



# Eco-Friendly Non-Toxic Biomaterial Extracted from Waste Cow Bones

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## *Author's contribution*

The sole author designed, analyzed, interpreted and prepared the manuscript.

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## ABSTRACT

**Aims:** Exploitation of bone waste to extract a vital material of high economic value.

**Study Design:** The type of research is experimental.

**Place and Duration of Study:** Department of Physics Education, Kristen Indonesia University, Jakarta, between March 2024 and September 2024.

**Methodology:** The calf bones used in this study were boiled for six hours to remove fat, after which they were cleaned with aquades, sun-dried, and completely dried. The bones were dried and then calcined for eight hours at 1000°C. After the bones were dried, they were pulverized into a fine powder in a mortar. Phosphoric acid was added to the resultant CaO powder to create hydroxyapatite using a wet precipitation technique. After that, the CaO powder and phosphoric acid mixture was sintered for five hours at 900°C. XRD (X-ray Diffraction) and SEM (Scanning Electron Microscopy) measurements were used to determine the hydroxyapatite phase.

**Results:** The hydroxyapatite (HAp) phase, with a hexagonal crystal system and space group P63/m, was effectively produced, according to the XRD (X-ray Diffraction) analysis results. A different chemical, whitlockite ( $\text{Ca}_{57}\text{Mg}_6\text{O}_{168}\text{P}_{42}$ ), with a trigonal crystal structure and space group R3c, was also created during this synthesis technique. The generated hydroxyapatite displayed

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agglomeration, according to SEM (Scanning Electron Microscopy) analysis, with most of the individual particles having a spherical shape. The generated hydroxyapatite particles ranged in size from 500 to 900 nm, which is in the nanoscale.

**Conclusion:** Utilizing cow bones as the starting material for bone filler, the hydroxyapatite (HAp) biomaterial was effectively synthesized utilizing the wet precipitation method. The synthesis results show that the generated HAp has a space group P63/m and a crystalline phase with a hexagonal crystal structure. The generated hydroxyapatite particles range in size from 500 to 900 nm, which is in the nanoscale.

*Keywords: Cattle bones; hydroxyapatite; synthesis process.*

## 1. INTRODUCTION

Cattle are one of the livestock species widely cultivated in Indonesia. In 2023, beef and buffalo meat consumption in Indonesia is estimated to reach 816.79 thousand tons, with a population of around 278.84 million people [1]. The large number of cattle slaughtered results in significant waste, including bones. Traditionally, cattle bones have been used as raw material for handicrafts and processed into flour, which serves as a mineral supplement in fish feed production. The abundance of this waste presents specific challenges for local researchers. Nevertheless, cattle bones contain high levels of calcium and phosphorus, which are essential elements in the formation of apatite compounds. One type of apatite that can be extracted from cattle bones is the biomaterial hydroxyapatite (HAp) [2].

Hydroxyapatite is a bioceramic material that constitutes a key component of hard tissues in living organisms, such as bones and teeth [3]. It has a crystal structure similar to that of bone and exhibits bioactive, biocompatible, and bioresorbable properties, making it suitable for use as a bone implant [4]. The bioactive characteristic indicates that hydroxyapatite bound to bone tissue can stimulate specific biological responses [5]. Its biocompatibility suggests that hydroxyapatite can integrate with the body without causing rejection reactions [6]. The bioresorbable nature means that the porous surface of hydroxyapatite can serve as a site for bone cell growth. Hydroxyapatite is a calcium phosphate compound with the chemical formula  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$  and has a hexagonal structure. To form hydroxyapatite, a molar ratio of Ca/P of 1.67 is required. It is one of the most stable calcium phosphate compounds and shows high potential in tissue engineering. The crystalline calcium phosphate compounds consist of four phases: dicalcium phosphate ( $\text{CaHPO}_4$ ), octacalcium phosphate ( $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$ ),

tricalcium phosphate ( $\text{Ca}_3(\text{PO}_4)_2$ ), and hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ) [7].

So far, most of the demand for hydroxyapatite has been met through imports from countries such as Japan, the United States, and Switzerland, due to the high prices of hydroxyapatite in Indonesia. However, Indonesia produces 101.25 tons of hydroxyapatite per year, which has not been fully utilized. Considering that cattle are one of the major livestock species in Indonesia, research on the production of hydroxyapatite from cattle bone waste can make a significant contribution to addressing this issue. Cattle bones consist of 93% hydroxyapatite (HA) and 7%  $\beta$ -tricalcium phosphate ( $\text{Ca}_3(\text{PO}_4)_2$ ,  $\beta$ -TCP) (Brakat et al., 2008). They contain a high calcium content of approximately 85.84% [8]. This substantial calcium content allows for its utilization in the synthesis of hydroxyapatite.

Hydroxyapatite is an apatite mineral that is currently under extensive development, although its availability remains limited. With the increasing demand for this biomaterial, further research is needed to advance the synthesis of hydroxyapatite. The synthesis process can utilize natural calcium sources [5]. Natural calcium sources for hydroxyapatite synthesis include limestone [9] crab shells [10] and cattle bones. This study uses cattle bones as the raw material for hydroxyapatite synthesis. To transform cattle bones into hydroxyapatite powder, they must be heated to 1000°C until a weight loss occurs, indicating the release of elements from the bones [4]. This research aims to synthesize hydroxyapatite through a precipitation method using cattle bones as the raw material.

## 2. EXPERIMENTAL DETAILS

This study was conducted through several stages, including the formation of cattle bone powder (CaO), synthesis of hydroxyapatite, and characterization of hydroxyapatite using XRD (X-ray Diffraction) and SEM (Scanning Electron Microscopy).

## 2.1 Formation of Cattle Bone Powder (CaO)

Cattle bones used in this study were sourced from waste generated by meatball vendors around Universitas Kristen Indonesia, Jakarta. The bones were cut into small pieces and washed with aquades. Subsequently, the bones were boiled for 6 hours to remove any adhering fat. After boiling, the bones were washed again with aquades, sun-dried, and thoroughly dried. The mass of the dried bones was measured before the calcination process. In the next stage, 500 grams of crushed, dried cattle bones were heated to 1000°C for 8 hours. The resulting material was then ground using a mortar.

## 2.2 Synthesis of Hydroxyapatite

CaO and  $(\text{NH}_4)_2\text{HPO}_4$  were weighed using an analytical balance, with 2.83 grams of CaO powder from cattle bones and 3.97 grams of  $(\text{NH}_4)_2\text{HPO}_4$ , resulting in a Ca/P ratio of 1.67:1. Each material was then placed into 250 mL beakers. Next, 100 mL of aquades was added to each beaker containing CaO and  $(\text{NH}_4)_2\text{HPO}_4$ , and the mixture was stirred. The  $(\text{NH}_4)_2\text{HPO}_4$  solution was then introduced via infusion and flowed into the CaO/aquades mixture at a flow rate of 10 mL/min, accompanied by stirring at a speed of 350 rpm using a magnetic stirrer. The mixture was allowed to stand at room temperature for 24 hours. After that, the mixture was filtered, and the resulting precipitate was dried in an oven at 110°C for 3 hours, followed by heating in a furnace at 900°C for 5 hours. The final product was then weighed using an analytical balance.

## 2.3 Characterization of Hydroxyapatite Using XRD (X-ray Diffraction) and SEM (Scanning Electron Microscopy)

The obtained hydroxyapatite was then characterized using XRD (X-ray Diffraction) and SEM (Scanning Electron Microscopy) methods. XRD (X-ray Diffraction) was employed to evaluate the crystal structure, phases, and

structural properties of the material through the analysis of the X-ray diffraction patterns produced by the sample. Meanwhile, SEM (Scanning Electron Microscopy) was used to assess the surface morphology and microstructure of the sample.

## 3. RESULTS AND DISCUSSION

### 3.1 Efficiency of Hydroxyapatite from Cow Bones

The CaO compound used in the synthesis process is derived from cow bones and has been calcined for 10 hours. The product of this synthesis is hydroxyapatite in powder form. The efficiency level of the hydroxyapatite produced from the synthesis process can be seen in Table 1.

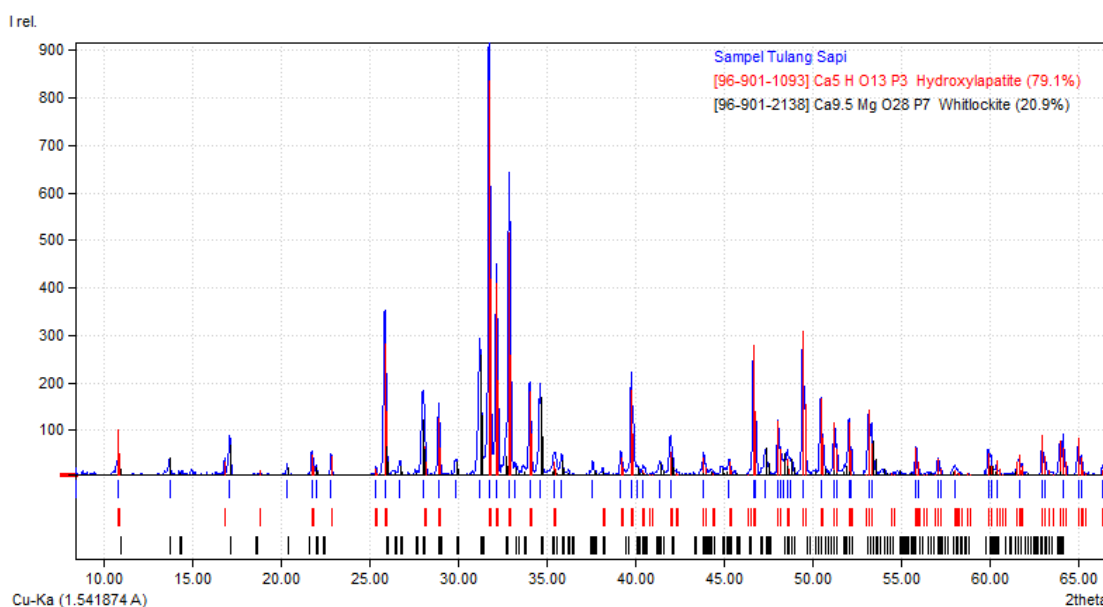
The mass of hydroxyapatite (HAp) produced is smaller than the total mass of CaO and  $(\text{NH}_4)_2\text{HPO}_4$ . This is due to heating in the oven during the mixing process, which causes evaporation of liquid components and reduces the final mass of HAp. Additionally, HAp that has been precipitated is filtered before drying, leading to some mass loss along with aquades. Using a filter with smaller pores might improve the efficiency of the obtained hydroxyapatite. The recorded efficiency of HAp is 34.85%, indicating that the yield is not yet optimal. The very fine texture of HAp causes some material to pass through the filter paper. For comparison, from 2.83 grams of CaO used in the synthesis, 2.37 grams of hydroxyapatite were obtained.

### 3.2 Results of XRD (X-ray Diffraction) Analysis on Cow Bones

Hydroxyapatite produced from CaO derived from cow bones and  $(\text{NH}_4)_2\text{HPO}_4$  has a final mass of 2.37 grams. This hydroxyapatite compound was then analyzed using XRD (X-ray Diffraction). The diffraction pattern of hydroxyapatite obtained from the XRD (X-ray Diffraction) analysis is shown in Fig. 1.

Table 1. Efficiency level of hydroxyapatite produced

Source of CaO	Mass (grams)			Efficiency (%)
	CaO	$(\text{NH}_4)_2\text{HPO}_4$	HAp	
Cow Bones	2.83	3.97	2.37	34,85



**Fig. 1. XRD (X-ray Diffraction) Diffraction Pattern of Hydroxyapatite Produced from CaO Derived from Cow Bones, After Calcination for 10 Hours**

**Table 2. Structural Parameters of Hydroxyapatite Compound in the COD Database with ID 9011092**

Mineral name	Hydroxylapatite
Formula	Ca <sub>5</sub> H O <sub>13</sub> P <sub>3</sub>
Calculated formula	Ca <sub>10</sub> H <sub>2</sub> O <sub>2</sub> O <sub>i24</sub> P <sub>6</sub>
Title of publication	Significant precision in crystal structural details: Holly Springs hydroxyapatite Locality: Holly Springs, Cherokee County, Georgia, USA Sample: X-23-6
Authors of publication	Sudarsanan, K.; Young, R. A.
Journal of publication	Acta Crystallographica, Section B
Year of publication	1969
Journal volume	25
Pages of publication	1534 - 1543
a	9.424 Å
b	9.424 Å
c	6.879 Å
α	90°
β	90°
γ	120°
Cell volume	529.086 Å <sup>3</sup>
Number of distinct elements	4
Hermann-Mauguin symmetry space group	P 63/m

The measurement of the X-ray diffraction pattern and phase identification of the synthesized HAp sample using the precipitation method (Fig. 1) is characterized by diffraction peaks between 2θ angles of 10–65°. Phase identification of the sample was performed by comparing its diffraction pattern with hydroxyapatite

compounds in the COD (Crystallography Open Database). The measurement of the X-ray diffraction pattern and phase determination for the hydroxyapatite (HAp) sample obtained via the precipitation method (as shown in Fig. 1) reveals the presence of diffraction peaks at 2θ angles between 10° and 65°. Phase identification

was conducted by comparing the obtained diffraction pattern with hydroxyapatite data from the COD (Crystallography Open Database). The peaks visible in Fig. 1 were compared using software, and the results indicate that the diffraction pattern corresponds to the hydroxyapatite compound listed in the COD with ID 9011092. Data from the COD indicate that the produced hydroxyapatite is a crystalline phase with structural parameters listed in Table 2.

The crystal peaks in the XRD (X-ray Diffraction) data for the hydroxyapatite compound were identified at  $2\theta$  angles of  $25.88^\circ$ ,  $31.76^\circ$ ,  $32.18^\circ$ ,  $32.89^\circ$ ,  $46.68^\circ$ , and  $49.47^\circ$ . However, very high-intensity peaks that do not correspond to hydroxyapatite appeared at  $2\theta$  angles of  $17.12^\circ$ ,  $28.01^\circ$ ,  $31.24^\circ$ , and  $34.63^\circ$ . After comparing these peaks using software, the diffraction pattern was found to correspond to the Whitlockite compound listed in the COD (Crystallography Open Database) with ID 9012137. Information from the COD indicates that the formed Whitlockite is a crystalline phase with structural parameters listed in Table 3.

The peaks with the highest intensity indicate that hydroxyapatite is the dominant component, while the whitlockite compound has lower intensity

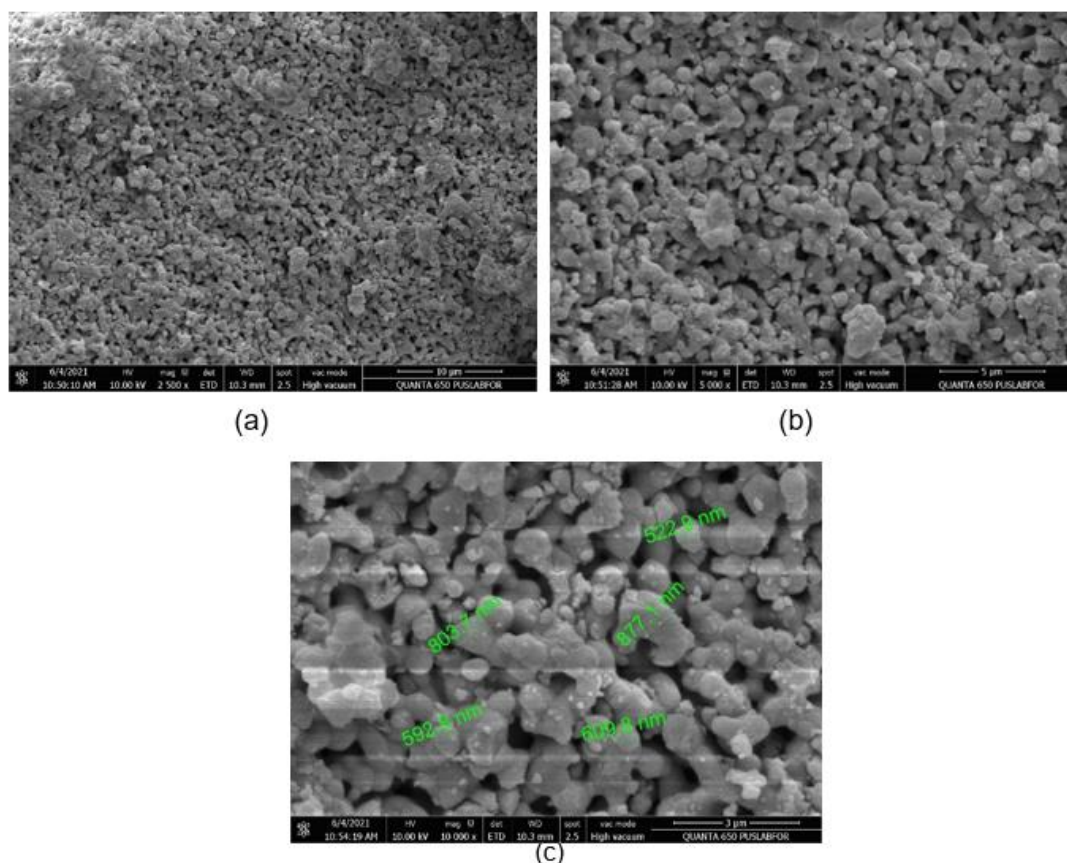
peaks. The XRD (X-ray Diffraction) characterization graph shows that the percentage of hydroxyapatite obtained reaches 79.1%, significantly higher than the 20.9% for whitlockite. The presence of whitlockite may be due to pH instability during synthesis; if the pH drops to acidic levels ( $\text{pH} < 4.2$ ), the formation of whitlockite can occur more readily (Cheng, 1988). Nonetheless, the presence of whitlockite is not harmful to humans, as this compound is often used in pathology, particularly in dental calculus. Additionally, whitlockite has higher mechanical strength compared to hydroxyapatite, which can enhance compressive strength when applied in the medical field.

### 3.3 Results of SEM (Scanning Electron Microscopy) Analysis on Cow Bones

Scanning Electron Microscopy (SEM) was utilized for the characterization of the synthesized sample to evaluate the surface morphology of HAp particles. Using SEM (Scanning Electron Microscopy), various physical characteristics of HAp, such as size, shape, structure, and crystal morphology, can be identified. The surface morphology of hydroxyapatite is displayed in Fig. 2.

**Table 3. Structural Parameters of Whitlockite Compound in the COD Database with ID 9012137**

Mineral name	Whitlockite
Formula	Ca <sub>9.5</sub> Mg O <sub>28</sub> P <sub>7</sub>
Calculated formula	Ca <sub>57</sub> Mg <sub>6</sub> O <sub>168</sub> P <sub>42</sub>
Title of publication	Crystallographic studies of the role of Mg as a stabilizing impurity in beta-Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> . II. Refinement of Mg-containing beta-Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> Locality: synthetic Sample: x = 0.29 Mg
Authors of publication	Schroeder, L. W.; Dickens, B.; Brown, W. E.
Journal of publication	Journal of Solid State Chemistry
Year of publication	1977
Journal volume	22
Pages of publication	253 - 262
a	10.337 Å
b	10.337 Å
c	37.068 Å
$\alpha$	$90^\circ$
$\beta$	$90^\circ$
$\gamma$	$120^\circ$
Cell volume	3430.2 Å <sup>3</sup>
Number of distinct elements	4
Hermann-Mauguin symmetry space group	R 3 c :H



**Fig. 2. SEM (Scanning Electron Microscopy) Results of Hydroxyapatite from CaO Source of Cow Bones at Magnifications: (a) 2500x, (b) 5000x, (c) 10000x.**

Fig. 2 shows that the hydroxyapatite particles exhibit agglomeration. This finding is consistent with the research by Hui et al. (2010), which also noted agglomeration in the observed particles. Individual particles generally have a spherical shape, with sizes varying from micro to nano scale. For the samples derived from cow bones, the formed hydroxyapatite has a size range of 500 to 800 nm. The observed sizes of individual particles varied, with the largest measuring up to 877 nm and the smallest around 522 nm (see Fig. 2). The size difference between the largest and smallest particles is not significant, allowing us to conclude that the characteristics of the individual particles formed are relatively uniform.

#### 4. CONCLUSION

The synthesis of hydroxyapatite (HAp) biomaterial using cow bones as a base material for bone filler was successfully carried out using the wet precipitation method. The synthesis results indicate that the produced HAp possesses a crystalline phase with a hexagonal

crystal system and space group P63/m. In addition to HAp, the synthesis process also yielded another compound, whitlockite ( $\text{Ca}_5\text{Mg}_6\text{O}_{16}\text{P}_4$ ), which has a crystalline phase and a trigonal crystal system (hexagonal axis) with space group R3c. The formed hydroxyapatite exhibits agglomeration, with individual particles generally having a spherical shape. The size of the produced hydroxyapatite particles is in the nanoscale, ranging from 500 to 900 nm.

#### DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

#### COMPETING INTERESTS

Author has declared that no competing interests exist.

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