

Natural Hydroxyapatite Extracted From Bovine Bone

Nazia Bano¹, Suzi Salwah Jikan^{1*}, Hatijah Basri¹,
Sharifah Adzila S. Abu Bakar², Awwal Hussain Nuhu¹

¹Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia, Johor, Malaysia

²Faculty of Mechanical and Manufacturing Engineering, Universiti Tun Hussein Onn Malaysia, Johor, Malaysia

Received 30 September 2017; accepted 14 December 2017; available online 18 December 2017

Abstract: The aim of this study is to extract natural hydroxyapatite (HAP) from bovine bone. Hydrothermal method followed by calcination treatment at different temperatures is used in this current research. Bovine bone has potential for producing hydroxyapatite, a chief component present in bone and teeth of vertebrates. HAP is an excellent material used in bone restoration and tissue regeneration. Characterizations of the bovine bone natural HAP powder were done by scanning electron microscopy (SEM), Fourier transforms infrared (FTIR) and energy dispersive x-ray (EDX) spectroscopy. The FTIR result confirms the presence of phosphate (PO_4^{3-}), hydroxyl (OH^-) and carbonate (CO_3^{2-}) groups in the powder. SEM displays the surface morphology whereas EDX provides calcium (Ca) to phosphorous (P) ratio in the HAP samples. The results of the EDX analysis indicate that the Ca/P ratio of HAP varies from 1.67 to 1.83. The extracted HAP material is found to be thermally stable up to 1100°C.

Keyword: Hydroxyapatite, Bovine femur bone, Hydrothermal, Calcination

1. Introduction

HAP is one of the most attractive and well-known biomaterials for teeth and bone tissues implant. Its composition and biological structure resemble to natural bone. The chief constituent of bone mineral is hydroxyapatite (HAP) with a chemical formula of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ [1–4]. Natural HAP has all the distinctive features of biomaterials, such as bioactive, osteoconductive, non-inflammatory, biocompatible, non-toxic, and non-immunogenic assets. Moreover, the close resemblance of HAP with calcium phosphates components of human bone provokes researchers to use it for different biomedical applications [5, 6]. It is considered that HAP plays a dynamic role for numerous applications [7] such as restoration of skull defects [8], restoration of huge area bone defect [9], bone tissue engineering scaffolds [10–12], removal of heavy metals and drugs delivery [13]. In this study, bovine bones were used to extract natural HAP by hydrothermal followed by calcination at different temperatures. Through this extraction method, organic components in bones can be thermally decomposed and removed. Every single

genome sign of disease can be removed and thus provides high biological safety factors [14]. Extraction of natural HAP from bovine femur bones is done at different calcination temperature and different particle sizes [15–17]. The use of bovine bone to generate hydroxyapatite will decrease the pollution effect of the waste and the subsequent conversion of the waste into an extremely valuable product. The process of converting bovine bone into HAP is an environmentally friendly process.

2. Materials and Methods

The fresh cortical bone of mature bovine (2-3 years old) was used as raw material purchase from local market of Parit Raja Malaysia.

2.1 Bone samples preparation

Bones were cleaned with water and soft parts inside the bones were manually detached from compact part manually by stiletto. Then the bones were cut into small pieces using cutter. The bones were boiled with water for 2 to 3 hours. Subsequently, the fluids in bone

*Corresponding author: suzi@uthm.edu.my
2017 UTHM Publisher. All right reserved.
penerbit.uthm.edu.my/ojs/index.php/jst

marrow and residual soft material were removed with knife and washed with distilled water several times. The bone fragments were dried in an oven at 80°C for 72 hours (3-days) to denaturalize the protein. After that, dry fragments of bone were abridged to small particles by means of crusher. The crushed bone was subjected to ball milling process for 24 hours until the powder was fine enough. Bone powder was obtained by sieving. 25 μ m particle size of powdered bone sample was used in this study. Fig. 1 shows the flow chart of extraction of HAP from bovine bone.

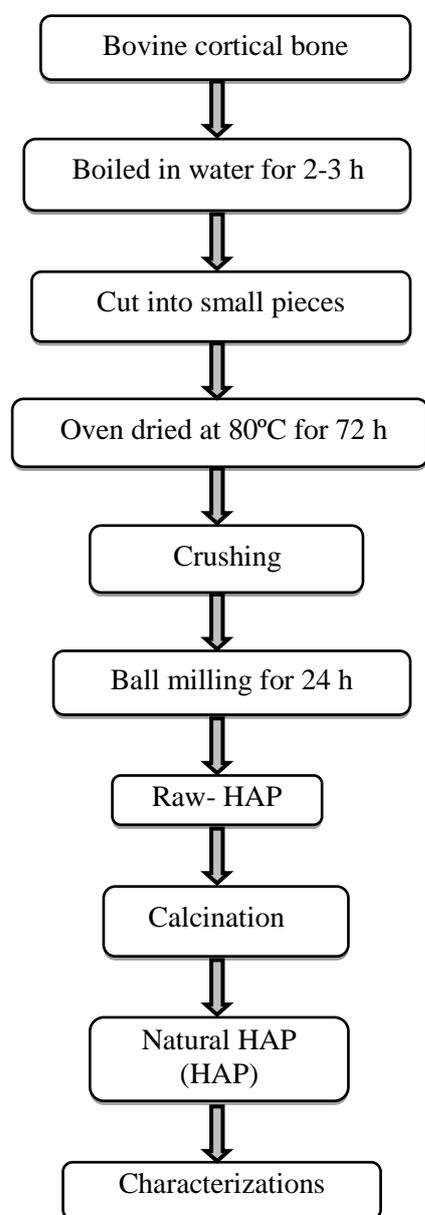


Fig. 1 Flow chart of extraction of HAP from bovine bone

2.2 Calcination of HAP powder

Pure natural-HAP (mineral phase of the bone) free from fat and protein was achieved by calcination. The powder samples of HAP were calcined in a furnace at 600°C, 700°C, 800°C, 900°C, 1000°C and 1100°C, which named as HAP-600, HAP-700, HAP-800, HAP-900, HAP-1000, HAP-1000 and HAP-1100 respectively with 3 hours holding time at a heating and cooling rate of 5 °C/min. Natural HAP was produced by decomposition of the organic phase. These temperatures were designated after a Thermo gravimetric analysis (TGA). The entire bone powder samples were cooled inside the furnace.

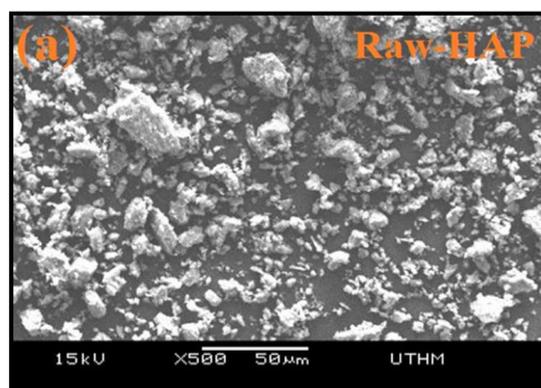
2.3 Characterizations of HAP powder

The characterizations of HAP before and after calcination were performed using SEM, FT-IR and EDX. FTIR was used to obtain information about the functional groups present in HAP powders in the range of 4000 cm^{-1} - 450 cm^{-1} . Morphology of HAP particles were obtained by SEM (JSM 6490LA, Jeol, Japan) and the element compositions of the powder sample was determined by EDX.

3. Results and Discussions

3.1 SEM analysis

Fig. 2 shows the SEM micrographs of the HAP powder before and after calcination at different temperature. The particles are agglomerated in some parts and have irregular shapes including small spheres and rods as reported in literatures [18–20]. It is due to the van der Waals attraction between HAP particles during calcination [21].



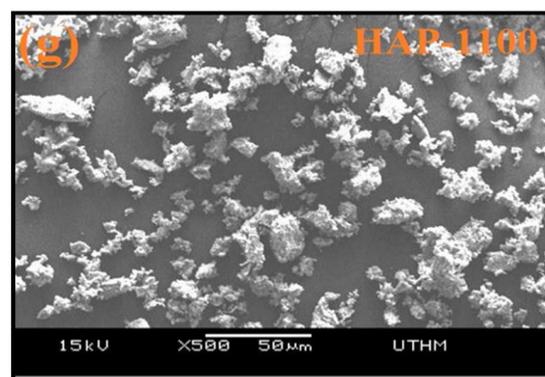
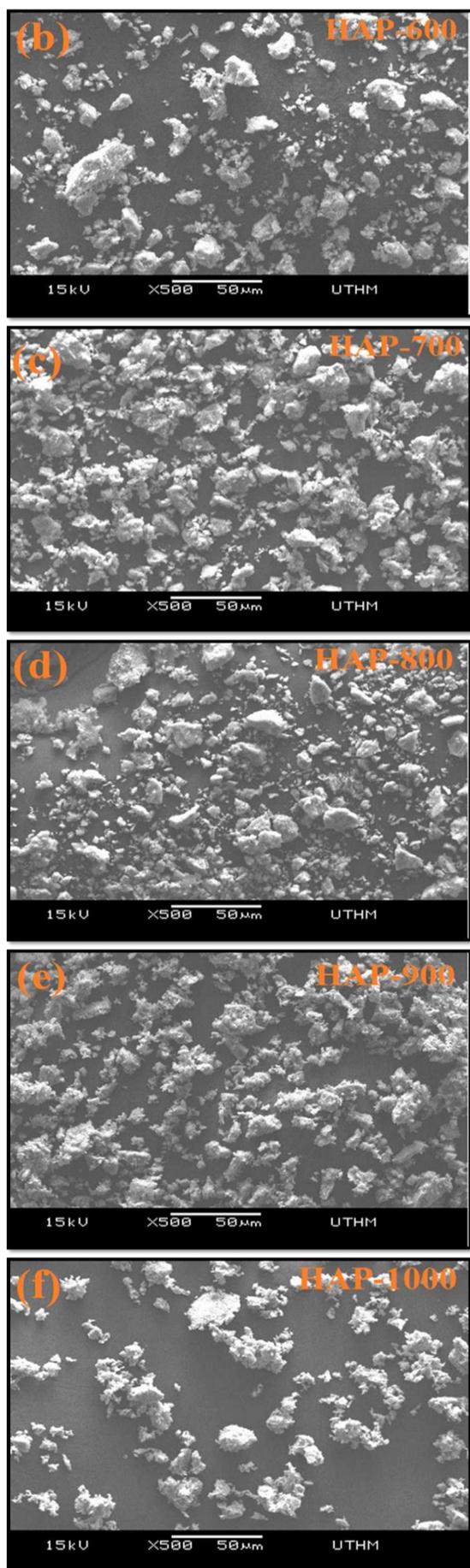


Fig. 2 SEM micrographs of uncalcined powder sample (raw-HAP) (a) and calcined samples at different temperature (b) HAP-600 (c), HAP-700, (d) HAP-800, (e) HAP-900, (f) HAP-1000 and (g) HAP-1100 respectively.

3.2 FTIR analysis

Fig. 3 reveals the FTIR spectra of uncalcined sample (raw-HAP) and calcined HAP at different temperatures. The FTIR spectra indicate the presence of phosphate (PO_4^{3-}), hydroxyl (OH^-) and carbonate (CO_3^{2-}) groups. These spectra are more clearly seen in calcined HAP samples because the calcination process has destroyed the cross linked structure in the raw HAP bovine bone powder. In the spectra of HAP, many bands matched with the HAP reference spectrum and are in agreement with reported data on HAP [22, 23].

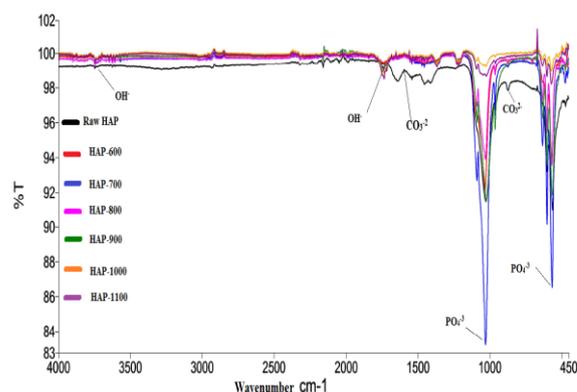


Fig. 3 FTIR spectra of extracted HAP powders of uncalcined sample (raw-HAP) and calcined samples at different temperature.

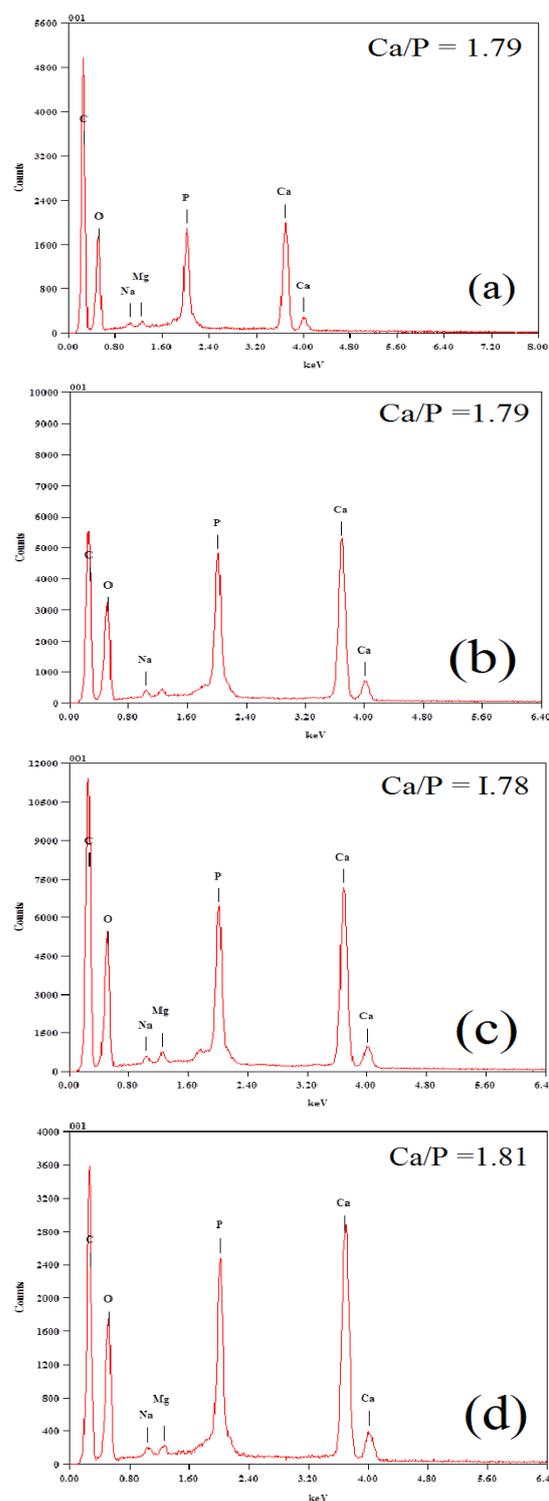
As shown in Fig. 3, the bands of raw HAP and calcined n-HAP are apparently different due to the alterations in their chemical bonds during calcination at different temperature. The color of raw HAP changes from yellowish white to

white after calcination. This color changes indicates the decomposition of organic substance which are collagen and proteins in calcined HAP bone powder [24, 25]. The FTIR band at 1637 cm^{-1} corresponds to Amide I of collagen [19] that is present in the raw HAP of bovine bone powder and is completely removed in calcined HAP samples. A strong and broad band due to symmetric stretching vibration that appears at 961.66 cm^{-1} , 962.99 cm^{-1} , and asymmetric stretching vibration bands at 1023 cm^{-1} and 1092 cm^{-1} in calcined HAP bone samples are associated with the phosphate group (PO_4^{3-}). The raw HAP bone powder exhibit a wide band near 3285.48 cm^{-1} which corresponds to the absorbed water molecule. Low intensity peaks of carbonate groups are identified in the FTIR spectra at 1460 cm^{-1} and 1520 cm^{-1} due to asymmetric stretching ν_3 of (CO_3^{2-}) groups. The sharp narrow band at 630.92 cm^{-1} , 634.47 cm^{-1} and wide band at 3525.94 cm^{-1} are associated with hydroxyl group that verify the presence of HAP phase. In addition, after calcination most of the bands have largely increased in intensity due to the phosphate vibrations of hydroxyapatite as described by other authors [25, 26].

3.3 EDX analysis

The chemical compositions of HAP powder before and after calcination at different temperatures were evaluated by using EDX analyzer. Fig. 4 shows the EDX analysis of HAP powders prepared by calcination at different temperatures. The Ca/P ratios of the extracted HAP samples were calculated and the ratios are 1.79, 1.79, 1.78, 1.81, 1.67, 1.75 and 1.83 for HAP-600, HAP-700, HAP-800, HAP-900, HAP-1000, HAP-1100 for calcined samples and uncalcined sample (raw-HAP) respectively. The ratios are equivalent with previously study [25]. As the Ca/P weight ratio of the extracted HAP calcined at different temperatures did not show any significant difference, it can be concluded that Ca/P weight ratio does not depends on the calcination temperature. The higher Ca/P value of bovine bone derived HAP powder indicates that its chemical composition is close to the

composition of human bone [27]. The higher Ca/P values may be due to the occurrence of either calcium and phosphorus compounds other than hydroxyapatite or Ca compounds deprived of phosphorus [28]. In fact, Figueiredo *et al* articulated that natural HAP is non-stoichiometric having Ca/P ratio greater than 1.67 [29].



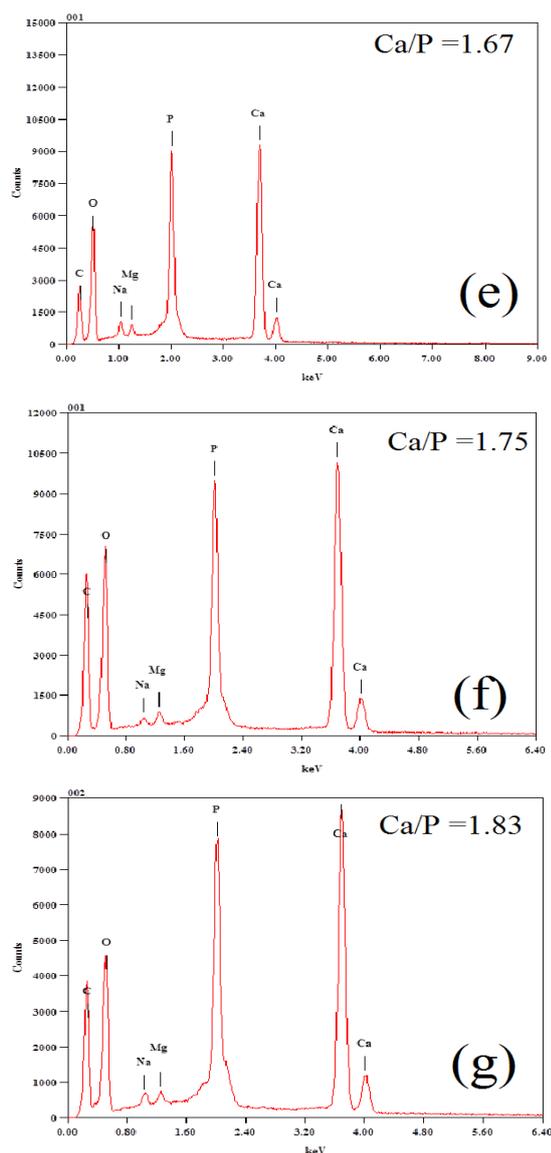


Fig. 4 EDX analysis of HAP powders prepared by calcination at different temperature (a) HAP-600, (b) HAP-700, (c) HAP-800 (d) HAP-900 (e) HAP-1000 (f) HAP-1100 and (g) uncalcined sample (raw-HAP) respectively.

5. Conclusion

The study confirms that HAP is able to be extracted from bovine bone wastes. So, bovine bone wastes have great potential to be used in extracting the HAP deprived of any impurities as indicated by FTIR analysis. The methods involved hydrothermal followed by calcination of bovine bone powder at different temperature to remove its organic components thus the produced HAP which is suitable for the use in orthopedic and dental applications.

Powder samples of HAP of bovine bone have close Ca/P molar ratio (1.67 - 1.83) to the stoichiometric HAP (1.67). The HAP extraction methodology is simple and low cost.

Acknowledgements

This study was partially supported by Center of Graduate Studies (CGS), Universiti Tun Hussein Onn Malaysia from the UTHM scholarship (UTHM/PS/500-14/8).

References

- [1] C. V. M. Rodrigues *et al.*, (2003) "Characterization of a bovine collagen-hydroxyapatite composite scaffold for bone tissue engineering," *Biomaterials*, vol. 24, no. 27, pp. 4987–4997.
- [2] H. Eslami, M. Solati-Hashjin, and M. Tahriri, (2009) "The comparison of powder characteristics and physicochemical, mechanical and biological properties between nanostructure ceramics of hydroxyapatite and fluoridated hydroxyapatite," *Mater. Sci. Eng. C*, vol. 29, no. 4, pp. 1387–1398.
- [3] A. Rogina, M. Ivanković, and H. Ivanković, (2013) "Preparation and characterization of nano-hydroxyapatite within chitosan matrix," *Mater. Sci. Eng. C*, vol. 33, no. 8, pp. 4539–4544.
- [4] W. L. Suchanek, P. Shuk, K. Byrappa, R. E. Riman, K. S. TenHuisen, and V. F. Janas, (2002) "Mechanochemical-hydrothermal synthesis of carbonated apatite powders at room temperature," *Biomaterials*, vol. 23, no. 3, pp. 699–710.
- [5] G. Chen, T. Ushida, and T. Tateishi, (2001) "Development of biodegradable porous scaffolds for tissue engineering," *Mater. Sci. Eng. C*, vol. 17, no. 1–2, pp. 63–69.
- [6] Y. Z. Wan *et al.*, (2007) "Biomimetic synthesis of hydroxyapatite/bacterial cellulose nanocomposites for biomedical applications," *Mater. Sci. Eng. C*, vol. 27, no. 4, pp. 855–864.
- [7] M. Aizawa *et al.*, (2000) "Fabrication of Porous by Sintering Ceramics of Fibrous with Open Pores Hydroxyapatite Particles," *J. Ceram. Soc. Japan*, vol.

- 108, no. 3, pp. 249–253.
- [8] P. F. Tang, G. Li, J. F. Wang, Q. J. Zheng, and Y. Wang, (2009)“Development, characterization, and validation of porous carbonated hydroxyapatite bone cement,” *J. Biomed. Mater. Res. - Part B Appl. Biomater.*, vol. 90 B, no. 2, pp. 886–893.
- [9] Y. Liu *et al.*, (2013)“Integration of a calcined bovine bone and BMSC-sheet 3D scaffold and the promotion of bone regeneration in large defects,” *Biomaterials*, vol. 34, no. 38, pp. 9998–10006.
- [10] M. B. Nair, S. Suresh Babu, H. K. Varma, and A. John, (2008)“A triphasic ceramic-coated porous hydroxyapatite for tissue engineering application,” *Acta Biomater.*, vol. 4, no. 1, pp. 173–181.
- [11] S. V. Dorozhkin, (2009)“Calcium orthophosphates in nature, biology and medicine,” *Materials (Basel)*, vol. 2, no. 2, pp. 399–498.
- [12] M. J. Coughlin, J. S. Grimes, and M. P. Kennedy, (2006)“Coralline hydroxyapatite bone graft substitute in hindfoot surgery,” *Foot ankle Int.*, vol. 27, no. 1, pp. 19–22.
- [13] S. McGrellis., J.-N. Serafin, J. JeanJean, J.-L. Pastol, and M. Fedoroff, (2001)“Influence of the sorption protocol on the uptake of cadmium ions in calcium hydroxyapatite,” *seperation Purif. Technol.*, vol. 24, no. 1, pp. 129–138.
- [14] E. Hosseinzadeh, M. Davarpanah, N. H. Nemati, and S. A. Tavakoli, (2014)“Fabrication of a hard tissue replacement using natural hydroxyapatite derived from bovine bones by thermal decomposition method,” *Int. J. Organ Transplant. Med.*, vol. 5, no. 1, pp. 23–31.
- [15] C. F. Ramirez-Gutierrez, A. F. Palechor-Ocampo, S. M. Londoño-Restrepo, B. M. Millán-Malo, and M. E. Rodríguez-García, (2016)“Cooling rate effects on thermal, structural, and microstructural properties of bio-hydroxyapatite obtained from bovine bone,” *J. Biomed. Mater. Res. B. Appl. Biomater.*, vol. 104, no. 2, p. 339–344.
- [16] S. M. Londoño-Restrepo, C. F. Ramirez-Gutierrez, A. Del Real, E. Rubio-Rosas, and M. E. Rodriguez-García, (2016)“Study of bovine hydroxyapatite obtained by calcination at low heating rates and cooled in furnace air,” *J. Mater. Sci.*, pp. 1–11.
- [17] S. S. Rahavi, O. Ghaderi, A. Monshi, and M. H. Fathi, (2017)“A comparative study on physicochemical properties of hydroxyapatite powders derived from natural and synthetic sources,” *Russ. J. Non-Ferrous Met.*, vol. 58, no. 3, pp. 276–286.
- [18] W. Khoo, F. M. Nor, H. Ardhyananta, and D. Kurniawan, (2015)“Preparation of Natural Hydroxyapatite from Bovine Femur Bones Using Calcination at Various Temperatures,” *Procedia Manuf.*, vol. 2, pp. 196–201.
- [19] N. A. M. Barakat *et al.*, (2008)“Physiochemical characterizations of hydroxyapatite extracted from bovine bones by three different methods: Extraction of biologically desirable HAp,” *Mater. Sci. Eng. C*, vol. 28, no. 8, pp. 1381–1387.
- [20] I. Hilmi, M. Rinastiti, and M. K. Herliansyah, (2011)“Synthesis of hydroxyapatite from local bovine bones for biomedical application,” *Proc. - Int. Conf. Instrumentation, Commun. Inf. Technol. Biomed. Eng. 2011, ICICI-BME 2011*, no. November, pp. 409–411.
- [21] A. Ruksudjarit, K. Pengpat, G. Rujijanagul, and T. Tunkasiri, (2008)“Synthesis and characterization of nanocrystalline hydroxyapatite from natural bovine bone,” *Curr. Appl. Phys.*, vol. 8, no. 3–4, pp. 270–272.
- [22] S. Jinawath, D. Pongkao, and M. Yoshimura, (2002)“Hydrothermal synthesis of hydroxyapatite from natural source,” *J. Mater. Sci. Mater. Med.*, vol. 13, no. 5, pp. 491–494.
- [23] R. Murugan, T. S. S. Kumar, and K. P. Rao, (2002)“Fluorinated bovine hydroxyapatite: Preparation and characterization,” *Mater. Lett.*, vol. 57, no. 2, pp. 429–433.
- [24] E. Kusriani, A. R. Pudjiastuti, S. Astutiningsih, and S. Harjanto, (2012)“Preparation of Hydroxyapatite from Bovine Bone by Combination Methods of Ultrasonic and Spray Drying,” *Int. Conf. Chem. Bio-Chemical Environ. Sci. December 14-15, 2012*

Singapore Prep.

- [25] C. Y. Ooi, M. Hamdi, and S. Ramesh, (2007)“Properties of hydroxyapatite produced by annealing of bovine bone,” *Ceram. Int.*, vol. 33, no. 7, pp. 1171–1177.
- [26] S. E. Etok *et al.*, (2007)“Structural and chemical changes of thermally treated bone apatite,” *J. Mater. Sci.*, vol. 42, no. 23, pp. 9807–9816.
- [27] E. Kusri and M. Sontang, (2012)“Characterization of x-ray diffraction and electron spin resonance: Effects of sintering time and temperature on bovine hydroxyapatite,” *Radiat. Phys. Chem.*, vol. 81, no. 2, pp. 118–125.
- [28] A. Doostmohammadi, A. Monshi, M. H. Fathi, and O. Braissant, (2011)“A comparative physico-chemical study of bioactive glass and bone-derived hydroxyapatite,” *Ceram. Int.*, vol. 37, no. 5, pp. 1601–1607.
- [29] M. Figueiredo, A. Fernando, G. Martins, J. Freitas, F. Judas, and H. Figueiredo, (2010)“Effect of the calcination temperature on the composition and microstructure of hydroxyapatite derived from human and animal bone,” *Ceram. Int.*, vol. 36, no. 8, pp. 2383–2393.