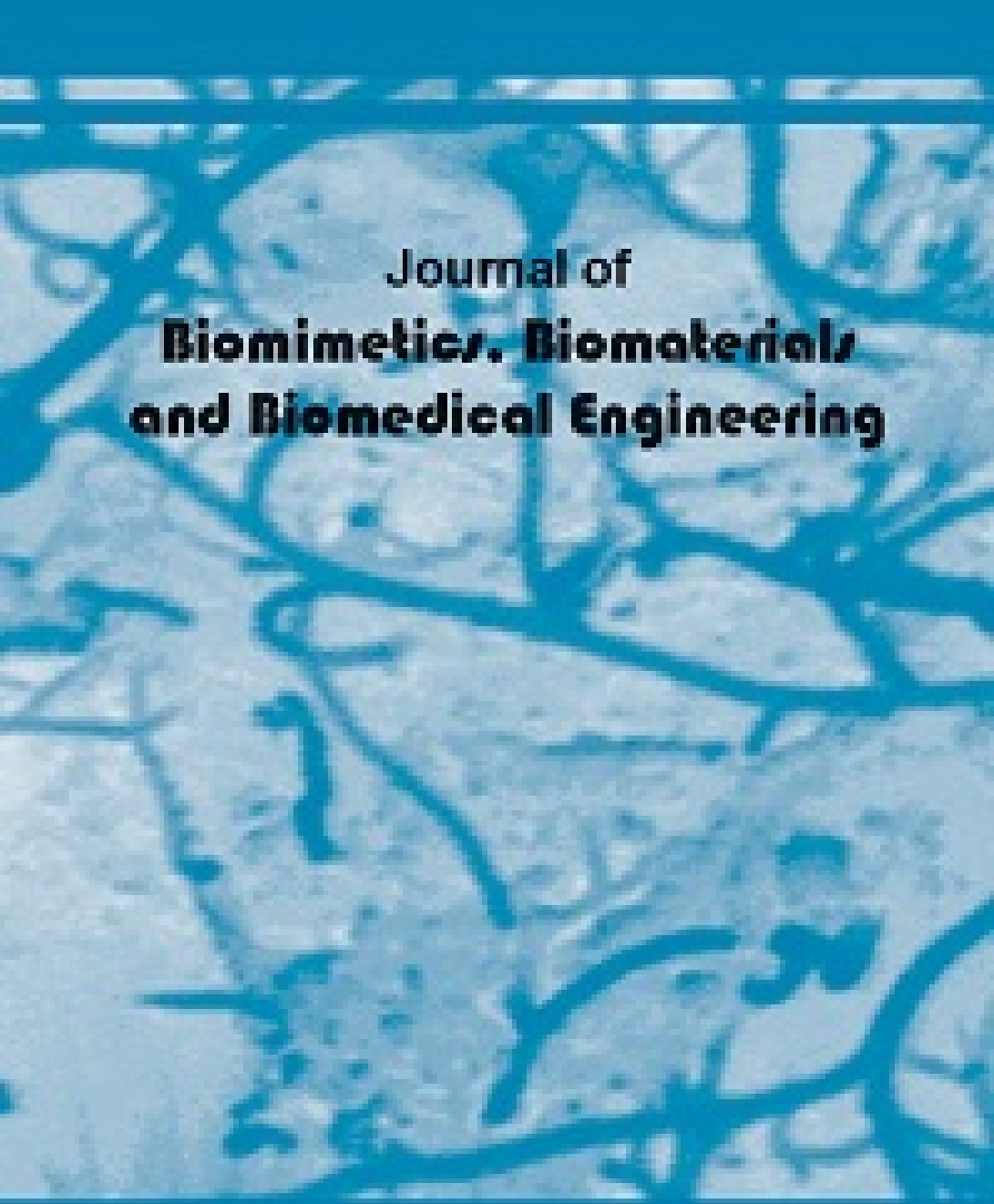

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The Characterization of Bovine Bone-Derived Hydroxyapatite Isolated Using Novel Non-Hazardous Method

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Keywords: Bovine bone, extraction, hydroxyapatite, calcination, non-hazardous, fourier transform infrared, scanning electron microscopy, X-ray diffraction analysis, tissue engineering, scaffold.

Abstract. Bovine bone is a considerable source for the production of hydroxyapatite. The recent study reported a novel method to extract hydroxyapatite from bovine bone without producing hazardous residue. The bovine bones were cut and boiled in the opened chamber followed by boiling in pressurized tank. The bones were then soaked into 95% ethanol. Calcination was then conducted in 800°C, 900°C and 1,000°C, for 2 hours. The result was then grinded and sieved. The powder then was characterized using Fourier transform infrared (FT-IR), Scanning electron microscopy (SEM) and X-ray diffraction analysis (XRD) to measure the purity of hydroxyapatite. It is concluded that the hydroxyapatite derived from this process showed 100% purity, resulting 35.34 ± 0.39% w/w from the wet bone weight and 72.3% w/w from the dried weight. The present extraction method has been proven to yield high amount of pure hydroxyapatite as well as reducing the use of hazardous reagent.

Introduction

Hydroxyapatite, Ca10(PO4)6(OH)2), is known as a medical material for teeth and bone tissue implant. Its composition and biological structure resemble natural bone [1]. Hydroxyapatite exhibits interesting properties such as having good biocompatibility and bioactivity as well as exhibiting a non-inflammatory and non-immunogenic properties [2]. Thus, hydroxyapatite is the bioceramic that is widely synthesized using many methods. Some of the methods are precipitation, microwave irradiation, and calcium hydroxide and phosphate reaction [3-5]. Another conventional method to synthesize hydroxyapatite is a wet method that is comprised of precipitation, hydrothermal technique and hydrolysis [6]. However, these synthesis processes might be complicated or biologically unsafe. Natural hydroxyapatites contain carbonates group and a small proportion of magnesium, sodium and metal traces. In addition, natural hydroxyapatite exhibits a Ca/P stoichiometric ratio higher than 1.67. These facts suggest that natural hydroxyapatite is more preferable for the use in medical application [7]. Recent development in the synthesis of hydroxyapatite suggests the extraction of natural hydroxyapatite bioceramics by simple calcinations of several bio-waste, for instance, salmon fishbone, animal bones [8-10]. However, these extraction processes involve the use of hazardous materials such as sodium hydroxide and potassium hydroxide. Moreover, the process takes a longtime and results in a non-eco-friendly waste product. The extraction of the natural cubical form of hydroxyapatite has been well-performed using hexane as a fat removal solvent and hydrogen peroxide as a protein-disintegrating agent. However, the use of these reagents is ultimately hazardous because of their carcinogenic feature. Thus, the development of effective and eco-friendly methods to extract hydroxyapatite from natural source is still needed.

Our unpublished trials in extracting natural hydroxyapatite from bovine bone show that the use of pressurized boiler and recyclable ethanol may effectively removes the fat and protein residues. The present study was conducted to optimize the calcination process, evaluate the yield and characterize the obtained hydroxyapatite.

Materials and Methods

Hydroxyapatite preparation

Around 3.5 to 4 kg fresh cortical bone of mature bovine was used as raw material. The bone was purchased from Surabaya official slaughterhouse in Indonesia. Three replications for hydroxyapatite extraction were conducted. Bones were cut and cleaned with water. The spongy parts and bone marrow were removed. The bone was boiled with distilled water for 5 hours. The distilled water was changed every hour to prevent the saturation from fat and protein. The bone was then boiled in the pressurized tank for 3 hours with water changing every hour. The boiled bone was dried in the temperature of 60°C for 3 hour. The dried bone was then shanked in absolute ethanol (Brataco Chemika, Surabaya, Indonesia) and shaken for 24 hours with ethanol changing every 12 hours. The purpose of the process is mainly to remove fat and protein residues. The calcination process was conducted by combustion using furnace for 2 hours. The process was optimized using 3 temperatures which were 800°C, 900°C and 1000°C. The product was then grinded and the powder was subjected to 80-mesh sieve.

Characterizations

The characterization of the hydroxyapatite products was done with Fourier Transform Infrared (FT-IR) spectroscopy (Perkin Elmer, MA, USA). The hydroxyapatite product sample was pelleted with potassium bromide. The measurement was done in 400- 4,000 cm^{-1} wave number range. The surface morphology was identified using energy dispersive X-ray (EDX) equipped SEM (Inspect S-50, FEI, Japan). The solid phase and crystalline morphology were assessed using X –Ray diffractometer (XRD; X'Pert Pro, Malvern panalytical Ltd., Royston, UK).

Results and Discussion

Table 1. The percentage of product weight during extraction process compared to wet weight of bovine bone materials

Replic.	Wet weight kg (%)	Weight after five hours boiling kg (%)	Weight after pressurized boiling kg (%)	Weight of dry materials kg (%)	Weight after Calcification kg (%)	% to dry material
1	3.722 (100)	3.147 (84.6)	2.956 (79.4)	1.892 (50.8)	1.332 (35.6)	70.4
2	3.416 (100)	3.001 (87.9)	2.897 (84.8)	1.544 (45.2)	1.192 34.9	77.2
3	3.941 (100)	3.469 (88.0)	3.114 (79.6)	1.989 (50.5)	1.401 (35.5)	70.4
Means	3.693±0.264 (100)	3.206±0.239 (86.8±1.9)	2.989±0.112 (81.3±3.0)	1.808±0.234 (48.8±3.1)	1.308±0.107 (35.3±0.4)	72.3

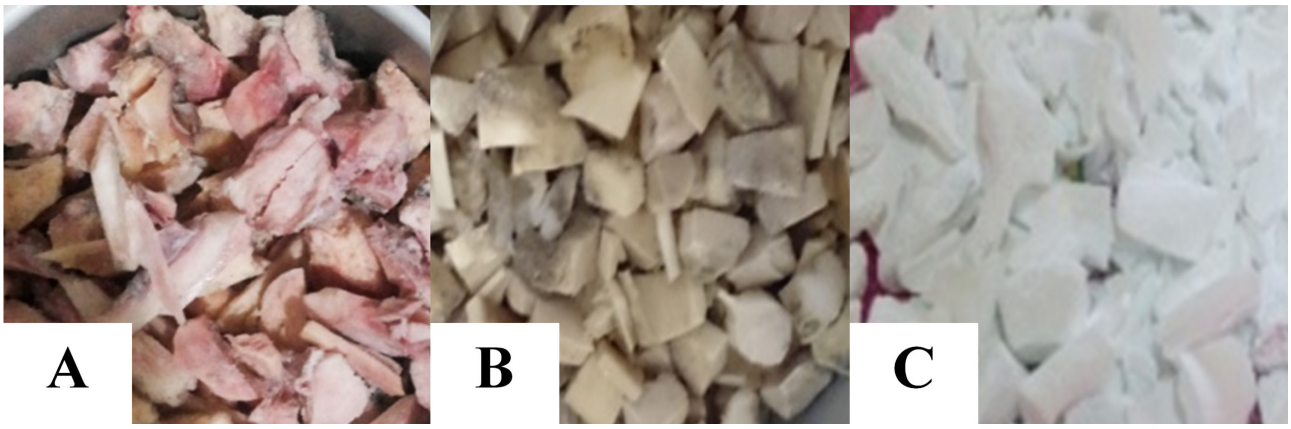

Fig 1. The representative physical appearances of raw bone materials before boiling process (A) and after pressurized boiling process (B), and end hydroxyapatite product after calcification (C).

The non-hazardous process prevents the use of carcinogenic materials that may endanger the operators as well as the environment. The result of present study showed that the use of distilled water boiling effectively removed the fat and protein residues and lowered the bone weight to 86.8±1.9 %. The appearance of the product was in red-yellowish colored bone (Fig. 1A). The further process by which the bone was boiled in the pressurized tank removes the fat and proteins attached to the bone and lowered the product weight to 81.3 ± 3.0%. The process gained a yellowish product (Fig. 1B). The use of organic solvent was to effectively remove the residues of fats. Ethanol is chosen as the organic solvent extractor for the process for it is recyclable, cost-effective and eco-friendly. The immersion and continuous shaking of the product in ethanol for 24 hours reduced the weight of the product to 48.8 ± 3.1%. The calcination process using furnace combustion removed the remaining fats and proteins and changed the appearance of the product into a clear white product (Fig. 1C). The present study showed that the final yield of hydroxyapatite product as compared to the wet weight of raw material is 35.3 ± 0.4 % (Table 1). The result showed that the process produced weight of 72,3 % of the dry raw materials.

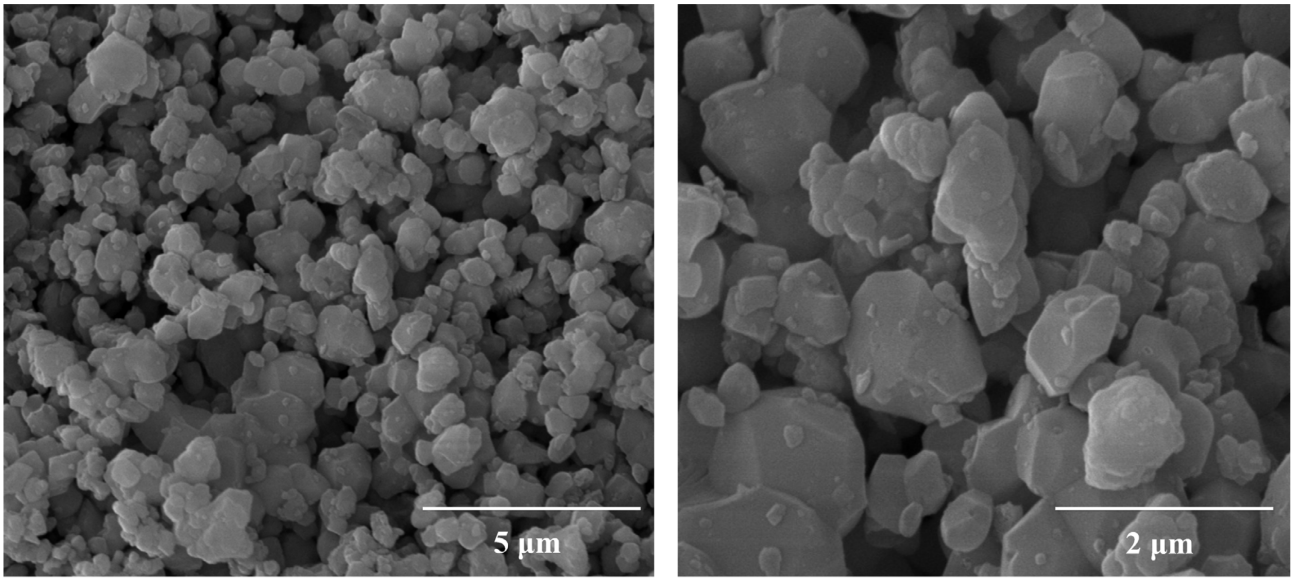

Fig 2. The representative SEM scanning result of the hydroxyapatite product. The particle shape analysis showed that the extracted BHA particles exhibit a hexagonal shape.

The result showed that the hydroxyapatite powder yielded by the process exhibited hexagonal particle shape (Fig. 2). This is in agreement with the previous report showing that the natural BHA exhibits hexagonal particle shape [11].

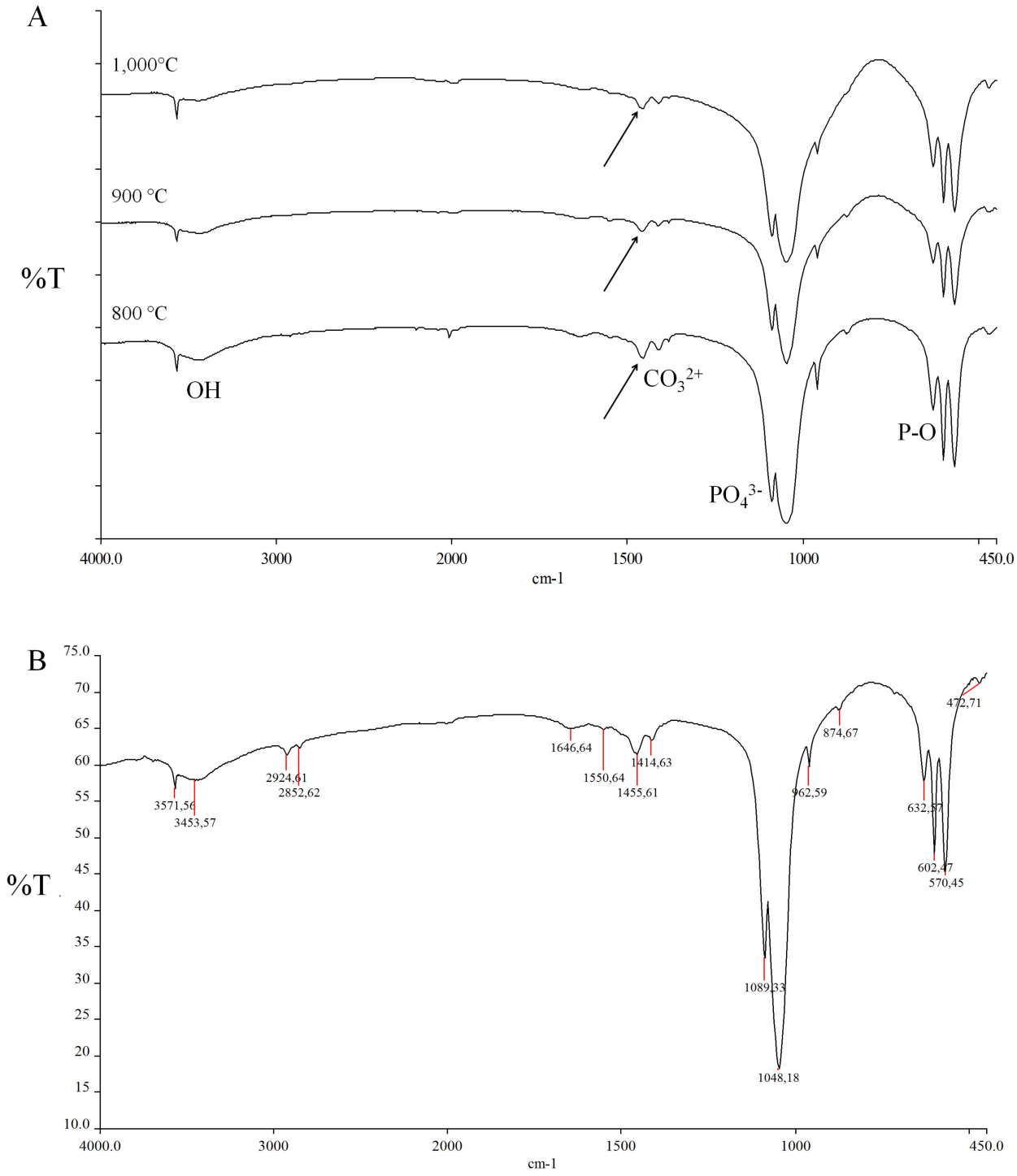

Fig 3. The representative infrared spectrum profile of extracted bovine hydroxyapatite (A) and reference profile of bovine hydroxyapatite; Ca_{10} $(PO_4\text{-})_6$ $(OH)_2$ (B). The reference profile shows asymmetric bending vibrations of P-O chain at 570 and 602 cm^{-1} and peak of tetrahedral PO_4 at 1,048 cm^{-1}. All profile shows specific peak for carbonate ($-CO_3^{-2}$) group at 1,455 cm^{-1} as the trace of natural BHA extraction.

The infrared spectroscopy was used to characterize the extracted BHA and compare their infrared spectrum to the reference spectrum for natural BHA. The results showed that the extracted samples exhibited asymmetric bending vibrations from P-O chains at 570 and 602 cm^{-1}, and sharp peak representing tetrahedral PO_4 at 1,048 cm^{-1}. The profiles from extracted BHA and reference similarly showed the carbonate group ($-CO_3^{-2}$) absorption at 1,455 cm^{-1} (Fig 3A-B). The carbonates group absorption in the BHA infrared profile resembles the specific functional group trace for natural BHA [7]. This indicates that the extracted BHA in this study similar to the reference natural BHA.

Furthermore, the difference in the calcination temperature resulted a slight difference in infrared profiles. It is shown that the absorbance at 1,455 cm^{-1} was shortened due to the increase of calcination temperature. The result showed that 1,000°C furnace calcination resulted the shortest peak at carbonate group absorption, which may reflect the small amount of carbonate traces remained in the sample (Fig. 3A; top profile). The present finding suggests that the 1,000°C calcination results an effective production of BHA with small portion of carbonate residues.

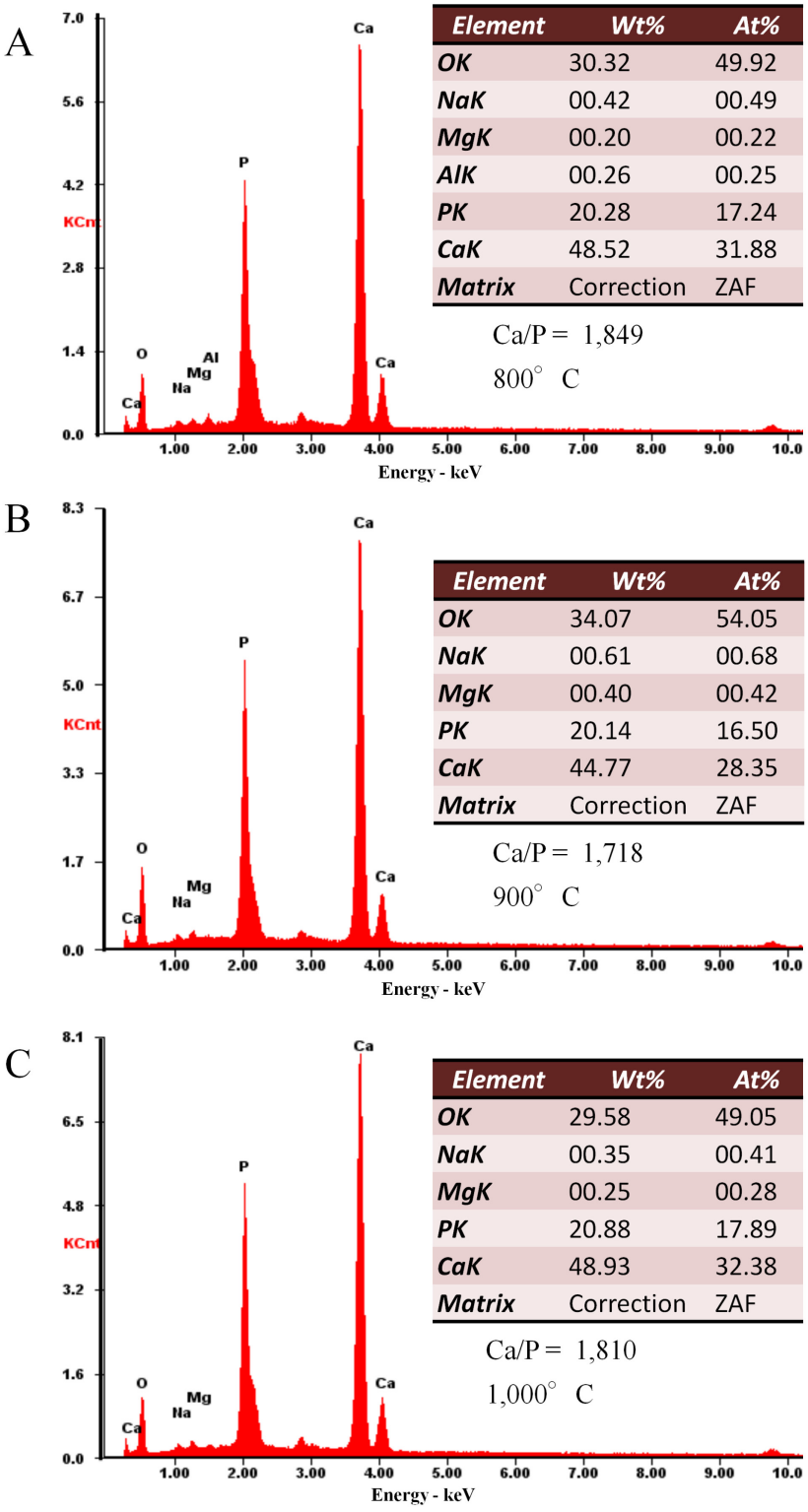

Figure 4. The representative EDX results of extracted BHA with different calcination temperature. The calcination was conducted by furnace combustion at 800°C (A), 900°C (B) and 1000°C (C).

It is known that the stoichiometric comparison between calcium and phosphate for BHA is commonly at Ca/P ratio 1.67. The result of the present study showed that the Ca/P ratio of extracted BHA combusted at 800°C, 900°C and 1,000°C were 1.849, 1.718 and 1.810, respectively (Fig 4A-C). It is reported that BHA exhibiting Ca/P ratio more than 1.67 is more suitable for medical application [7]. In addition, the EDX results in this study showed that there were low level of sodium remained in the extracted BHA. The Na residues for extracted samples with combustion temperature of 800°C, 900°C and 1,000°C were respectively 0.42, 0.61, and 0.35 % w/w. It is reported that the extraction of HA from the natural source by base extraction method using sodium hydroxide produces a high amount of sodium residue around 0.99% w/w [8]. The present study suggests that the present extraction method may reduce the sodium residue in the BHA as well as diminish the use of sodium hydroxide as a hazardous reagent in the conventional extraction method.

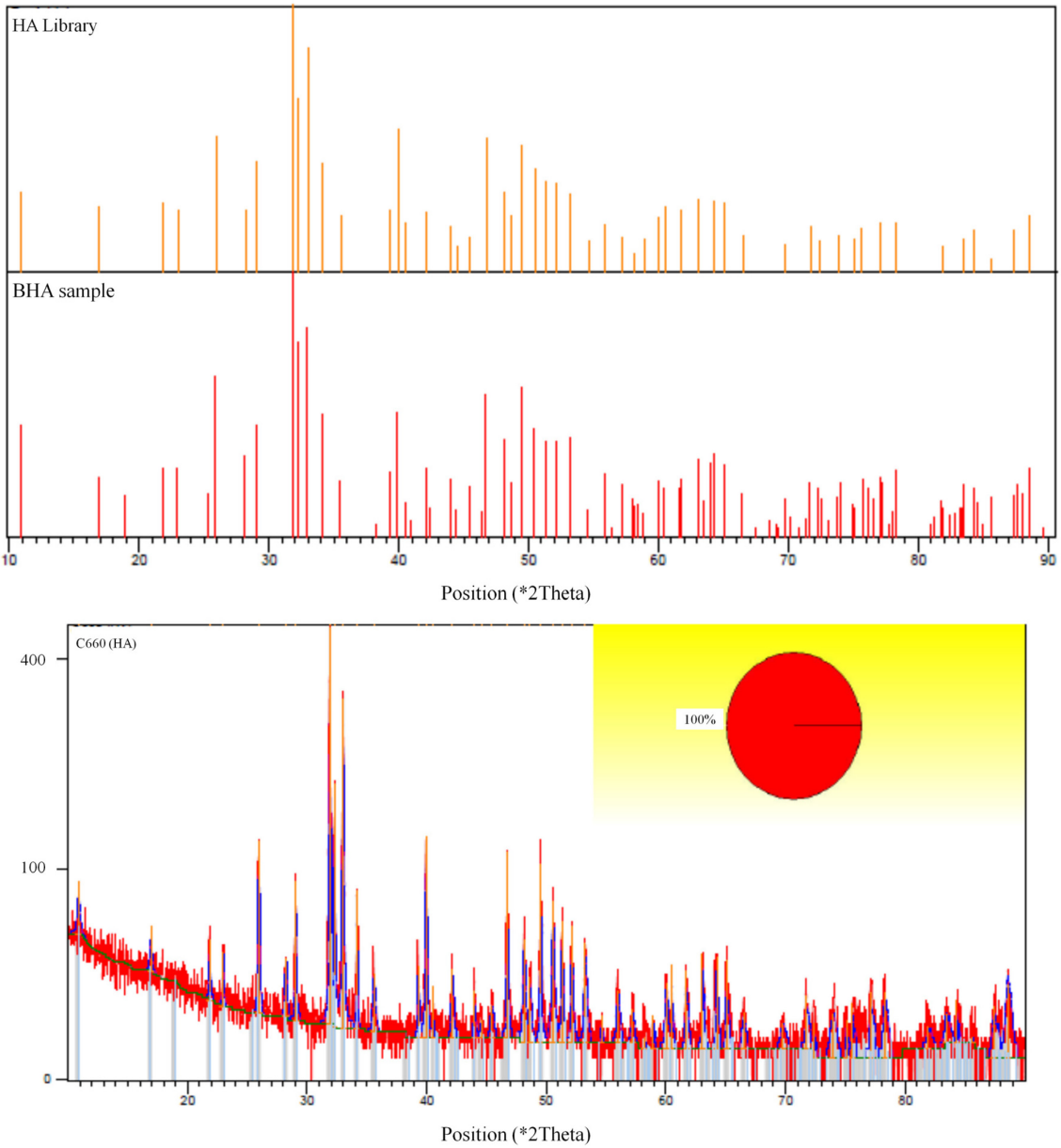

Figure 5. The representative X-ray diffractogram of extracted BHA. The diffractogram of extracted sample is compared to that of hydroxyapatite based on the library database. Upper panel is the peak list of the diffractogram of the samples (lower graph; red) and the reference from database library (upper graph; orange).

It is known that natural based BHA is playing important role in the production of bone cement and other bone remodeling supporting products, due to the influence carbonate substitution that is known could stimulates the activity of osteoclast and osteoblast [12]. Our finding showed that the

purity of BHA in the samples were 100% as compared to the XRD profile of the BHA reference material (Fig 5). It is known that XRD profile is commonly utilized to examine the similarity of the compound as compared to the standard or reference material based on their crystalline status. The present study suggests that the crystalline state of extracted compound was similar to that of the reference BHA material.

However, the complication of the method and environment aspect of the extraction method still being the challenge for the pharmaceutical industry to provide good quality, safe and effective BHA raw materials. It is remain to be explored whether the synthetic BHA with high osteoconductivity may be produced in a cost effective and eco-friendly way.

Conclusion

The present results suggest the effectiveness of the proposed BHA extraction method to yield high amount of BHA and lower use of hazardous materials. Moreover, the method produces a high purity and low traces residue based on FTIR, SEM, EDX and XRD measurements. Together, the results indicate that the non-hazardous extraction method using pressurized boiling and high temperature furnace effectively gains a high yield and purity of BHA.

Authors Contribution

Study conception and design by ASB, S, CA; Acquisition of data by S, WPN; Analysis and interpretation of data by S, WPN, CA; Drafting of manuscript by ASB, CA, MAG; Critical revision by ASB, S, WPN, CA, MAG.

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Conflict of Interest

The authors declare no conflict of interest in the conduct of this study.

Reference

[1] Bano, N., Jikan, S. S., Basri, H., Bakar, S. A. A., Nuhu, A. H. (2017). Natural Hydroxyapatite Extracted from Bovine Bone. *Journal of Science and Technology*, 9(2):22-28

[2] Barakat, N. A. M., Khil, M. S., Omran, A. M., Sheika, F. A., Kim, H. K. (2009). Extraction of pure natural hydroxyapatite from the bovine bones bio waste by tree different methods. *Journal of Materials Processing Technology*, 209(7):3408-3415

[3] Cao, L. Y., Zang, X., Huang, J. F. (2015). Synthesis of hydroxyapatite nanoparticles in ultrasonic precipitation. *Ceramic International*, 31(8):869-872

[4] Isobe, T., Nakamura, S., Nemoto, R., Senna, M., Sfihi, H. (2002). Solid-state double nuclear magnetic resonance study of the local structure of calcium phosphate nanoparticles synthesized by a wet-mechanochemical reaction. *Journal of Physical Chemistry B*, 106(20):5169-5176

[5] Kumar, G. S., Sathish, L., Govind, R., Girijab, E. K. (2015). Utilazation of Snail shells to synthesise hydroxyapatite nanorods for orthopedic applications. *RSC Advances*, 5(49)39544-39548

[6] Pankaew, P., Hoonnivathana, E., Limsuwan, P., Naemchanthara, K. (2010). Temperature effect on calcium phosphate synthesized from chicken eggshells and ammonium phosphate. *Journal of Applied Sciences*, 10(24):3337-3342.

[7] Brzezinska-Miecznik, J., Haberko, K., Sitarz, M., Bucko, M. M., Macherzynska, B. (2015). Hydroxyapatite from animal bone- Extraction and Properties. *Ceramic International*, 41(3):4841-4846

[8] Alparslan, Y and Baygar, T. (2017). Extractin, Characterization and Antimicrobial Activity of Hydroxyapatite from Seabass and Seabrean Scale. *Journal of Food and Health Science*, 3(3):90-96

[9] Sobczak, A., Kowalski, Z., Wzorek, Z. (2009). Preparation of hydroxyapatite from animal bones. *Acta of Bioengineering and Biomechanics*, 11(4):23-28

[10] Venkatesan, J., Lowe, B., Manivasagan, P., Kang, K. H., Chalisserry, E. P., Anil, S., Kim, D. G., Kim, S. K. (2015). Isolation and characterization of nano-hydroxyapatite from salmon fish bone. *Materials*, 8(8):5426-5439

[11] Costescu, A., Pasuk, I., Ungureanu, F., Dinischiotu, A., Costache, M., Huneau, F., Galaup, S., Coustumer, P., Predoi, D. (2010). Physico-chemical properties of nano-sized hexagonal hydroxyapatite powder synthesized by sol-gel. *Digest Journal of Nanomaterials and Biostructures*, 5(4):989-1000.

[12] Germaini M, Detsch R, Grünewald A, Magnaudeix A, Lalloue F, Boccaccini R, Champion E. (2017). Osteoblast and osteoclast responses to A/B type carbonate-substituted hydroxyapatite ceramics for bone regeneration. *Biomedical Materials*, 12(3), 035008.

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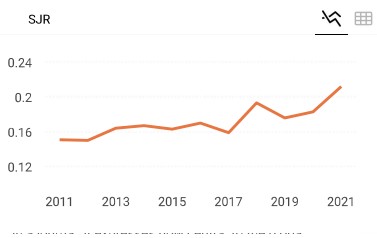

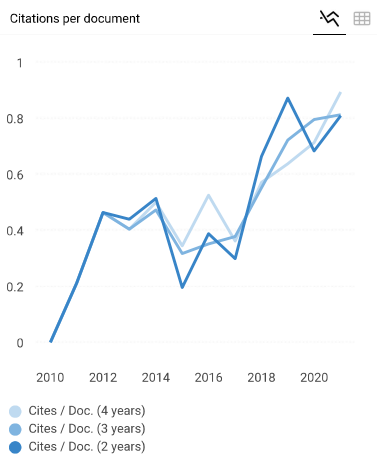

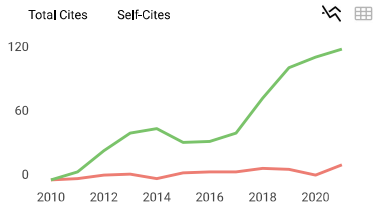

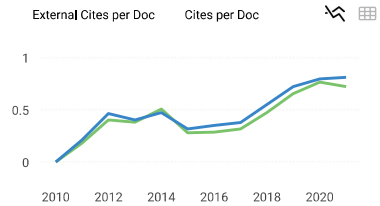

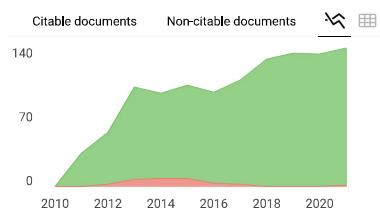

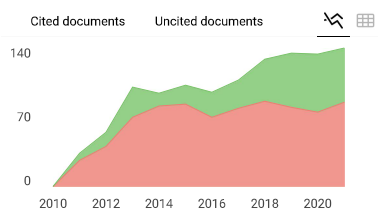

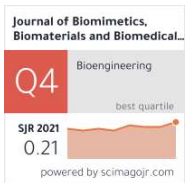

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