

## Obtaining and characterization of hydroxyapatite derived from bovine bone for the manufacture of bone cement

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### ABSTRACT

The production of stoichiometric hydroxyapatite (HAp) from animal by-products, such as bovine femur, represents a sustainable and innovative alternative, as it enables the utilization of raw materials that would otherwise be discarded. In the biomedical field, HAp is a highly relevant bioceramic, particularly for bone regeneration, due to its excellent biocompatibility, osteoinductive properties, and structural similarity to the mineral component of human bones. However, its high cost limits its application in certain cases. For this reason, the objective of this study was to synthesize stoichiometric hydroxyapatite from biogenic sources using hydrothermal methods, evaluating the production process, its characterization, and the preparation of bone cement. The study showed an average yield of 4.1% by weight, which could increase up to 8.5% under ideal conditions, when the raw material is free of organic matter. Although this yield is higher than that reported in other studies, it remains relatively low, limiting its viability on a small scale. X-ray fluorescence (XRF) analysis revealed the Ca and P contents of 40.02% and 15.43%, respectively, with a Ca/P ratio of 2.59 – higher than the values typically reported in the literature – suggesting an excess of calcium in the final product. To evaluate the quality of the material, bone cement was prepared and subjected to compressive strength tests at intervals of 1, 3, 7, 11, and 28 days. The tests showed a maximum compressive strength of 537.78 kPa, significantly below the minimum required for biomedical applications. These results highlight the need to optimize the synthesis and processing of HAp to improve its feasibility for future applications.

**Keywords:** hydroxyapatite, HAp, XRF, Ca/P, bone cement.

### INTRODUCTION

Cattle slaughtering plays an important part in food supplies production and contributes significantly to human nutrition. This process provides approximately 15% of food energy source and 25% of protein intake worldwide (Santana, 2020). In Latin America, an important producer

and exporter of cattle meat, the period between 2019 and 2021 being the highest in export volume and profit. During this period the region exported more than 1.5 billion of dollars in cattle meat, with a total of 2 million cattle heads (Food & Agriculture Org. United Nations, 2024).

The process of cattle slaughtering produces considerable waste. Studies show that at least

27.5% of animal weight is discarded in form of sub-products, as hair, bones and secretion (Singh & Singh, 2022). Waste management and treatment present a considerable challenge from an economic and environmental points of view. For this reason, it is necessary to improve the practice of waste management in the meat industry and lessen its environmental impact.

The literature argues for various alternative methods for bone recycling from meat industry, such as bio-ceramic production (hydroxiapatita) and its use in medicine due to its osteoinductive without local toxicity; the use of catalysts for the production of methyl esters from fatty acids with applications in fuel, bioplastic improvement, ethanol production, as well as bone flour production for the bioremediation of tributaries as coagulants, as a chemical adjuvant for sewage, for obtaining collagen, etc. (Chaveanghong et al., 2017; Clementz et al., 2019; Adeleke et al., 2016; Mondal et al., 2023). Obtaining HAp is a process of great interest which can be carried out in different ways, such as precipitation, sol-gel chemical synthesis and hydro-thermic methods (Adhikara et al., 2024; Ferrairo et al., 2023). HAp is a material with diverse properties which are useful in many fields; in addition, the material can be obtained from cattle resources, thus, besides being a polyfunctional material, it is possible to mitigate its environmental impact (Mondal et al., 2023; Borges et al., 2022).

Time and technological progress have made it more convenient to use byproducts and waste to increase the added value of products, reduce costs and minimize pollution. In this context obtaining HAp from synthetic means is costly in terms of production and long-term synthesis (Adhikara et al., 2024). For this, obtaining HAp through natural means may offer higher flexibility for operational costs, depending on the raw materials that are used, and, thus it is a more eco-friendly and cheaper method (Londoño et al., 2018).

The high demand for hydroxyapatite (HAp) is due to its notable properties, such as biocompatibility, bioactivity, low polymerization exotherms, non-toxicity, and rapid curing time, which make it a key material for various current applications. Additionally, it possesses osteoinductive and osteoconductive properties, enabling bone regeneration and promoting integration with surrounding tissue (Londoño et al., 2020). However, despite its excellent properties, pure HAp has limitations regarding mechanical strength and brittleness. A

strategy to overcome these drawbacks is the addition of tricalcium phosphate (TCP) to the solid phase, as this bio-ceramic helps enhance the compressive strength and stiffness of material (Reyes et al., 2018). This research addresses two key aspects: resource reuse, specifically the exploitation of bovine bone through hydrothermal methods, and the evaluation of the resulting HAp for manufacturing bone cement, ensuring compliance with current regulations.

## MATERIALS AND METHODS

The selection of bovine femur as the raw material is based on its high bone density, making it an ideal choice to maximize HAp yield. This bone has a more compact and resistant structure compared to other parts of the animal (Šromová et al., 2023), and it produces more yield from extraction. At the same time, a statistical analysis was carried out in the waste collection area to identify which part of the bovine is most frequently discarded. The results indicate that the femur possesses low commercial value in the locality, which makes it an ideal material for analyzing its recycling potential.

### Summary of the process

The preparation of the raw material involved two stages of cleaning and weighing throughout the entire process, with the aim of determining the yield per kilogram of bone. The first cleaning stage consisted of immersing the bone in boiling water to denature the proteins and facilitate the removal of most of the flesh, blood, fat, and other soft tissues. The second stage involved an alkaline hydrothermal process, in which a 2% w/v NaOH solution at 3.12 M and 100 °C was used to remove residual surface fat. The next phase was drying at 160 °C in a Genesis LT3140 oven for 7 hours – a crucial step to weaken the outer layers of the bone and facilitate subsequent crushing and grinding processes (Adhikara et al., 2024).

As a secondary phase, the bone was crushed using a knife mill (1000A) to obtain millimeter-scale particles, which facilitated subsequent bleaching with a 10% w/v oxalic acid (C<sub>2</sub>H<sub>2</sub>O<sub>4</sub>) solution under agitation for 2 hours. This process effectively removed most of the organic and microbial matter. The sample was then washed with distilled water, filtered through a 2 µm porous

bed, and dried in the previously mentioned oven at 160 °C for 2 hours. Afterward, a sufficiently small particle size was achieved to improve the calcination process; for this purpose, the sample was ground using a disc and blade mill (Wiley TE-680) and sieved with a No. 200 mesh to isolate the finest particles (Ferrairo et al., 2023; Palma et al., 2024).

Finally, during the calcination phase, a *Thermolyne* muffle furnace (FB1410M) was used to heat the sample to 720 °C at a rate of 9 °C/min for 8 hours, with the aim of eliminating residual organic matter such as collagen. This temperature threshold was not exceeded, as previous studies indicate that at this level, nanocrystalline HAp is formed; these crystals have a size that allows for more efficient powder compaction, thereby enhancing the mechanical properties of the material for future use (Londoño et al., 2020; Londoño et al., 2018; Haugen et al., 2019).

### XRF characterization

HAp samples, X-ray fluorescence (XRF) spectroscopy was used to identify and characterize the components present in the HAp samples. This analysis was conducted at the Extractive Metallurgy Laboratory of the Escuela Politécnica Nacional (EPN) main campus in Quito, Ecuador. XRF was selected for its ability to detect elements with low atomic numbers, allowing for the determination of the average atomic number of the samples. This approach was essential for accurately identifying the components of the biological material, particularly calcium (Ca), the predominant element in bone composition. One of the main advantages of the XRF technique is that it is non-destructive, which enabled the analysis of samples without compromising their physical integrity (Pessanha et al., 2019).

### Bone cement

The preparation of the cement consisted of two phases. In the solid phase, a mixture of TCP and HAp was prepared in different proportions to evaluate compressive strength. Tests were conducted using three different ratios: 1:39, 1:1.85, and 1:1, corresponding to weight ratios of 2.5% HAp and 97.5% TCP; 35% HAp and 65% TCP; and 50% HAp and 50% TCP, respectively. The liquid phase consisted of a 2.5% w/v aqueous solution of Na<sub>2</sub>HPO<sub>4</sub> in distilled water, used as

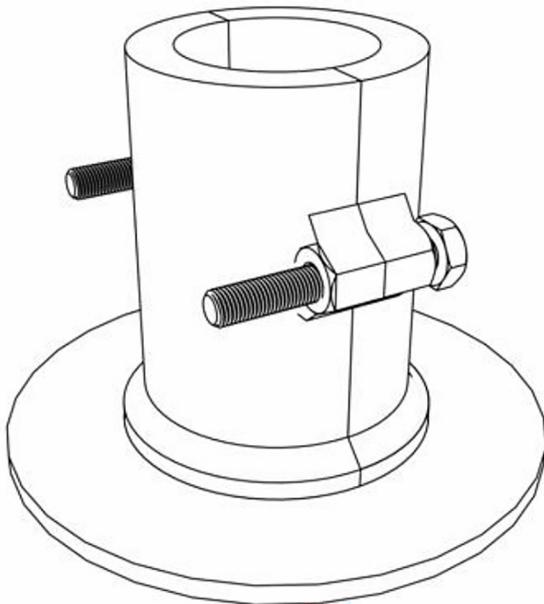
a setting accelerator to promote the hardening of the mixture. According to (Acuña et al., 2015), an optimal liquid-to-powder (L/P) ratio is 0.5 mL/g; however, this ratio does not provide sufficient consistency for placement into molds. Therefore, additional aqueous solution was added until a paste with the necessary consistency and workability was achieved, reaching an average L/P ratio of 0.71 mL/g.

The compression tests were carried out in accordance with the INEN 1573 standard. Although this standard is originally designed for testing the compressive strength of construction cements, it was adapted in this study to evaluate the compressive strength of bone cement, as it provides a standardized methodology for measuring material strength under compression. The procedure followed the specifications of the standard, with appropriate adjustments to accommodate the biocompatible materials used. Molds measuring 3.5 cm in height and 1.75 cm in diameter were used, maintaining a height-to-diameter ratio (H/D) of 2 (Ecuadorian Institute of Standardization, 2010). These molds were designed with the required dimensions and subsequently 3D printed using polylactic acid (PLA) material. A coupling system with M3x25 mm screws was incorporated to facilitate the demolding of the cement (Figure 1).

The test was conducted using a *Trúmax* SG-5T electric compression machine. Three samples were evaluated to measure compressive strength under the following conditions: 48 hours of setting and 24 hours of curing at a controlled room temperature of 25 ± 1 °C, with the aim of determining the optimal mix ratio based on the results obtained. Once the most effective ratio was identified, additional compression tests were performed after 3, 7, 11, and 28 days of curing, in accordance with the aforementioned standard (Ecuadorian Institute of Standardization, 2010).

## RESULTS AND DISCUSSION

The analysis conducted through X-ray fluorescence (XRF), using the X S8 Tiger equipment along with the Spectra Plus software, enabled the determination of the solid-state particle composition, with results expressed as weight percentages. A key parameter evaluated was the calcium-to-phosphorus (Ca/P) ratio, a fundamental indicator of the quality of the synthesized HAp. The results revealed a high concentration of both



**Figure 1.** Visual representation of the mold used for obtaining cement

calcium and phosphorus – essential elements in HAp composition – with a Ca/P ratio of 2.59 (Table 1). This value is significantly higher than the stoichiometric ratio of 1.67, which is considered optimal for HAp according to previous studies (Borges et al. 2022; Londoño et al. 2020; Lu et al. 2018). A Ca/P ratio higher than 1.67 is desirable, as it contributes to greater thermal stability during the calcination phase, promoting the growth of HAp nanocrystals while minimizing dehydroxylation and material decomposition. Deviations in the Ca/P ratio can significantly impact the physical properties of HAp, particularly its mechanical

**Table 1.** Weight percentage of HAp

Elements	Weight percentage (%)
Ca	40.02
P	15.43
Na	0.94
Mg	0.67
Al	0.21
Ce	0.11
Si	0.08
Cl	0.08
Sr	0.06
Fe	0.04
S	0.03
K	0.02
Zn	0.02

behavior and dissolution capacity. The difference observed in this study is likely attributable to the nature of the raw material used (Figure 2).

Another crucial factor in the process was the yield of HAp obtained, which was estimated at 4.1% by weight of the final material. This means that approximately 40.52 g of HAp can be extracted from each kilogram of processed bovine bone. However, it is important to note that this yield may vary depending on the amount of organic matter present in the bones, such as meat, fat, and other non-mineral components. To obtain a more accurate figure for the HAp yield per gram of bovine bone, it is essential to consider the bone weight after a second cleaning phase, which removes most of the residual organic matter. Following this secondary cleaning process, the yield improves significantly, reaching 8.5%, which corresponds to 86.52 g of HAp per kilogram of clean bovine bone (Table 2).

Previous studies report that in the extraction of HAp it is possible to obtain a yield of 13.5% when considering the dry fraction (Borges et al., 2022). This suggests that the extraction processes of HAp from biogenic sources such as animal bones typically yield between 10% and 20%, depending largely on the organic matter content of the raw material. These variations in yield highlight the importance of thorough cleaning procedures to maximize the recovery of high-quality HAp.

On the basis of the above, the solid mixture was formulated in three different proportions to evaluate its durability and hardness. Sample MA3 yielded the best results in the compression test, as shown in Table 3. This formulation consisted of a 1:1 ratio of HAp to TCP, combined with a 2.5% w/v aqueous solution of  $\text{Na}_2\text{HPO}_4$ , and a liquid-to-powder ratio of 0.71 ml/g. Four replicates were prepared, each undergoing a curing process of 3, 7, 11, and 28 days prior to their respective tests (Table 4).

The results indicate that compressive strength increased with curing time. However, the maximum value recorded at 28 days reached only 0.53778 MPa, which is significantly below the minimum requirement of 40 MPa established by the INEN 1573 standard (Ecuadorian Institute of Standardization, 2010). It is important to note that ISO 5833 (International Organization for Standardization, 2002), applicable to surgical implants made of acrylic bone cements, establishes a minimum compressive strength of 70 MPa. However, this standard was not considered in the present study, since the material used is not an acrylic resin. In this case, the setting time is longer, as

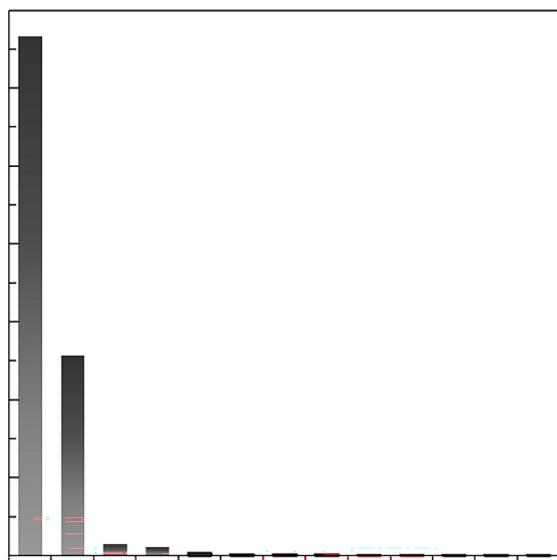


Figure 2. Frequency of elements that compose HAp

the hardening process involves a hydration reaction occurring through micropores. This behavior more closely resembles that of reinforced concrete, due to the use of a solid phase combined with an additive ( $\text{Na}_2\text{HPO}_4$ ), rather than the rapid polymerization characteristic of acrylic-based systems (Figure 3).

Data analysis allowed the identification of three factors that may have influenced the final

results. First, the Ca/P ratio in the cement composition, as the experimentally obtained ratio from bovine bone was 2.59. Previous studies indicate that Ca/P values above 3 tend to result in lower compressive strength compared to the formulations with ratios of 1.67 or 1.55, which are closer to the stoichiometric ideal for hydroxyapatite and are associated with improved mechanical performance (Borges et al., 2022; Londoño et al., 2020; Lu et al., 2018), which suggests that an elevated Ca/P ratio negatively affects the strength of the bone cement. Secondly, the particle size distribution of the raw material may have played a role, as the sieving process in this study was limited to a N. 200 mesh ( $70\ \mu\text{m}$ ). This resulted in larger particle sizes compared to those reported in the literature, which typically reference particle sizes around  $10\ \mu\text{m}$ . Larger particles reduce surface area and homogeneity in the mix, potentially impairing the setting and mechanical properties of cement (Londoño-Restrepo et al., 2020; Lu et al., 2018; Tugrul et al., 2020). This size difference – approximately seven times greater than the recommended particle size – leads to voids between particles, which reduce the overall strength of the cement. Finally, the purity of the TCP reagent also played a role in the mixing process, as the reagent used did not meet the necessary purity standards.

Table 2. Yields obtained from each stage of HAp synthesis

Treatment	Identification	Input*	Output *	Mass loss *	Mass loss (%)
1	Reception of raw materials	8.648	-	-	-
2	Primary cleaning	8.648	5.390	3.258	37.67
3	Secondary cleaning	5.390	4.05	1.340	24.86
4	First drying process	4.05	3.09	0.960	23.70
5	Raw material crushing	3.09	2.90	0.190	6.15
6	Bleaching	2.90	9.344	-	-
7	Filtration and washing	9.344	2.53	6.814	72.92
8	Second drying process	2.53	2.233	0.297	11.74
9	Sieving and milling	2.233	0.558	1.675	75.01
10	Calcination	0.558	0.350	0.208	37.28

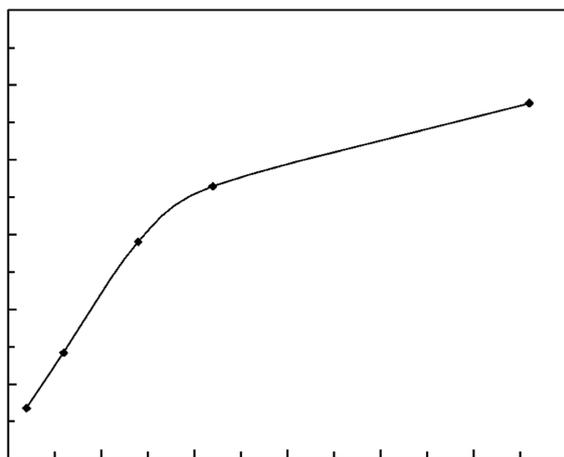
Note\*: kg.

Table 3. Variations in the mix with their respective compression strength

	HAp (%)	TCP (%)	L/P (mL/g)	Lb/F	MPa
MA 1	2.5	97.5	0.62	6	0.11126
MA 2	35	65	0.70	17	0.31525
MA 3	50	50	0.71	18	0.33379

**Table 4.** Compression essay from sample MA3

Sample MA3	Start date	Day of test	Days of curing	Lb/F	MPa
1	03/05/2023	05/05/2023	1	18	0.333794
2	03/05/2023	08/05/2023	3	20	0.370882
3	03/05/2023	12/05/2023	7	24	0.445059
4	03/05/2023	16/05/2023	11	26	0.482147
5	03/05/2023	02/06/2023	28	29	0.537779

**Figure 3.** Visual representation of resistance to compression vs cement age from HAp

This deficiency may have caused incomplete hydration, significantly compromising the compressive strength of the cement.

To obtain a Ca/P ratio closer to the stoichiometric value, the process that needs optimization is the calcination phase, by subjecting the sample to temperatures between 700 and 720°C, with a heating rate of 9°C/min, for at least 24 hours. In this case, it was only carried out for 8 hours due to the limited resources available for the research.

In general terms, the factors attributed to the low compressive strength were: the inadequate granulometry, in which a large particle size affected the strength of the bone cement by providing little surface contact between particles; additionally, the purity of the reagents used. Finally, the origin of the raw material influenced the results, since, being obtained from a slaughtered living organism, variables such as its health condition, diet, and age at the time of slaughter were not systematically controlled.

In the work conducted by Londoño et al. (2020), the authors mention properties such as biocompatibility, bioactivity, low exothermic polymerization, non-toxicity, and rapid setting time, which make it a key material today. Moreover,

it exhibits osteoinductive and osteoconductive properties, enabling bone regeneration and promoting tissue integration.

The origin of the raw material was not systematically controlled, because the aim of the study was to reuse a livestock by-product that is normally discarded; therefore, standard samples from selected cattle were not used as raw material, which is considered to have negatively influenced the results.

This study did not address the industrial scalability of the production process; it only considered the feasibility of obtaining HAp through the use of livestock waste. In this regard, it is important to clarify that, theoretically, this method could compete with the chemical synthesis of HAp due to the low cost of raw materials; however, since the present work does not include operational costs at an industrial scale, no definitive conclusion can be drawn.

## CONCLUSIONS

This study enabled the quantification of hydroxyapatite (HAp) obtained from bovine bone, yielding an average of 4.1% by weight of the processed raw material. This value can increase to 8.5% under ideal cleaning conditions and effective removal of organic matter. However, the amount of HAp obtained per kilogram of bovine bone in this study was relatively low. Combined with the significant resources required for processing, this makes small-scale HAp production using this method economically and operationally unfeasible.

XRF analysis revealed an excess of calcium in the recovered material, as indicated by a calcium-to-phosphorus (Ca/P) ratio of 2.59 – considerably higher than the stoichiometric value of 1.67, which is considered optimal for HAp. This imbalance in mineral composition suggests that the quality of the HAp may have been compromised, likely due to the nature of the raw material

used. An excess of calcium in HAp can alter its physicochemical properties, affecting both its stability and performance in biomedical application.

Compression test results were not favorable, as the sample was unable to withstand axial loads greater than 537.78 kPa, while the minimum acceptable value is 40,000 kPa. This outcome is attributed to several limitations of the study, including inadequate particle size distribution, which compromised the strength of the material, and the low purity of the reagents used. Additionally, the origin of the raw material had a significant impact, since it was derived from a slaughtered animal whose health condition, diet, and age at the time of slaughter were not systematically controlled. These variables introduce a degree of unpredictability in the quality of the final product.

Thus, although the method for obtaining HAp is viable, it presents challenges and inherent variability in product quality. In conclusion, further studies are recommended to optimize both the HAp extraction process and the selection of raw materials, in order to improve yield and material quality, making it more suitable for future applications.

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