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Bovine hydroxyapatite extraction from cow bone waste as raw material for Bone Screw

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ABSTRACT

This study was aimed to extract Bovine Hydroxy Apatite (BHA) from bone waste which was the sorting result of waste from the Animal Slaughtering Center, PPH. The method used was the boiling method using a high-pressure pan followed by washing with alcohol then sintered, crushed and sieved with an 80-mesh sieve. The extracted BHA was used as a bio-screw raw material by adding 10% gelatin and glutaraldehyde crosslinker. Extracted BHA was characterized by FTIR, SEM-EDAX and XRD. The results of bio-screw printing were then characterized its compressive strength. From 3.693 ± 0.264 kg of bone waste, it was obtained 1.308 ± 0.107 kg of BHA extraction result which could be calculated approximately 35.3% of the initial weight of bone waste. FTIR BHA extraction test results identified the presence of hydroxyl (OH^-) functional groups at wave numbers of 570, 602, 3571 cm^{-1}, carbonate function groups ($-CO_3^{2-}$) at wave numbers of 1412, 14575 cm^{-1} and phosphate function groups (PO_4^{3-}) at 473, 962,1049,1089 cm^{-1} wave number. SEM-EDAX test resulted particles with <1 µm size which were distributed evenly, with 1.74 Ca / P ratio. The result of X-Ray diffractometer (XRD) identified hydroxyapatite $\{Ca_{10}(PO_4)_{5.52}(HPO_4)_{0.15}(SiO_4)_{0.33}(OH)_{1.66}O_{0.19}\}$ hexagonal structure with a lattice constant a = b = 9.4180Å, and c = 6.8835 Å. Bio-screw was successfully printed and tested for compressive strength which was then known that the result range from 9.56 to 11.36 MPa. The value was suitable for cancellous bone repair.

Key words: *Bone waste, Bovine hydroxy Apatite, Bone screw compressive strength*

Introduction

Badan Pusat Statistik (BPS- Statistics Indonesia) has recorded that Indonesia's meat production reached 490,420.77 tons and the live cowimport reached 550,000 (BPS, 2019). Pusat Pemotongan Hewan (Animal Slaughter Center), also known as PPH, is a service unit that is authorized to guarantee the availability of meat according to national standards, such as safe, healthy, intact and halal. Nearly 16.6% of the total weight of average-300-kg cow, which was slaughtered, produced bone waste. PPH Pegirian Surabaya, for example, cuts about 200 cows every day (BPS East Java, 2019). By that number, the unit could produce 99 tons of cow bone waste each month. As the matter of fact, the cow bone waste consists of rich collagen protein compounds and calcium mineral. That composition is similar with the chemical composition, morphology, distribution, function and pathology of human pro-

tein and mineral compounds (Stock, 2018). Through a good sorting process, cow bone waste could be utilized as raw material for hydroxyapatite. Hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, is inorganic substances that makes up bone, along with other inorganic substances, such as Na^+, Mg^{2+}, CO_3^{2-} (carbonate) and F^- (fluoride).

Some researchers have reported the methods of hydroxyapatite extraction synthesis, which included the precipitation method (Siswanto *et al.,* 2020), microwave irradiation (Kumar *et al.,* 2015) as well as other extraction methods using NaOH and KOH solutions. Those technique took a long duration of time and produced the dangerous synthesis result and waste for the environment. Thus, recently natural hydroxyapatite bioceramics has been able to be extracted by normal calcinations of some biowastes, e.g. Salmon Fish Bone (Venkatesan *et al.,* 2015), animal bones (Miecznik *et al.,* 2015). Another alternative method was boiling the bones in a high pressure pan (Persto Pot) (Burmawi *et al.,* 2015), soaking them in the alcohol to remove fat and protein, and eventually sintering the HA at 1000 ^oC. The result of HA synthesis from natural materials usually contains carbonate groups and magnesium, sodium and metal alloys in small amounts with the Ca/P stoichiometric ratio higher than 1.67, which is more suitable for medical applications (Miecznik *et al.,* 2015).

Hydroxyapatite (HA) $\{Ca_{10}(PO_4)_6(OH)_2)\}$ is known as a material for medical applications, especially for teeth and bone tissue implants. Its composition and biological structure resemble to natural bone (Bano *et al.,* 2017). There are interesting properties of HA, which are the good biocompatibility, bioactivity, nonimflammatory and non immunogenicity (Pourhashemi *et al.,* 2019). Hydroxyapatite is also the main ingredient in biomaterials for handling bone fracture cases, the case in which the continuity of bone structure is broken. There has been 77% of internal bone fracture fixed with bone screw (Camathias *et al.,* 2014). Internal fixation by surgery and installation of biodegradable and biocompatible bioscrew has many advantages because it does not require the surgical removal process. It is because the screw could be integrated with the bone. The degraded bioscrew is safe for human body since itwould not cause irritation and created holes in the bones (Chao *et al.,* 2015).

Screw is categorized according to each part, such as threads, face angles, pitch, depth and width of the screw (Stahel *et al.,* 2017). Screws on sponge bones have a higher thread depth than cortical bone screws (compact bones) to increase contact area. The pitch leads to the linear distance that the screw passes after one full turn, so that the smaller the pitch, the more threads there are on the screw. (Stahel *et al.,* 2017). Kanno *et al* (2019) reported a mechanical study of a recently-developed novel augmentation method using hydroxyapatite (HA) granules for PPS fixation. This study was to evaluate the strength and stiffness of PPS fixation augmented with HA granules using an osteoporotic bone model. Percutaneous Pedicle Screws (PPS) could provide internal fixation of the thoracolumbar spine through a minimal invasive surgical procedure. PPS fixation has been widely used to treat various spinal diseases. Rigid fixation of PPS is essential for managing osteoporotic spine in order to prevent the risks of screw loss and implant failure.

Materials and Methods

Bovine bone preparation was obtained from fresh femoral cortical bone of mature bovine. The material used was purchased from the Animal Slaughtering Center, PPH Pegirian Surabaya, Indonesia. First of all, the purchased bones were cut off and cleaned with water. Then, the spongy part and bone marrow were removed before eradicating the fat and protein. The removal of fat and protein was done by boiling the material in water for 4 hours in which the water was changed every hour, so that the water used would not get saturated with fat and protein released from the bones. The next step was boiling the bones in a pressure cooker for 2 hours and change the water every hour. The boiled bones were then dried in an oven at 600 ^oC temperature for 3 hours. After being dried, the bones were soaked and shook constantly in alcohol for 3 hours.The used alcohol had to be replaced every hour in order to keep the sample clear from the dat. Eventually, Bovine Hydroxyapatite (BHA) was obtained after a 5-hour sintering process in Furnace at 1000^oC. After the sintering process, BHA was ground and sieved with 80-mesh sieves to produce a pure white powder. The BHA obtained became the main raw material for making bio-screw with the addition of Gelatin with ratio 9:1 of BHA: Gelatin, which was made into 5 samples, according to the Federer formula.

Characterization of BHA samples included functional group identification test with FT-IR (Perkin

Elmer) spectroscopy, at wave numbers 400 - 4000 cm^{-1}, surface morphology test with SEM equipped with energy dispersive X-ray (EDX) FEI TYPE INSPECT S-50. Moreover, phase and crystal structure identification was done by PANalytical Type X'Pert MPD X-Ray diffractometer (XRD) test. Bio-screw was tested its compressive strength using an Autograph.

Results and Discussion

BHA extraction was carried out in 3 extraction periods. The weighing of sample results in each process shown in Table 1. The average initial weight is about 3.693 ± 0.264 kg. The boiling process, which was carried out to removethe fat and protein in bovine bones, reduced the sample weight to 86.808 ± 1.957%. Subsequently, when the sample was boiled in a high-pressure pan so that the bones could be softened while removing the remaining protein and fat, the weight was reduced to 81.290 ± 3.048%. In order to lose the fat completely from the bones, the sample was dissolved in alcohol because it is environmentally friendly, inexpensive and easily obtained. Soaking in alcohol carried out for 3 hours several repetitions to obtain a sample weight of only 48.834 ± 3.153%. HA formation was obtained after sintering at 1.000 °C for 5 hours. The pure white sample obtained was milled and then sifted with 80-mesh sieves until the remaining samples weighed 1.308 ± 0.107kg or it was about 35.341 ± 0.387%.

The result of the BHA extraction synthesis was tested by FTIR to identify the functional groups presented in the sample. FTIR test result is shown in Figure 1. In the FTIR spectra, the hydroxyl (OH -) functional groups were identified at the wave number at 570, 602, 3571 cm^{-1}, carbonate (- CO_3^{2-}) function groups at wave numbers 1412, 14575 cm^{-1} and the phosphate (PO_4^{3-}) functional group at wave number 473, 962, 1049, 1089 cm^{-1}.

The presence of phospor and calcium atoms as the main elements of BHA was identified through

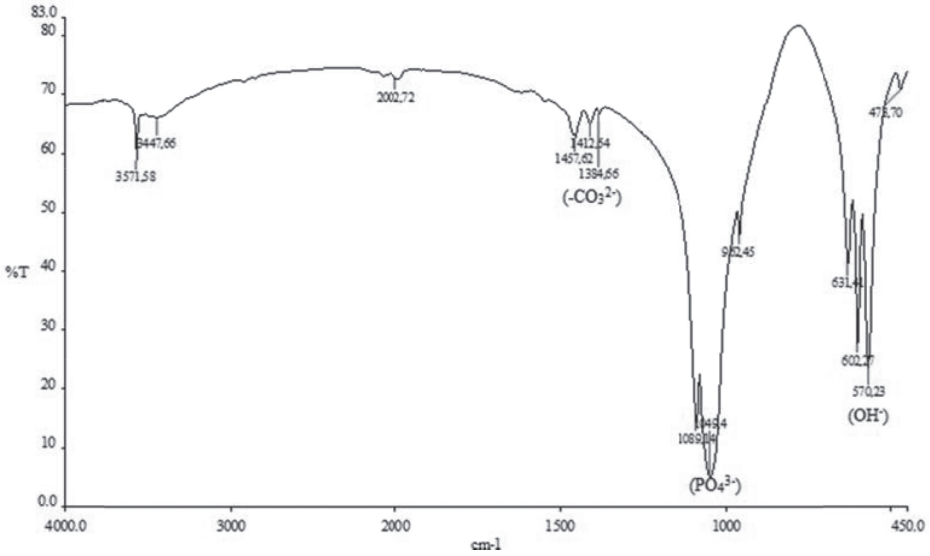

Fig. 1. FTIR Spectra of Bovine Hydroxyapatite (BHA) $\{Ca_{10}(PO_4\text{-})_6(OH)_2\}$

Table 1. The Hydroxyapatite extraction result from femoral bovine bone

No	Sample Weight (Kg)/ %				
	Initial Bone	Boiling 5 hours	High pressure-steam 3 hours	Alcohol immersion	Calcination
1	3.722/ 100	3.147 /84.551	2.956/79.420	1,892/50.833	1.332/35.579
2	3.416/100	3.001/87.851	2.897/84.807	1.544/45.199	1.192/34.895
3	3.941/100	3.469/88.023	3.114/79.642	1.989/50.469	1.401/35.549
	3.693±0.264/100	3.206±0.239/ 86.808±1.957	2.989±0.112/ 81.290±3.048	1.808±0.234/ 48.834±3.153	1.308±0.107/ 35.341±0.387

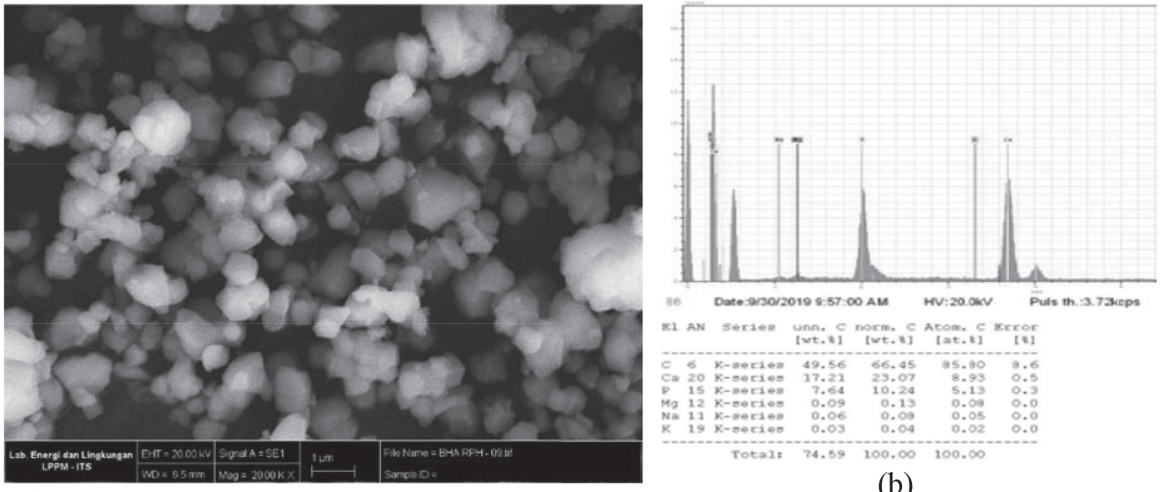

Fig. 2. The result of SEM-EDAX from BHA sample with magnificent of 20,000x.

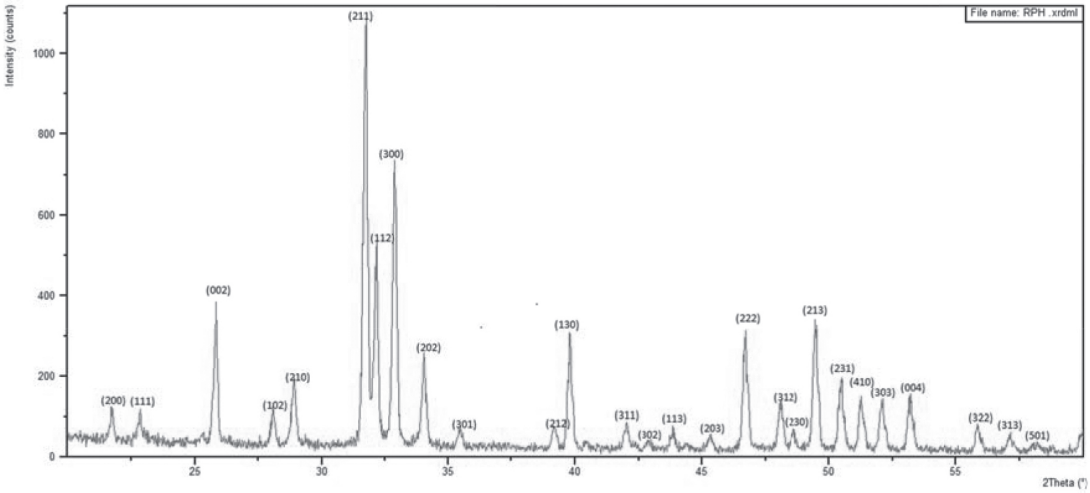

Fig. 3. XRD testing data of BHA sample

SEM-EDAX morphology test. Test results are shown in Figures 2 (a) and (b). It is shown in the morphological image that the resulted BHA particles was evenly distributed with size <1 µm. Based on EDAX data, the Ca / P ratio of BHA extract from the synthesis was 1.74.

For identifying the BHA produced, XRD test was done and identified by HighScore Plus software through the search match process. It then could be proven that the synthesized BHA extract was identified having the diffraction peaks from hydroxy apatite according to Ref Code 01-074-7566 namely $\{Ca_{10}(PO_4)_{5.52}(HPO_4)_{0.15}(SiO_4)_{0.33}(OH)_{1.66}O_{0.19}\}$ with hexagonal structure and lattice constants a = b = 9.4180Å, and c = 6.8835 Å. No peak was detected as a hazardous material impurity. The XRD test result that has been identified is shown in Figure 3.

Extracted Bovine hydroxyapatite (BHA) was used as a raw material for making bioscrew by adding gelatin. The printing results are shown in Figure 4(a). Then a compressive strength test was performed until the bio-screw sample was broken, as shown in Figure 4(b). The compressive strength test resultobtained from the bio-screw was of 9.56 to 11.36 MPa which wassuitable for cancellous bone repair. Ficai *et al.*, 2011 reported cancellous bone strength range of 2-12 MPa.

Conclusion

BHA extraction from PPH bone waste was success-

fully carried out from 3.693 ± 0.264 kg of bones and obtained 1.308 ± 0.107 kg of BHA extract or about 35.3% of the initial weight of bone waste. Furthermore, the BHA extract was successfully made bio-screw. FTIR test result identified the presence of hydroxyapatite functional groups namely hydroxyl (OH-) functional groups at wave numbers at 570, 602, 3571 cm^{-1}, carbonate function group ($-CO3^{2-}$) at wave numbers 1412, 14575 cm^{-1} and the phosphate functional group ($PO4^{-3}$) at wave number 473, 962, 1049, 1089 cm^{-1}. From EDAX SEM test result, it was obtained hydroxyapatite particles with the size of <1 µm and evenly distributed, with a Ca / P ratio of 1.74. X-Ray diffractometer (XRD) test resulted the diffraction peaks of hydroxyapatite crystal ($Ca_{10}(PO_4)_{5.52}(HPO_4)_{0.15}(SiO_4)_{0.33}(OH)_{1.66}O_{0.19}$} with hexagonal structure and lattice constant a = b = 9.4180Å, and c = 6.8835 Å. Moreover, the bio-screw compressive strength test resulted the compressive strength valuesbetween 9.56-11.36 MPa and known to be suitable for cancellous bone repair.

(a) (b)

Fig. 4. The printed bio-screw (a) before and (b) after the compressive strength test

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