

Comparing Properties of Bovine Bone Derived Hydroxyapatite and Synthetic Hydroxyapatite

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Some waste materials from natural sources are interesting resources for extraction to a more valuable compound. Specific techniques for the extraction of these waste materials are required. Hydroxyapatite (HAp) is one of the most important biomaterials that can be extracted from natural Bovine Bone waste. Natural Hydroxyapatite has been extracted from bovine bone by heating it at different temperatures, namely 850°C and 900°C. The properties of this natural hydroxyapatite have been compared with synthetic hydroxyapatite obtained from a commercial product. The influence of the HAp extraction method on several critical properties such as Ca/P ratio, crystal structure, crystallinity, crystallite sizes, microstrain, and morphology have been discussed. The hydroxyapatite obtained from the bovine bone was around 51% wt. The volume of the unit cell, crystallinity, crystallite size, ratio Ca/P, and morphology were affected by the extraction process. The conclusion is that HAp obtained from bovine bone is comparable to commercial products.

Keywords: Hydroxyapatite, Ca/P, Bovine bone

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1. Introduction

Bone is a part of the human body that plays a significant role in mechanical integrity for movement and protection. It is also involved in metabolic processes related to mineral homeostasis. Generally, bone consists of calcium phosphate (Ca/P) nanocrystals arranged homogeneously in collagen fibrils. Bone tissue is a metabolically active connective tissue composed of extracellular matrix (ECM), osteoclasts, osteoblasts, and osteocytes. Therefore, bones can regenerate bones. Bone tissue is the only tissue that can be reconstructed without leaving a scar. However, bone regeneration depends on the type of bone damage in which as time goes by, the amount of calcium in the bones will differ. Thus, it requires appropriate methods to increase the capacity of tissue repair.

The process of bone regeneration can be done using calcium phosphates such as amorphous calcium phosphates (FCA), brushite ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$), monetite (CaHPO_4), tricalcium phosphates (TCP), α -TCP, β -TCP, and hydroxyapatite. Calcium phosphate is suitable for bone regeneration because it has biocompatible, resorb ability, and bioactivity [1]. The type of calcium phosphate that attracts the attention of many researchers is hydroxyapatite because hydroxyapatite has a thermodynamic equilibrium in physiological conditions and body fluids [2, 3].

Hydroxyapatite is a bioceramic material formed from strong chemical bonds, derived from calcium apatite, and is a constituent of living organisms [4]. In bones, 65% - 70% are inorganic minerals in the form of hydroxyapatite, and 5% - 8% are bone water. The rest are organic minerals

such as glycoprotein, collagen, sialoprotein, and proteoglycans [5]. Hydroxyapatite, which has bioactive properties in repairing damaged bone tissue, is biocompatible, osteoconductive, osteoinductive, non-inflammatory, and not immunogenic. In addition, it can integrate with bone without causing local poisoning or inflammation [6].

Natural hydroxyapatite has advantages when compared with synthetic hydroxyapatite. Synthetic hydroxyapatite is less osteoinductive and exhibits poor mechanical properties, especially if exposed to wet environments [7]. Stoichiometric HAp has calcium and phosphorus with a molar ratio of Ca/P equal to 1.67, which has been the most effective in promoting bone regeneration. Natural hydroxyapatite is non-stoichiometric because there is usually a lack of calcium (Ca/P <1.67) or phosphorus (Ca/P >1.67). If there is a calcium vacancy in the hydroxyapatite crystal structure, the vacancy will be filled by cations such as Na⁺, Mg²⁺, dan Al³⁺.

Meanwhile, the deficiency of phosphate ions will be replaced by carbonate ions, and the lack of hydroxyl ions will be replaced by fluoride ions. The advantage of natural hydroxyapatite is that it can obtain apatite which is more similar to apatite in human bones compared to synthetic hydroxyapatite. This is due to the lack of Na⁺, Mg²⁺, dan Al³⁺, Fe²⁺, Si²⁺ dan F⁻ ions in synthetic hydroxyapatite, which are characteristic of osseous apatite [8, 9].

Since 1950, hydroxyapatite has been renowned for bone regeneration as a scaffold to fill bone defects. Hydroxyapatite has osteoinductive properties, promising broad application of hydroxyapatite [10]. Currently, hydroxyapatite is widely applied in the biomedical field, namely as bone filler material, orthopedic implant coating material, coating material for teeth, additional components in toothpaste to be filled with microcrack on the enamel surface to make it easier to remove plaque, can inhibit proliferation, induce apoptosis of cancer cells and so forth [1, 11].

Natural hydroxyapatite can be extracted from biological sources of waste such as aquatic sources (for example, fish-bone and fish scale), shell sources (for example, eggshell), mineral sources (for example, limestone), plants and algae, and also mammalian bone (for example, bovine, horse and camel.). Methods used to extract hydroxyapatite from biological sources depends on the type of biological sources. For mammalian sources, the methods used are calcination or alkaline hydrolysis or hydrothermal, or a combination of methods.

In the literature, the extraction of HAp from bovine bone was usually compared to the other BioSource but never compared to commercial products. The femoral bone of bovine bones is commonly used for extraction of HAp

because they have morphology and structure similar to human bone [12].

Extraction of the HAp from natural resources can also have many advantages such as an economical process, and environmentally friendly, and sustainability due to availability in large quantities. This may have a positive contribution to the economy and general health.

A simple calculation can show how cheap it is to extract Hydroxyapatite from bovine bone compared to commercial products. The sourcing cost to produce Hydroxyapatite comes from the price of raw materials and equipment used. In general, the commercial HAp uses calcium nitrate and ammonium phosphate, which cost higher than the cost of waste bovine bone. Equipment to produce synthetic HAp is also usually expensive. Hence, it can be estimated that the HAp extracted from bovine bone is cheaper than the commercial product synthesized from a chemical compound.

This research aims at knowing the properties of the HAp extracted using the calcination method from bovine bone compared to HAp from the commercial product synthesized commonly from chemical products. It is also to know whether the process is controllable or not, for example, to obtain the ratio Ca/P =1.67. These similar properties and the less cost of the HAp production compared to commercial products, might cause the HAp extracted from Bovine bone can be an alternative to replace an existing HAp commercial product.

2. Experimental Procedure

2.1. Sample Preparation

The fresh bovine bone was cut into pieces and cleaned to remove most of the dirt attached to the bone. Then the bones were boiled in water for 8 hours and dried. After that, the trabecular bone was removed to get a solid bone sample and solid bone samples were cut into small pieces. Bovine bone samples were calcined at 850°C and 900°C to obtain natural hydroxyapatite.

The choice of temperature was in accordance with the thermogravimetric analysis (TGA). Experiments with other calcination temperatures have also been carried out but the results are not presented. After calcination, the sample cooled naturally at room temperature. The number of samples was five for each calcination temperature. Next, the sample was mashed into powder using mortar and pestle. Hydroxyapatite commercial from sigma-Aldrich was compared with hydroxyapatite produced from bovine bones.

2.2. Sample Characterization

The phases formed after calcination and commercial HAp were identified by XRD (Rigaku RINT 2000 with Cu K α radiation). High Score Plus software was used to analyze XRD patterns. The degree of crystallinity (X_c), corresponding to the crystalline phase fraction present in the examined volume, was calculated by the relation below:

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

Crystallite sizes were obtained with the Williamson Hall method.

$$\beta \cos \theta = (k\lambda/D) + (4\epsilon \sin \theta) \quad (2)$$

In this expression, D is the crystallite size, k is the Scherrer constant of 0.9, λ is the x-ray wavelength of CuK α radiation of 0.15406 nm, β is the full width at half maximum intensity (FWHM), θ is the Bragg angle in radian and ϵ is the strain.

Fourier transform infrared spectroscopy (FTIR) was used to investigate functional groups in calcined samples at different temperatures and commercial HAp. FTIR spectra were obtained using Nicolet Magna IR-750. The spectrum was listed at 400-4000 cm^{-1} wave number. SEM (VEGA TESCAN) equipped with energy dispersive X-ray (EDX) was used to observe the morphology of the samples.

3. Result discussions

3.1. Thermal Analysis

Thermogravimetric curves from bovine bone samples and their derivative curves are shown in Fig. 1:

TGA pattern showed a decrease in bone mass of bovine as the temperature increased. It showed three stages of bone mass reduction at temperatures below 200°C, at temperatures between 200°C - 600°C, and temperatures above 600°C. In the first stage, at temperatures below 200°C, there was a reduction in mass. This decrease was associated with the level of hydration on the bone surface and decomposition of the organic compound, especially collagen.

In the third stage, carbonate decomposition occurred due to the release of CO₂ from the apatite lattice [7, 13, 14]. As mentioned by Kunio et al. and Khoo et al., the number of carbonate ions in bone was around 6-9 wt%. This carbonate ion will fill the phosphate void in the hydroxyapatite lattice because hydroxyapatite from natural materials usually results in a lack of calcium or phosphate [8, 15]. Three stages of cow bone mass reduction are presented in Table 1. Based on the results of thermogravimetric analysis, the calcination temperature was carried out at 850°C and 900°C.

Table 1. The weight percentage of the cow bone sample is based on the graph in Fig. 1(a).

	Bovine Bone
H ₂ O (wt%) (T < 200°C)	20.4 %
Organic Compound (wt%) (200°C < T < 600°C)	20.2 %
CO ₃ ²⁻ (wt%) (600°C < T)	7.9 %
Rendemen	51.5%

3.2. XRD Analysis

Fig. 2 shows the X-ray diffraction pattern of the samples. The pattern only shows the crystalline phase, indicating that the organic compound had been decomposed.

From Fig. 2, the hydroxyapatite peaks of the three samples were almost the same diffraction angle.

By doing linear fitting based on Eq. (2), the value of the intercept found that the average size of the crystallite and the slope shows the strain in the crystallite [13]. The intercept and slope values in the two-sample are shown in Fig. 3.

The calcination temperature affected the crystallite size and the degree of crystallinity. In the XRD results, an increase in intensity with increasing temperature indicated an increase in the degree of crystallinity. This is compatible with crystallite growth and carbonate decomposition [7]. Based on XRD phase identification, no β -tricalcium phosphate (β -TCP) was found at both calcination temperatures.

3.3. FTIR Analysis

Fig. 4 shows the FTIR spectra of the samples. It can be observed from both samples that increasing calcination temperature caused a more decomposed organic phase. The decomposition of organic compounds could be seen physically through changes in powder color, as the color of the raw bone powder is yellowish-brown [16].

At around 1793 cm^{-1} spectrum was identified as C=O, which was caused by adsorption of ethyl acetate [17]. The number of organic compounds detected in commercial HAp samples was greater than the calcined hydroxyapatite from bovine bones. It was also supported by the ratio Ca/P < 1.67 compared to calcined hydroxyapatite.

The carbonate groups in the samples 850°C were identified at 880 cm^{-1} , 1411 cm^{-1} , and 1457 cm^{-1} . In 900°C, the samples were identified at 880 cm^{-1} , and 1458 cm^{-1} . Whereas in the commercial HAp samples, there were no carbonate groups detected. However, a K₂CO₃ compound was detected to play a role in replacing the phosphate ion because the presence of potassium would be absorbed by the blood, leaving carbonate ions left.

In other words, at higher calcination temperatures, the

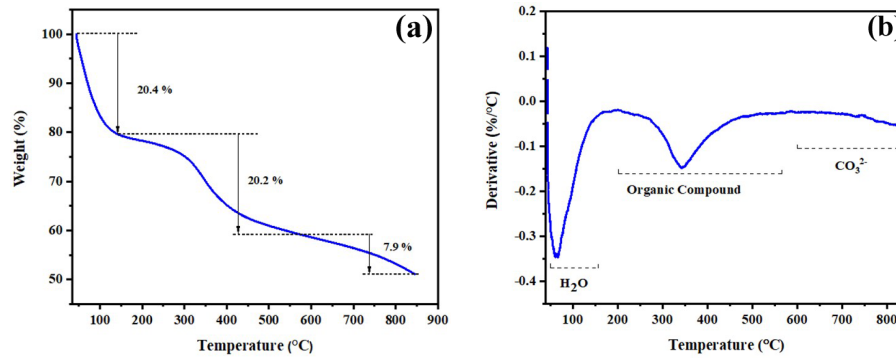


Fig. 1. (a) Bovine bone TGA curve (b) Bovine bone TGA derivative curve.

Table 2. Hydroxyapatite crystallographic parameters of calcined bovine bone samples at 850°C, 900°C, and commercial HAp.

Structural Parameters	Sample Code		
	850 °C HAp	900°C HAp	Commercial HAp
Crystal System	Hexagonal	Hexagonal	Hexagonal
Space Group	P 6 ₃ /m	P 6 ₃ /m	P 6 ₃ /m
Lattice Parameter (Å)	a = 9.4487	a = 9.4287	a = 9.4290
	b = 9.4487	b = 9.4287	b = 9.4290
	c = 6.8838	c = 6.88351	c = 6.8758
Volume Å ³	532.24	529.96	529.41
Crystallite size (W-H) (nm)	57.77	72.59	66.34
Strain	0.039	0.199	0.002
Crystallinity (%)	87.34	88.18	87.75

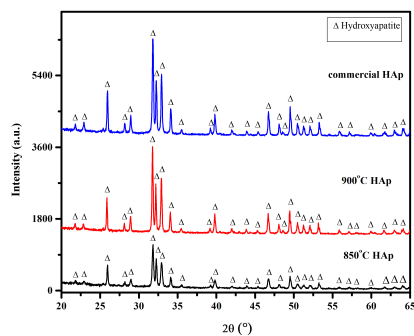


Fig. 2. X-RD pattern of bovine bone samples calcined at 850°C, 900°C, and commercial HAp.

amount of carbonate detected was decreasing. A large amount of carbonate in HAp would increase the Ca/P ratio. In HAp 850°C phosphate, the samples were detected on IR spectra 472 cm⁻¹, 568 cm⁻¹, 601 cm⁻¹, 960 cm⁻¹, 1026 cm⁻¹, and 1085 cm⁻¹. The 900°C samples were at 474 cm⁻¹, 567 cm⁻¹, 600 cm⁻¹, 962 cm⁻¹, 1030 cm⁻¹, and 1087 cm⁻¹. The commercial HAp samples were at 473 cm⁻¹, 560 cm⁻¹, 600 cm⁻¹, 961 cm⁻¹, and 1021 cm⁻¹.

3.4. SEM-EDS Analysis

Fig. 5 shows the morphology of the three samples. It shows the irregular shape of the particles. Hydroxyapatite synthesis from bovine bones by the calcination method has an irregular shape [18]. Commercial hydroxyapatite has a rod-like shape. The mean particle diameter of HAp 850°C is 437 nm, HAp 900°C is 462 nm, and commercial HAp is 108 nm as presented in Table 3.

Based on the average Ca/P ratio obtained, the 850 °C HAp sample was the closest to the stoichiometry compared to the 900 °C HAp sample. However, according to stoichiometry, the sample 850°C HAp Ca/P ratio was slightly higher than the Ca/P ratio. This was caused by the presence of more carbonate ions (CO₃²⁻) compared to the 900°C HAp sample in the FTIR analysis results in Fig. 4.

Table 4 shows properties comparison of HAp obtained from bovine bone with other reports. Different results might come from the origin of the bovine bone.

4. Conclusions

Natural hydroxyapatite (n-HAp) can be extracted from the bovine bone where the bovine bone is a waste material. The number of natural Hydroxyapatite (n-HAp) proper-

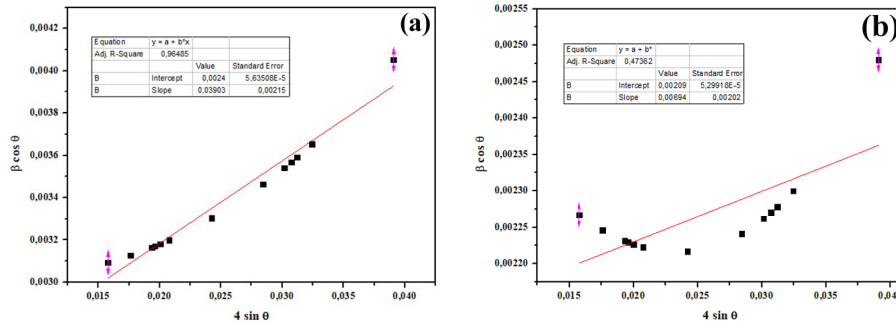


Fig. 3. Williamson-Hall sample plot (a) 850°C HAp (b) commercial HAp.

Table 3. Average particle diameter and the ratio Ca/P of the 850°C HAp, 900°C HAp, and commercial HAp.

	850°C HAp	900°C HAp	commercial HAp
Average Particle Diameter	437 nm	462 nm	108 nm
Average ratio Ca/P	1.689	1.609	1.523

Table 4. Properties Comparison of HAp obtained with other reports.

Source	Methods of Extraction	Ca/P ratio	Crystalline phases	Particle size	Reference
Bovine bone	Calcination	1.67	HAp (900°C)	< 420 μm	[16]
		1.5	HAp, β-TCP (850°C)	420-500 μm	[19]
Bovine bone	Calcination	1.689	HAp (850°C)	437 nm	author
		1.609	HAp (900°C)	462 nm	author
Camel bone	Calcination	1.66	HAp (1000°C)	79-0.9 nm	[20]
		2.036	HAp (700°C)	97 nm	[21]

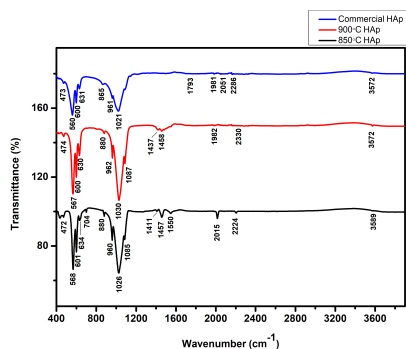


Fig. 4. FTIR of calcined bovine bone at 850°C, 900°C, and commercial HAp.

ties obtained, such as the ratio Ca/P, crystal system, and crystallite size can be considered comparable with the commercial product. It indicates that n-HAp can be produced from BioSource with a cheap, easy, and eco-friendly process. Moreover, it can be commercialized with cheaper than the commercial existing product.

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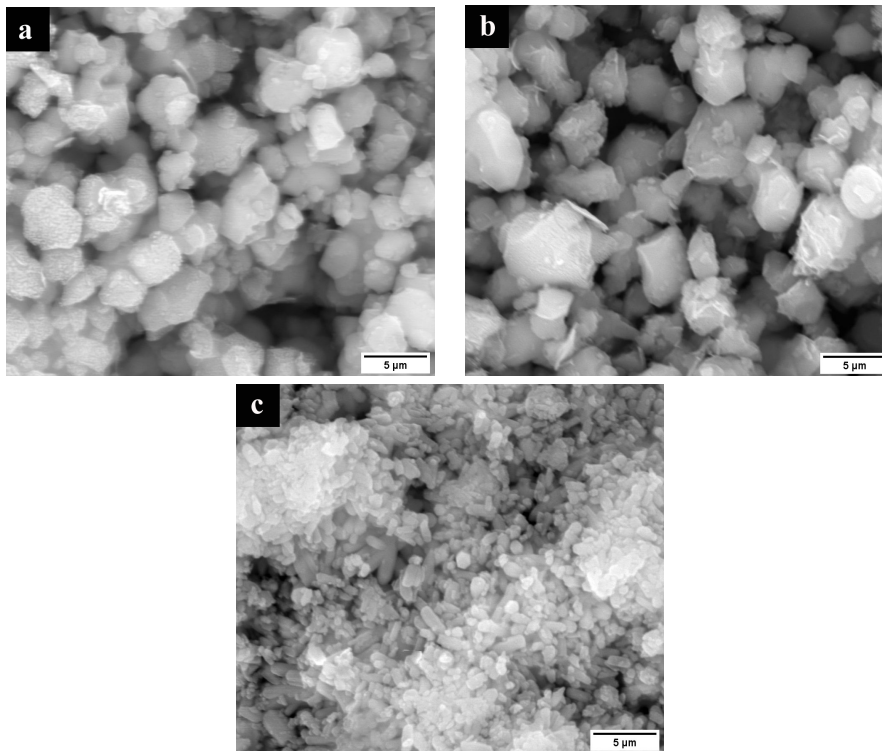


Fig. 5. (a) Morphology 850°C HAp, (b) 900°C HAp, and (c) commercial HAp.

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