp for both bones at 2000 magnification with no clear differences in surface morphology. The XRD diffractogram showed peaks at 2θ indicating that the synthesized HAps have hexagonal structures for both bones powder respectively. Also, FTIR spectra showed that the HAp synthesized from both bones had similar functional groups containing phosphate, hydroxyl and carbonate groups.

5. REFERENCES

[1] Wei, G. Ma, P.X. (2004). Structure and properties of nano-hydroxyapatite/polymer composite scaffolds for bone tissue engineering, Biomaters., vol. 25, (19), 4749

[2] Shi, H. Zhou, Z. Li, W. Fan, Y. Li, Z,. Wei, J. (2021). Hydroxyapatite based materials for bone tissue engineering: a brief and comprehensive introduction. Crystal, vol. 11, 149,

[3] Malla, K.P. Regmi, S. Nepal, A. Bhattarai, S. Yadav, R.J. Sakurai, S. Adhikari, R. (2020). Extraction and characterization of novel natural hydroxyapatite bioceramic by thermal decomposition of waste ostrich bone. Inter. J. Biomater., pp. 1-10.

[4] Vahdat, A. (2019). Removing Lead and Cobalt Ions from Aqueous Solution by Hydroxyapatite Adsorbent. Nanomed and Nanotechnol. Open Access. 4. 10.23880/nnoa-16000164.

[5] Salah, E. Marrane, K. Dänoun, D. Allouss, S. S, Badr-Eddine, C, Abdallah, R, and Mohamed, Z. (2022). A Novel Approach to Prepare Cellulose-g-Hydroxyapatite Originated from Natural Sources as an Efficient Adsorbent for Heavy Metals: Batch Adsorption Optimization via Response Surface Methodology, ACS Omega, vol. 7 (32), pp. 28076-28092.

[6] Nayak, A. Bhushan, B. (2021). Hydroxyapatite as an advanced adsorbent for removal of heavy metal ions from water: Focus on its applications and limitations. Materials Today: Proceedings. 46. 10.1016/j.matpr.

[7] Abideen, I.A. Edwin, A.O, Mopelola, A.I. Sarafadeen, O. K. Shappur, V. and Babu, B. R. (2018). Biowaste-Derived Hydroxyapatite for Effective Removal of Reactive Yellow 4 Dye: Equilibrium, Kinetic, and Thermodynamic Studies, ACS Omega, vol. 3 (2), pp. 1991-2000, DOI: 10.1021/acsomega.7b01768

[8] Vijayaraghavan, P. Rathi, M.A. Khalid S. A. Fatima, S. A. Yahya, B. E. (2022). Preparation and antibacterial application of hydroxyapatite doped Silver nanoparticles derived from chicken bone, J. of King Saud University – Sci., vol. 34, (2), 101749

[9] Ma, G. (2019). Three common preparation methods of hydroxyapatite. IOP Conference Series: Mater. Sci. Engr. Vol. 688, 033057.

[10] Odusote, J.K, Danyuo, Y., Baruwa, A.D., Azeez, A.A. (2019). Synthesis and characterization of hydroxyapatite from bovine bone for production of dental implants. J. Appl. Biomater. Funct. Mater. vol. 17, (2).

[11] Akpan, E.S., Dauda, M., Kuburi, L.S., Obada, D.O., Dodoo-Arhin, A. (2020). A comparative study of the mechanical integrity of natural hydroxyapatite scaffolds prepared from two biogenic sources using a low compaction pressure method, Results in Phy., vol. 17, 103051

[12] Saleh, O. (2018). Preparation of hydroxyapatite from natural resources literature review. Glo. Sci. J., vol. 6 (10), pp. 46-52.

[13] Challoob, D. A., and Taha, A. T. S. (2023). Characterization and In Vitro bioactivity of hydroxyapatite extracted from chicken bones for biomedical applications. Iranian J. Ichthyology, vol. 10 (1), pp. 125–131.