JMME, Vol.14, April, 2024
Pp 29 – 39

ISSN: 2006 - 1919

EFFECT OF HOLDING TIME ON EXTRACTION OF NATURAL HYDROXYAPATITE FROM GOAT BONE WASTE FOR BIOMEDICAL APPLICATIONS

[1, 2]Obayi C.S.*, [1]Elekwa C.B., and [1]Ugwu O.E.

chukwuemeka.elekwa.237949@unn.edu.ng, obinnamefuna@gmail.com, ,camillus.obayi@unn.edu.ng

[1]Department of Metallurgical and Materials Engineering, University of Nigeria, Nsukka, Nigeria.
[2]Department of Biomedical Engineering, University of Nigeria, Nsukka, Nigeria
**Corresponding Author: Dr. Camillus S. Obayi, e-mail: camillus.obayia@unn.edu.ng; Phone: +2348037506514*

ABSTRACT

Hydroxyapatite (HAP) is a very essential biomaterial used in orthopedics and dentistry to support bone healing processes as it shares chemical similarities with natural human and animal bones. Extraction of hydroxyapatite has been done both synthetically and naturally from animal bones. However, extraction of HAP from natural sources is challenged by the appropriate choice of processing parameters such as holding time and temperature that will enable the production of hydroxyapatite that meets the standard. This work investigated the influence of varying holding time on the physico-chemical properties of HAP obtained from goat bone (GB) waste by heat treatment. GB femur was obtained; washed; dried; pulverized and sieved to obtain GB powder of ≤ 500 µm that was calcined at 900°C and 1000°C and soaked for 1hr., 2 hrs. and 3 hrs. The composition, structure and morphology of the uncalcined and calcined powders were studied using Fourier Transform Infra-Red Spectroscopy (FTIR), X-ray diffractometry (XRD) and scanning electron microscopy (SEM), respectively. The FITR result confirmed presence of associated functional groups consisting of phosphate ions (PO_4^{3-}), hydroxyl ions (OH^-) and carbonate (CO_3^{2-}) ions in the synthesized HAP, but the phosphate ions (PO_4^{3-}) were most prominent. XRD analysis revealed similar crystallographic peaks of pure HAP. Increase in holding time led to decrease in crystallinity. SEM analysis revealed changes in the morphology of the samples with increase in holding time and temperature due to decomposition of organic compounds in calcined GB. The surface morphologies became finer with increase in holding time. Thus, holding time is an important parameter in extraction of HAP from goat bone waste.

Keywords: *Extraction, holding time, hydroxyapatite, goat bone, biomedical applications*

1.0 Introduction

Hydroxyapatite (HAP) is the most widely used calcium phosphate in biomedical applications due to its excellent biocompatibility and similarity in composition and structure to human bone and teeth [1-3]. Besides sharing similar composition to human bones, HAP has been found to be very bioactive and stable in supporting bone and teeth healing processes [4-8]. It is also used extensively for drug delivery and coating of implants [9, 10].

The importance of HAP in biomedical applications has led toits production, both synthetically and naturally from animal bones. Extraction of hydroxyapatite from

natural sources is preferred because natural HAP has essential trace elements found in the bones such as Sodium, Magnesium, Aluminium, Iron and minerals like carbonates that are beneficial for bone growth and supporting biological functions [1, 11, 12]. More so, extraction of HAP from natural sources is less expensive as staring materials are readily available.

Thus, the increased interest in synthesizing HAP from natural waste or biological sources that possess not only inherent inorganic minerals and important trace elements, but is environmentally friendly and economical. A good number of researchers has worked and are still working on synthesizing HAP from natural sources. The natural sources that are being investigated include bovine bones [13-17], chicken (galline) bones [10], fish bones [18,19], porcine bones [12,20], eggshells [21,22] and seashells [23,24]. Several methods have been used in preparing HAP from the above-mentioned animal sources. One popular technique used in obtaining HAP from natural sources is calcination and sintering [7, 10, 14, 25, 26]. Since calcination involves heating natural bone powders to high temperatures for certain time durations, calcination temperature and holding time are important parameters on evolution of HAP from natural bone powders. These parameters impact properties of HAP consequently [10].

Some authors who specifically evaluated goat (caprine) bones include [1, 10, 27-30], Ismail SA and Abdullah HZ [1], studied the evolution of HAP by subjecting goat bone samples to calcination process at 900℃, 1000℃, and 1100℃ at a constant holding time of 3 hrs. Ramesh et al [27] studied HAP evolution from cow, goat and chicken (galline) bones through calcination at 600-1000°C at a fixed holding time of 2 hrs. and compared the characteristics of the evolved HAP. Akhlis Rahman Sari Nurhidayat, et al [10] reviewed the effect of calcination temperature and holding time to isolate the effects of temperature and holding time on hydroxyapatite fabricated from the natural sources. They suggested that since the effect of temperature and holding time determines the yield of Ca/P ratio, there was the need to choose the appropriate temperature and holding time which will enable the production of hydroxyapatite with Ca/P ratio that meets the standard. The section of this review that considered goat bone focused on bone calcined in the range of 900-1300°C for a fixed holding time of 2 hrs. Barua *et al*[28] studied the extraction and physico-chemical properties of bioactive hydroxyapatite (HA) from goat bone waste by calcination at 700-1300°C with holding time of 2 hrs. They compared the change in properties of the calcined bone specimens with the raw bones. Bui Xuan Vuong [29] studied the extraction and characteristics of hydroxyapatite (HA) material from goat bone heat-treated at 750°C for 6 hours. Lin Linet al [30], calcined goat bone powder at four different temperatures of 800°C, 900°C, 1000°C and 1100°C in a muffle furnace for 3 hrs. The results indicated that percentage crystallinity and crystal size increased with increase in temperature; only a single phase of HAP was identified in XRD pattern at each treatment temperature, which eliminated organic compounds and collagen.

Considering that the above workers investigated the evolution of HAP from goat bone at varying calcination temperatures at constant holding times, this work focused on effects of variation of holding time at two constant calcination temperatures on the evolution of HAP from caprine bone. The results of this work will provide the data for optimization of these two parameters that have determining effects on the evolution of HAP by calcination process. Moreso, waste bones of goat are highly available in Nigeria. The worldwide bone waste generation stands at 130 billion kilograms per annum [31]. Nigeria produces more than 8% of this 130 billion kg of animal bone residue produced by slaughterhouses globally every year [32,

33, 34]. Also in Nigeria, out of an estimated total livestock production which stands at 52.4 x 10^6, goats constitute about 26.5 x 10^6 [35].

2.0 Materials and Methods

2.1a Materials

The materials used for the preparation of hydroxyapatite are goat bone femur, which was sourced from an abattoir at Ogige market, Nsukka, Nigeria; distilled water for washing and cleaning the bones and acetone for de-fatting the cleaned bones.

2.1b Equipment

The equipment used were pestle and mortar or crushing the goat bone into small pieces; grinding machine for pulverizing the bones into smaller particles; a sieve for separating the particles into particle sizes $\leq 500\ \mu m$ and a muffle furnace for heat treatment of the bone powder. Other equipment was Fourier-transform infrared spectrometer (FTIR) for determining the functional groups, scanning electron microscope (SEM) for viewing the morphology and X-ray diffractometer for ascertaining the structure of the uncalcinedand calcined goat bone powders, respectively.

2.2 Methods

2.2.1 Preparation of goat bone powder and physico-chemical characterization

The sourced goat bones were boiled in water for 1 hr. to enable easy defatting and removal of impurities. This was followed by washing and rinsing of the bones several times in water in order to remove meat, tendons, bone marrows and soft tissues. The washed goat bones were soaked in acetone for 2 hrs.; and washed again with distilled water many times to remove invisible fat. The bones were then sun dried for 4 weeks to evaporate adsorbed moisture and further dried in an oven at 105°C for 1 hour to remove absorbed water. The dried bones were ground using a milling machine and sieved to obtain particles sizes $\leq$ 500 μm.

One hundred and fifty grams each of the sieved goat bone powder was measured out for calcination. In order to reduce oxidation, the measured powder was placed separately in a small ceramic container with a lid sealed with clay material. The sealed ceramic containers were placed in a muffle furnace and heated at the rate of 10°C per min to 900^{0}C, 1000^{0}C, and held at three different holding times of 1hr, 2hrs and 3 hrs. for each sample. The calcined samples were then cooled slowly to room temperature, followed by evaluation of the physico-chemical properties.

The functional groups in the uncalcined and calcined goat bone powder samples were determined using Fourier-transform infrared spectroscopy (FTIR) with Cary 630 FTIR Spectrometer (Agilent Technologies, Inc.). The FTIR spectra were obtained using this spectrometer in the attenuated total reflection (ATR) mode. The spectra were registered in the 4000–650 cm^{-1} region using 64 scans and a spectral resolution of 16 cm^{-1}. The morphology of the uncalcined and calcined goat bone powder was viewed with a scanning electron microscope (SEM). SEM observations were carried out in a JSM 5900 Scanning Electron Microscope (JEOL, Japan) operating at 15 kV. The structure/phases of both the uncalcined and calcined goat bone powder samples were ascertained using a Siemens D5000 X-ray diffractometer, operated using Cu-Ka radiation (k = 0.15406 nm) at an accelerating voltage of 40 kV and a current of 30 mA. The scan rate was 0.05°s^{-1} in the range of 20–120° at a step size of 0.02°. The degree of crystallinity ((X_c)) was calculated using equation (1) used by Rana et al[36] in their work.

$$X_c(\%) = 1 - \frac{I_{112/300}}{I_{300}} \quad (1)$$

where;

$I_{112/300}$ - intensity of the valley (hollow part) between peaks (112) and (300);

I_{300} −intensity of the peak (300).

3.0 Results and Discussion

3.1 FTIR spectra of goat bone samples

Fourier Transform Infra-Red Spectroscopy (FTIR)was performed to identify the functional groups present in the as-sieved GB sample and GB powders calcined at 900°C and 1000°C using varying holding times of 1 hr., 2 hrs., and 3 hrs., to enable comparison with standard HAP. Figures 1Aand1B show the spectra absorption ranges (4000-650 cm^{-1}) of the calcined goat bone powders compared to the uncalcined one. The most prominent functional group in the calcined goat bone as can be seen in Figure 1A and 1B is the phosphate group ($PO4^{3-}$). This had also been observed by [37, 38] and this could be attributed to the elimination of the cross-linked structure in the raw goat bone due to the calcination process at high temperatures [4]. The phosphate absorption band are observed at wavenumbers 1416.4 cm^{-1}, 1013.6 cm^{-1} and 872.2 cm^{-1}. Phosphate is very essential for the formation of bone and teeth because the formation of bone and teeth requires phosphate to calcify the osteoid produced by osteoblasts [39].

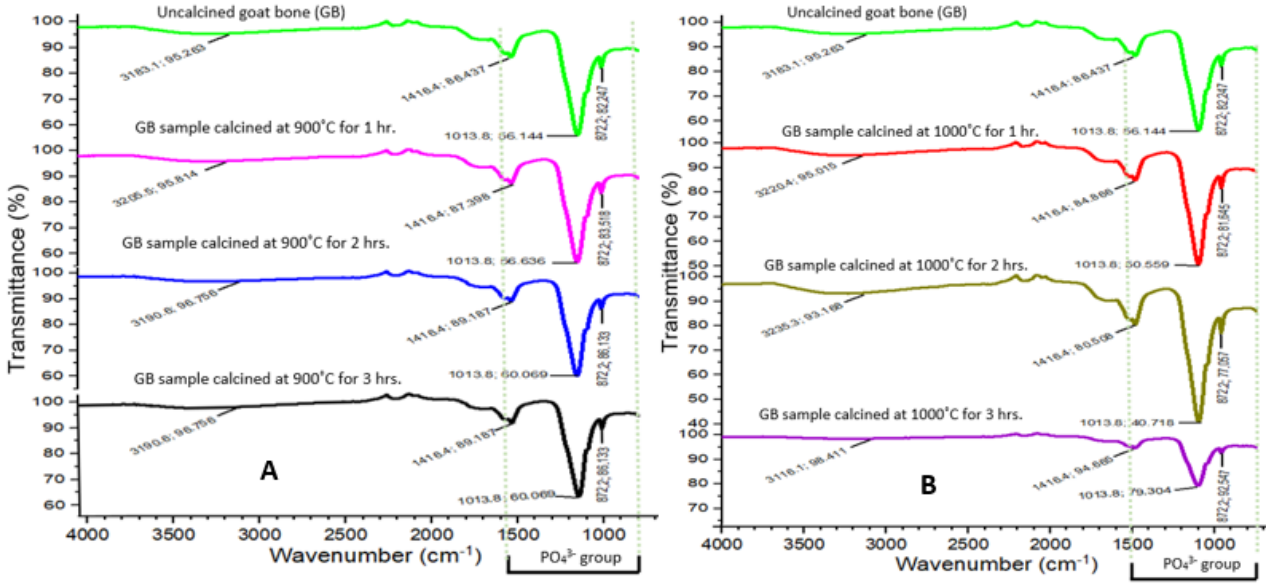

Figure 1A. FTIR spectra of the raw goat bone and goat bone samples calcined at 900°C for holding times of 1-3 hours. Figure 1B. FTIR spectra of the raw goat bone and goat bone samples calcined at 1000°C for holding times of 1-3 hours.

In addition, spectral bands of $CO3^{2-}$ and OH^- were also observed, which could be explained by CO_2 and water vapour absorption during sample storage since the samples were calcined at higher temperatures and it was expected all organic matter must have been expelled at these temperatures. The intensity of the band formation of the goat bone powder calcined at 1000°C was higher than those calcined at 900°C for the same time duration and increased with increase in holding time. The sample calcined at 1000C for 3 hrs. had the highest intensity of the phosphate group.

3.2 Crystallographic analysis of uncalcined and calcined goat bone samples

The X-ray diffractograms of the uncalcined and calcined goat bone powders at 900°C for 1, 2, 3 hrs. and at 1000°C for 1, 2, 3 hrs. in comparison with standard HAP diffractogram is shown in Figures 2a and 2b, respectively. The results show that the diffractograms of the calcined goat bone at different temperatures and similar holding times are in good agreement with the characteristic peaks of pure hydroxyapatite (JCPDS no. 09-0432). There were no new peaks formed and calcined samples have similar crystallographic planes obtainable in pure Hydroxyapatite. The identified characteristic crystallographic planes corresponding to pure HAP from Figures 2a and 2b include (002), (210), (211), (112), (310), (222), (213) and (004), but the most intense is the (211) plane.

It could be observed in Figures 2a and 2b that the uncalcined sample displayed an amorphous phase with a low intensity and a broader peak. This could be attributed to the significant presence of organic matter such as collagen in the uncalcined bone powder. However, the amorphous phase disappeared at the calcination temperatures of 900°C and 1000°C, indicating a complete removal of organic matter present in the bone matrix at these temperatures. This conforms with the works of Blanco et al. [40] and Ojo et al. [41], which indicated that animal bone calcined at high temperatures (≥ 700°C) displays intense and sharp peaks, proving crystallinity and complete removal of organic matter.

It has been established that the degree of crystallinity increases with increase in temperature [38]. The goat bone samples calcined at 1000°C were more crystalline than those calcined at 900°C. However, the degree of crystallinity decreased with increase in the holding time as can be seen in Table 1.

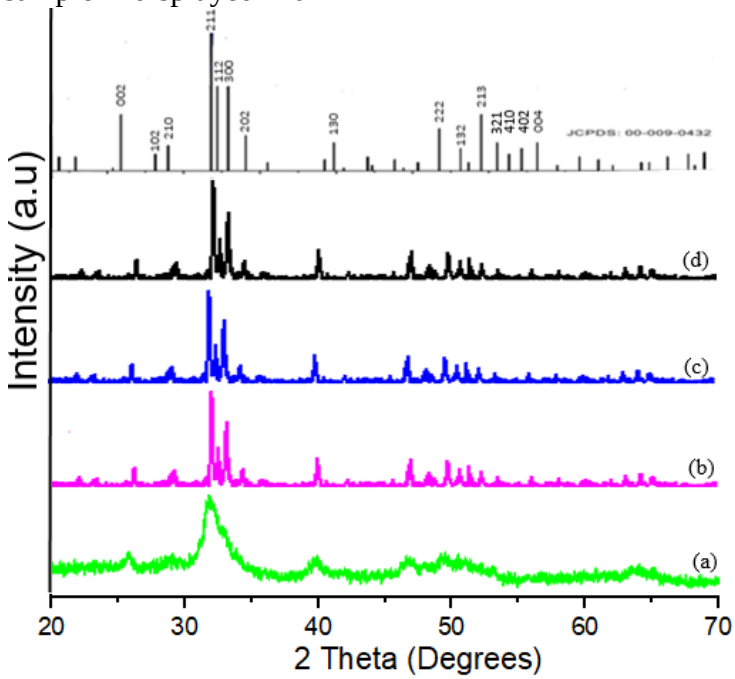

Figure 2a. *Diffractograms of the GB samples compared with standard Hydroxyapatite (JCPDS: 00-009-0432): (a) = uncalcined GB sample; (b) = GB calcined at 900°C for 1 hr.; (c) = GB calcined at 900°C for 2 hrs.; (d) = GB calcined at 900°C for 3 hrs.*

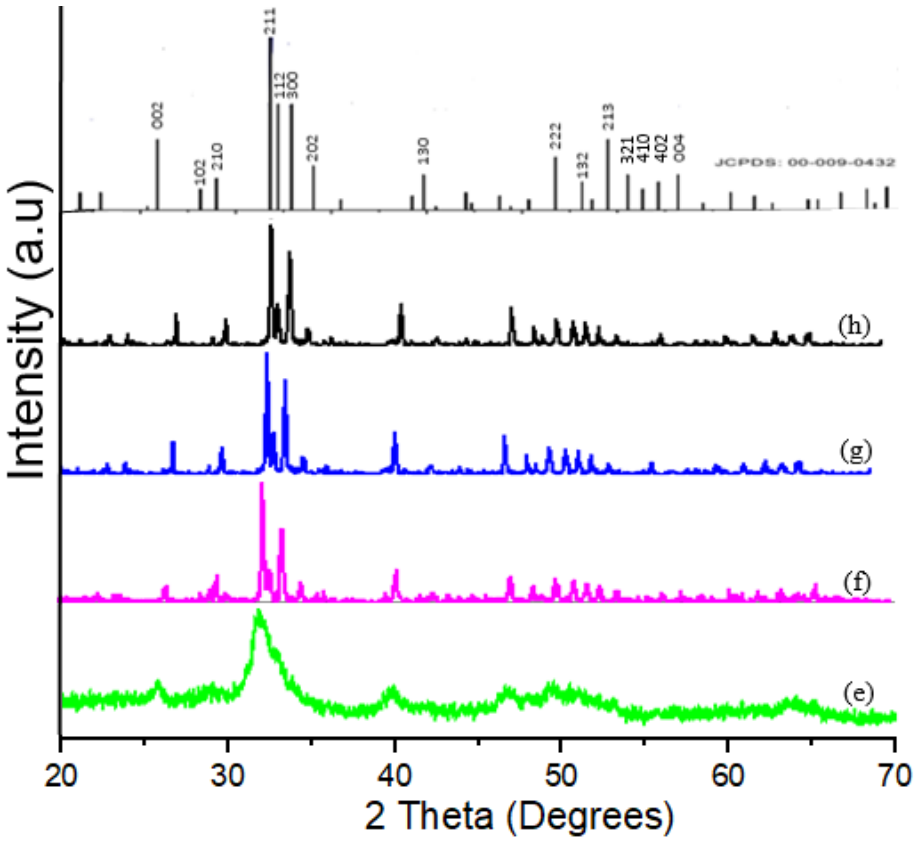

Figure 2b. *Diffractograms of the GB samples compared with standard Hydroxyapatite (JCPDS: 00-009-0432): (e) = uncalcined GB sample; (f) = GB calcined at 1000°C for 1 hr.; (g) = GB calcined at 1000°C for 2 hrs.; (h) = GB calcined at 1000°C for 3 hrs.*

Table 1. Degree of crystallinity of GB samples calcined at 900°C and 1000°C at various sintering duration

Temperature/holding time	Degree of crystallinity (%)
900°C/1 hr.	88.6
900°C/2 hrs.	85.9
900°C/3 hrs.	78.2
1000°C/1 hr.	90.6
1000°C/2 hrs.	85.5
1000°C/3 hrs.	82.3
Control/uncalcined CB	diffuse

3.3 SEM analysis of the goat bone samples

The surface morphologies of the uncalcined and calcined GB powder samples obtained with a scanning electron microscope (SEM) are shown in Figures 3 (a) and Figure(b). The surface morphologies varied with an increase in both holding time and temperature and became finer with increase in holding time.The crystallite size, though, not measured here, appeared to decrease with increase holding time at each calcination temperature as can be seen in the SEM micrographsof the calcined samples, suggesting grain refinement with increase in holding time.

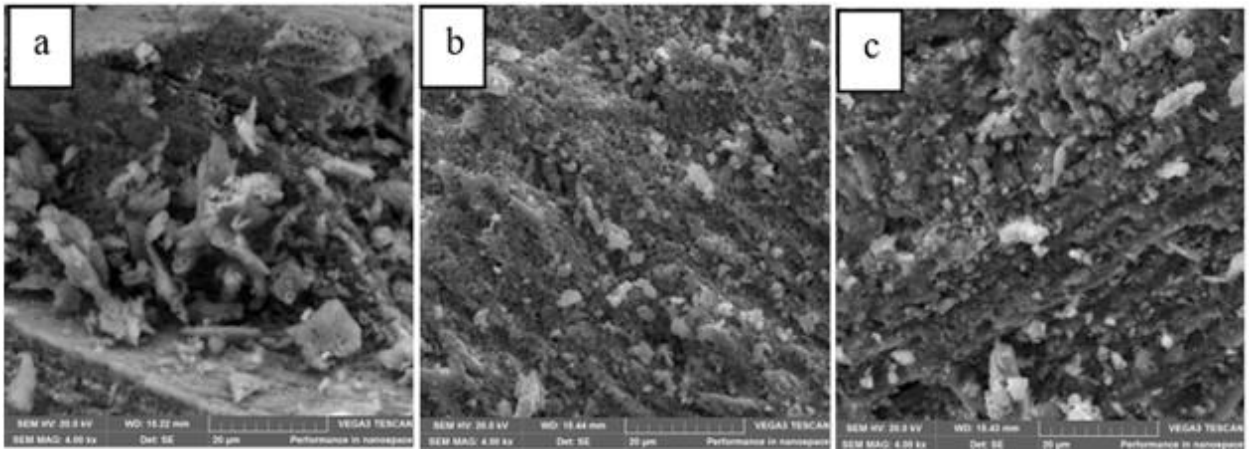

Figure 3a. Morphology of calcined goat bone powders:(a) = GB calcined at 900°C for 1 hr.; (b) = GB calcined at 900°C for 2 hrs.; (c) = GB calcined at 900°C for 3 hrs.

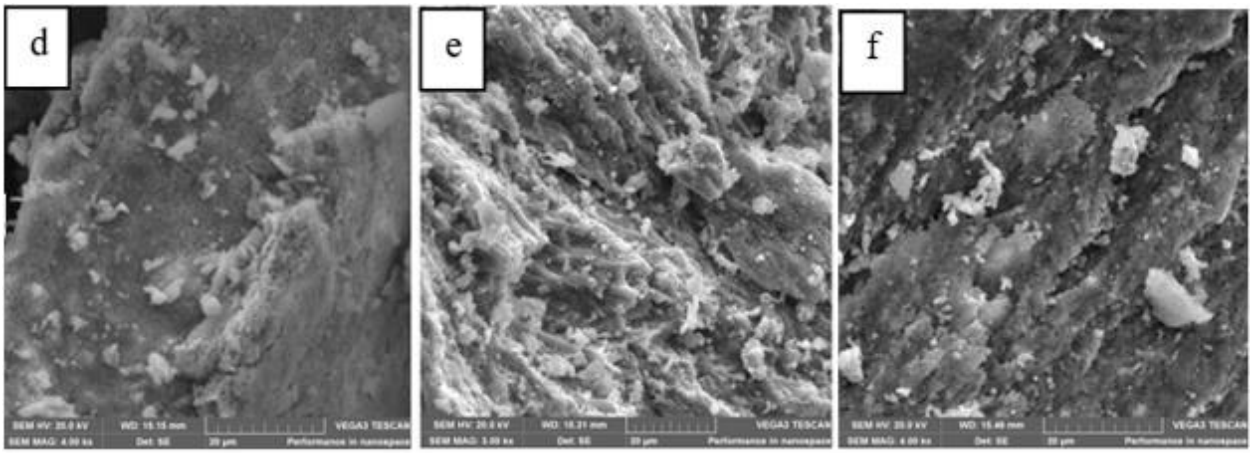

Figure 3b. Morphology of calcined goat bone powders (d) = GB calcined at 1000°C for 1 hr.; (e) = GB calcined at 1000°C for 2 hrs.; (f) = GB calcined at 1000°C for 3 hrs.

4.0 Conclusions

This work investigated the influence of varying holding time on the physico-chemical properties of HAP obtained from goat bone (GB) waste by heat treatment. In light of the results obtained, the following conclusions could be drawn:

1. The FTIR spectra indicated the presence of characteristic vibrational bands of hydroxyl (OH^-), phosphate (PO_4^{3-}), and carbonate (CO_3^{2-}) groups associated with hydroxyapatite. However, the most prominent functional group was phosphate (PO_4^{3-}).
2. The XRD results revealed diffraction patterns that were characteristic peaks of hydroxyapatite.
3. The degree of crystallinity of the hydroxyapatite decreased with an increase in holding time.
4. Based on the SEM results, the surface morphologies varied with an increase in both holding time and temperature and became finer with increase in holding time, suggesting grain refinement with increase in sintering duration.

5. This work has established that in addition to temperature, holding time is an important parameter in extraction of HAP from goat bone waste. Holding time affects the evolution of functional groups, crystallinity, morphology and grain size, which are major property requirements of hydroxyapatite extracted from animal bones for biomedical applications. However, further work is required to optimize the effects of both temperature and holding time to yield the best of combination of these properties for medical applications.

References

[1] S.A. Ismail, H.Z. Abdullah (2020), "Extraction and characterization of natural hydroxyapatite from goat bone for biomedical applications", Materials Science Forum,1010,573-578 doi: 10.4028/www.scientific.net/MSF.1010.573.

[2] E. Fiume, G. Magnaterra, A. Rahdar, E. Verné, F. Baino (2021), "Hydroxyapatite for biomedical applications: A short overview, Ceramics, 4, 542-563. https://doi.org/10.3390/ceramics4040039.

[3] A. Szcześ, L. Hołysz, E. Chibowski (2017),"Synthesis of hydroxyapatite for biomedical applications", Adv. Colloid Interface Sci., 249, 321-330. doi: 10.1016/j.cis.2017.04.007.

[4] W. Khoo, F. M. Nor, H. Ardhyananta, D. Kurniawan (2015),"Preparation of natural hydroxyapatite from bovine femur bones using calcination at various temperatures". Procedia Manufacturing 2, 196-201.

[5] S. Kanhed, S. Awasthi, S. Goel, A. Pandey, R. Sharma, A. Upadhyaya, Kantesh Balani (2017),"Porosity distribution affecting mechanical and biological behaviour of hydroxyapatite bio ceramic composites", Ceramics International, 43 (13), 10442-10449.

[6] C. Feng, K. Zhang, R. He, D. Ding, M. Xia, X. Jin, C. Xie (2020),"Additive manufacturing of hydroxyapatite bioceramic scaffolds: dispersion, digital light processing, sintering, mechanical properties and biocompatibility". Journal of Advanced Ceramics, 9, 360-373.

[7] C. Piccirillo, R. C. Pullar, E. Costa, A. Santos-Silva, M. M. E. Pintado, P. M. L. Castro (2015), "Hydroxyapatite-based materials of marine origin: A bioactivity and sintering study", Mater. Sci. Eng. C., 51, 309-315.

[8] J. Jeong, J.H. Kim, J.H. Shim, N.S. Hwang, C.Y. Heo (2019), "Bioactive calcium phosphate materials and applications in bone regeneration". Biomatter Res., 23, 4.https://doi.org/10.1186/s40824-018-0149-3

[9] S. Mazumder, A. K. Nayak, T. J. Ara, M. S. Hasnain (2018),"Hydroxyapatite composites for dentistry". Elsevier Inc., 2018.Sarmistha Mazumder, Amit Kumar Nayak, Tahseen Jahan Ara, M. Saquib Hasnain,7-Hydroxyapatite composites for dentistry, Ed(s): Abdullah M. Asiri, Inamuddin, Ali Mohammad, In Woodhead Publishing Series in Biomaterials, Applications of Nanocomposite Materials in Dentistry, Woodhead Publishing,2019, PP. 123-143,

[10] Akhlis Rahman Sari Nurhidayat, A.P. Bayuseno, Rifky Ismail, Rilo Berdin Taqriban(2021),"Review of the temperature and holding time effects on hydroxyapatite fabrication from the natural sources", Journal of Biomedical Science and Bioengineering. 1 (1), 27-31

[11] A. F. A. Yazdi, A. Yazdani, T. T. Khozani, M. Kalantar (2013),"Extraction and viability checking of various carbonated hydroxyapatite by Wharton's jelly mesenchymal stem cell", Sci. Int., 1(5), 132-138. DOI: 10.5567/sciintl.2013.132.138.

[12] M. Akram, R. Ahmed, I. Shakir, W. A. W. Ibrahim, R. Hussain (2014),"Extracting hydroxyapatite and its precursors from natural resources", J. Mater. Sci., 49, 1461-1475.

[13] A. Niakan, S. Ramesh, S.V. Naveen, S. Mohan, T. Kamarul (2017), "Osteogenic priming potential of bovine hydroxyapatite sintered at different temperatures for tissue engineering applications", Mater. Letts., 197, 83-86.

[14] M. Figueiredo, A. Fernando, G. Martins, J. Freitas, F. Judas, H. Figueiredo (2010), "Effect of the calcination temperature on the composition and microstructure of hydroxyapatite derived from human and animal bone", Ceram. Int., 36, 2383-2393.

[15] A. Niakan, S. Ramesh, P. Ganesan, C.Y. Tan, J. Purbolaksono, H. Chandran, S. Ramesh, W.D. Teng (2015),"Sintering behaviour of natural porous hydroxyapatite derived from bovine bone", Ceram. Int., 41, 3024-3029.

[16] W. Khoo1, F. M. Nor, H. Ardhyananta, D. Kurniawan (2015), "Preparation of natural hydroxyapatite from bovine femur bones using calcination at various temperatures", Procedia Manufacturing, 2, 196-201.

[17] J.K. Odusote, Y. Danyuo, A.D. Baruwa, A.A. Azeez (2019), "Synthesis and characterization of hydroxyapatite from bovine bone for production of dental implants" Journal of Applied Biomaterials & Functional Materials, 17(2), 1-7. doi:10.1177/2280800019836829.

[18] T. Goto, K. Sasaki (2014),"Effects of trace elements in fish bones on crystal characteristics of hydroxyapatite obtained by calcination", Ceram. Int., 7, 10777-10785.

[19] Anindya Pal, Sudeep Paul, Amit Roy Choudhury, Vamsi Krishna Balla, Mitun Dass, Arijit Sinha (2017),"Synthesis of hydroxyapatite from lates calcarifer fish bone for biomedical applications", Mater. Letts. 203, 89-92.

[20] S. Pramanik, A. K. Agarwal, K. N. Rai, A. Garg (2007),"Development of high strength hydroxyapatite by solid-state-sintering process". Ceram. Int.,33 (3), 419–426.

[21] P. Kamalanathan, S. Ramesh, L.T. Bang, A. Niakan, C.Y. Tan, J. Purbolaksono, Hari Chandran, W.D. Teng (2014),"Synthesis and sintering of hydroxyapatite derived from eggshells as a calcium precursor", Ceram. Int., 40 (10), 16349-16359.

[22] S. Ramesh, A.N. Natasha, C.Y. Tan, L.T. Bang, S. Ramesh, C.Y. Ching, Hari Chandran (2016), "Direct conversion of eggshell to hydroxyapatite ceramic by a sintering method", Ceram. Int., 42, 7824-7829.

[23] Shih-Ching Wu, Hsueh-Chuan Hsu, Shih-Kuang Hsu, Chien-Pei Tseng, Wen-Fu Ho (2017),"Preparation and characterization of hydroxyapatite synthesized from oyster shell powders", Adv. Powder Tech. 28,1154-1158.

[24] Emmanuel Jay M. Edralin, Jonyl L. Garcia, Francis M. dela Rosa, Eric R. Punzalan (2017), "Sonochemical synthesis, characterization and photocatalytic properties of hydroxyapatite nano-rods derived from mussel shells", Mater. Letts. 196, 33-36.

[25] P. V. Nam, N. Van Hoa, T. S. Trung (2019),"Properties of hydroxyapatites prepared from different fish bones: A comparative study", Ceram. Int., 45 (16), 20141-20147.

[26] N.A.S. Mohd Pu'ad, J. Alipal, H.Z. Abdullah et al. (2020),"Synthesis of eggshell derived hydroxyapatite via chemical precipitation and calcination method", Materials Today: Proceedings, https://doi.org/10.1016/j.matpr.2020.11.276

[27] S. Ramesh, Z.Z. Loo, C.Y. Tan, W.J. Kelvin Chew, Y.C. Ching, F.Tarlochan, Hari Chandran, S. Krishnasamy, L.T. Bang, A.D. Ahmed Sarhan (2018),"Characterization of biogenic hydroxyapatite derived from animal bones for biomedical applications", Ceramics International, 44 (9), 10525-10530.https://doi.org/10.1016/j.ceramint.2018.03.072.

[28] Emon Barua, Apurba Das, D. Pamu, Ashish B. Deoghare, Payel Deb, Sumit Das Lala, Sushovan Chatterjee (2019), "Effect of thermal treatment on the physico-chemical properties of bioactive hydroxyapatite derived from caprine bone bio-waste", Ceramics International, 45 (17), 23265-23277. https://doi.org/10.1016/j.ceramint.2019.08.023.

[29] Bui Xuan Vuong (2022), "Characterization of hydroxyapatite extracted from goat bone", Asian Journal of Applied Science and Technology (AJAST), 6 (2), 01-07.

[30] Lin Lin Naing, Ni Ni Sein, Thida Win (2018),"Effect of calcination temperature on morphological, structural and thermal properties of hydroxyapatite derived from goat bone". J. Myanmar Acad. Arts Sci. xvi (1a), 497-516.

[31] H. Fayaz, S. Alshahrani, M.M. Abbas, H.M. Khan, A. Jamil, H. Yaqoob, M.E.M. Soudagar, M. Imran, M. Ahmad, M. Munir(2021),"Waste animal bones as catalysts for biodiesel production: A mini review", Catalysts, 11, 630. https://doi.org/10.3390/catal11050630.

[32] R. Blench(1999), "The Nigerian national livestock resource survey: A personal account. In: Baroin, C. and Jean, B., Eds., Man and Animal in the Lake Chad Basin, Paris: IRD, Mega-Chad Network, Symposium, Orléans (FRA), 627-648.

[33] M. Clark, D. Tilman(2017), "Comparative analysis of environmental impacts of agricultural production systems, agricultural input efficiency, and food choice", Environmental Research Letters, 12, Article ID: 064016. https://doi.org/10.1088/1748-9326/aa6cd5.

[34] A.J. Oyejide,S.O. Adetola, A. Lawal (2022),"Machine design approach to bone waste utilization in slaughterhouses of developing countries with focus on Nigeria", World Journal of Engineering and Technology, 10, 444-457. https://doi.org/10.4236/wjet.2022.102026.

[35] G.O. Egwu; P.A. Onyeyili; G.A. Chibuzo; J.A. Ameh (1995), "Improved productivity of goats and utilization of goat milk in Nigeria", Small Ruminant Research,16(3), 195-201. doi:10.1016/0921-4488(95)00630-4.

[36] M. Rana, N. Akhtar, S. Rahman, H. Jamil, S.M. Asaduzzaman (2017), "Extraction of hydroxyapatite from bovine and human cortical bone by thermal decomposition and effect of gamma radiation: A comparative study", Int J of Compl. Alt. Med. 8 (3), 00263.

[37] Bui Xuan Vuong (2022), "Characterization of hydroxyapatite extracted from goat bone", Asian Journal of Applied Science and Technology, 6 (2), 1-7.

[38] L.J. Anita,K. Ravichandran, M. Sundareswari (2015),"The study on the synthetic methodologies for maneuvering the morphology crystallinity and particle size of hydroxyapatite" ,*J Chem Pharm Res.* 7(2): 231-239.

[39] E. Takeda, Y. Taketani, N. Sawada, T. Sato, H. Yamamoto. (2004), The regulation and function of phosphate in the human body", BioFactors, 21(1–4), 345–355.

[40] A. Blanco, M.C. Monte, C. Campano, A. Balea, N. Merayo, C. Negro(2018), Nanocellulose for industrial use: cellulose nanofibers (CNF), cellulose nanocrystals (CNC), and bacterial cellulose (BC). *In: C.M. Hussain,* Handbook of Nanomaterials for Industrial Applications Elsevier, 74–126.

[41] E.O. Olusola, I.S. Olatunde, M.O. Ilomuanya, P.G. Oluwashina,S.O. Adeosun (2022),"Structural and morphological evaluations of natural hydroxyapatite from calcined animal bones for biomedical applications", .*J Cast Mater Eng.* 6 (1): 14–2.