

Preparation and Characterization of Hydroxyapatite Obtained from Bovine Bones

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Abstract– According to certain research, feedlots are a major source of unseparated solid and liquid organic wastes and residues, which contaminate the air, surface waters, and groundwater. Cattle bones are typically discarded before being fully exploited, therefore hydroxyapatite can be extracted from this bio-waste in an affordable, environmentally responsible way that adds value. The current research was carried out to examine the influence of the particle size of hydroxyapatite sintered at 1000 °C obtained from bovine bone to estimate its compressive strength and porosity in order to offer it a practical approach and enhance its long-term mechanical properties. The stoichiometry of the bovine bone powder was determined by a thermogravimetric analysis (TGA), which demonstrated that it is suitable for producing hydroxyapatite. Cleaning, boiling, drying at 150 °C, burning at 400 °C, and calcining at 900 °C were all performed on the cow bones. They were further crushed and sieved using screens of 300, 150, 75, and 53 µm aperture diameters. By uniaxially compacting specimens of hydroxyapatite powder at a pressure of 1 MPa and sintering them at 1000 °C, the specimens were created. By analyzing the sintered hydroxyapatite powders using X-Ray Diffraction (XRD) and Scanning Electron Microscopy (SEM), the powders were given a specific character. When samples with various grain sizes were evaluated, those with a grain size of 53 µm and a porosity of 44.24% had a greater compressive strength of 2.53 MPa.

Keywords– Hydroxyapatite, bovine bone, bioresidue, porosity, compressive strength.

I. INTRODUCTION

Because they produce a large amount of organic waste and residues that aren't usually segregated into solid and liquid forms, cattle slaughterhouses have recently emerged as highly polluting sources. These biological waste spills cause air pollution, surface, and groundwater contamination, and are a source of environmental risks. It should be mentioned that since these wastes can be used as a source of raw materials for the creation of biomaterials, attempts to valorize them are a helpful strategy for addressing these issues [1].

Engineering has been greatly impacted by the rise in environmental consciousness, and as a result, biomaterials are now more and more in demand. Hydroxyapatite (HA) is one of the most alluring biomaterials that have been used for many biomedical applications because of its characteristics that resemble those of natural bone. It is a kind of bioceramic made from calcium orthophosphate, a substance found in teeth and bones [2].

Production of hydroxyapatite has been ongoing from natural sources, primarily from biowaste (mammalian skeletal remains, poultry eggshells, fish bones and scales). The extraction of hydroxyapatite from this bio-waste is cost-effective, ecologically benign, and adds value because bovine bones are typically thrown without being fully exploited [3]. Additionally, using trash to create biomaterial can be good for the environment as a method of managing biowaste and lowering waste output.

The qualities of the generated product will depend on the kind of precursor used, and the synthesis processes employed. There are various ways to make hydroxyapatite, including dry, wet, and high-temperature techniques. However, these synthesis processes can be challenging or dangerous for biological systems, therefore recently they have been extracted by synthesis from biological sources. The microstructure features, such as grain and pore size, which are dependent on the processing parameters (particle size reduction) and sintering temperature, are crucial because they may have an impact on the mechanical capabilities of the hydroxyapatite [4].

Using discarded chicken femur bone, reference [4] investigated the effects of calcination temperatures of 500 °C, 700 °C, and 900 °C on the characteristics of natural hydroxyapatite. This thermal treatment revealed that the morphology, content, and crystallinity of hydroxyapatite were substantially associated with the calcination temperature. Additionally, its crystallinity and crystallite size increased dramatically when the sintering temperature was raised. Furthermore, they said that 700 °C would be the ideal temperature to create hydroxyapatite and that temperatures between 250 and 800 °C totally eradicated the organic phase, making accurate temperature management of the calcination process crucial. This study contributes to the research by explaining the rationale for producing hydroxyapatite from food industry waste, realizing a thermal analysis of the bone to understand how it decomposes during the calcination process, and performing XRD and SEM tests to identify the composition and crystallinity of the produced hydroxyapatite.

A study published in Reference [5] compared the physical, chemical, and mechanical characteristics of natural hydroxyapatite made from catfish bones and unseparated animal bones that underwent 900 °C of heat treatment. Catfish bones and hydroxyapatite made from unseparated animal bones both showed Ca/P ratios of 1.58 and 1.63, respectively. Both hydroxyapatite formed from catfish bones and hydroxyapatite derived from unseparated animal bones had fracture toughness values of 2.35 MPa.m^{1/2} and 5.72 MPa.m^{1/2},

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compressive strengths ranging from 0.47 to 1.92 MPa, and hardness values of 0.65 GPa and 0.48 GPa, respectively. The hardness values were also discovered to be in the range of human cortical bone, increasing the viability of catfish bone-derived hydroxyapatite for use in biomedical applications where compressive pressures are present. The exploration of the low pressure approach (1 MPa) for specimen compaction and the connection between porosity and the enhancement of particular mechanical qualities are both furthered by this work.

Reference [6] assessed the characteristics of hydroxyapatite ceramics produced by direct thermal conversion from catfish bones (CB) and unseparated animal bones (NB). Cold compaction was used to create representative scaffolds, which were subsequently sintered at temperatures of 900, 1000, and 1100 °C. They discovered that the CB-derived hydroxyapatite showed an increase in hardness (0.73 GPa) at 1000 °C, followed by a reduction. While NB-derived hydroxyapatite's hardness characteristics became less robust as the temperature rose. All samples' fracture toughness decreased as the sintering temperature increased from 900 to 1100 °C. Phase changes that occur during heat treatment are the causes of these gradients in mechanical measurements. This paper explains the rationale behind the sintering temperature of hydroxyapatite specimens and the tendency of high temperatures to reduce mechanical characteristics.

Reference [1] examined the effects of various sintering temperatures (900 °C, 1000 °C, and 1100 °C) on the mechanical and microstructural characteristics of naturally occurring hydroxyapatite biowaste produced by a cold compaction technique under low pressure (500 Pa). The characterization process employed XRD and SEM measurements. The hydroxyapatite powder demonstrated thermal stability while sintering at 1000 °C. Both 0.93 and 1.09 GPa of hardness, 4.28 and 6.20 GPa of Young's modulus, 1.87 and 2.21 MPa.m^{1/2} of fracture toughness, and 0.84 and 0.69 MPa and 0.69 MPa, respectively, of compressive strength without pressure and with 500 Pa of compaction pressure were produced. Consistently observing increased mechanical qualities, except for compressive strength because of stress created during compaction of the hydroxyapatite particles.

Reference [7] produced natural hydroxyapatite from chicken, goat, and bovine bones using cold isostatic pressing techniques at temperatures of 1100 °C, 1200 °C, and 1300 °C. They were characterized using XRD and SEM analysis, and in terms of mechanical characteristics, their hardness and compressive strength were assessed both before and after sintering. According to the study, hydroxyapatite extracted from cattle had a higher production efficiency than that of chicken and goat; the greatest hardness was 3.7 GPa at 1300 °C, while the highest compressive strength (0.44 MPa) was for the sample sintered at 1200 °C. The relative density increased as the sintering temperature climbed, rising from 12.44% at 1100 °C to a maximum of 30.3% at 1300 °C. This study adds to the investigation of the natural source that, when compared to chicken, goat, and cattle bones, had the highest efficiency of

hydroxyapatite formation, with the bovine bone source having the best outcomes.

Reference [8] employed a heat treatment in an air atmosphere at temperatures ranging from 600 °C to 1000 °C to generate hydroxyapatite from animal bone biowaste, specifically bovine, goat, and chicken bones. Following heat treatment above 700 °C, tricalcium phosphate (TCP) residues were found in the hydroxyapatite generated from goat and chicken bones, indicating phase instability, whereas hydroxyapatite derived from bovine bone shown good thermal stability. Energy-dispersive x-ray spectroscopy (EDS) was used to analyze the hydroxyapatite and determine its chemical composition. This method revealed that the sample's elemental composition is similar to that of natural bone. When the bovine bones were sintered at particular temperatures, a porous hydroxyapatite body with hardness values comparable to those of human cortical bone was created. The value of 387 MPa was obtained at a temperature of 750 °C, whereas the hardness of human cortical and cancellous bone is 396 MPa and 345 MPa, respectively. In comparison to the other two types of bones, the sintered chicken bone sample had higher levels of porosity and lower levels of hardness (200 MPa). This work adds to the body of knowledge regarding the characterization of hydroxyapatite, and in this instance, bovine bone was the natural source that produced the greatest outcomes.

Reference [9] investigated how particle size and calcination temperature affected the structural characterization of hydroxyapatite. The hydroxyapatite powders were sintered using the chemical precipitation technique. XRD, SEM, and Fourier-transform infrared spectroscopy (FTIR) were used for characterization. They acquired the increase in particle size, as well as the appearance and growth of grains, as a result of the rise in calcination temperature. They used X-rays to observe the presence of distinctive hydroxyapatite peaks at each of the three calcination temperatures, but due to thermal factors, they were unable to identify any hydroxyapatite disintegration phases. This study adds to the body of knowledge regarding the behavior of the hydroxyapatite grain size as well as the resulting crystallinity.

As a result, the goal of this study was to ascertain how particle size influenced the compressive strength and porosity of HA made from bovine bones that were sintered at 1000 °C. There has been limited research on the physical and mechanical properties of HA as a function of bio-residue particle size, despite some studies being done on the production and sintering of hydroxyapatite.

II. MATERIAL AND METHODS

A. Material

The object of study in this research was hydroxyapatite obtained by calcination and grinding of bovine bones. For its physical and mechanical characterization, 32 cubes of 25 mm of side were elaborated by uniaxial pressing.

B. Experimental design

A one-factorial experimental design was applied where the independent variable was grain size with four study levels, 300, 150, 75 and 53 μm and the dependent variables were compressive strength (MPa) and porosity (%). All tests were performed in quadruplicate.

C. Synthesis

10 kg of cattle bones were taken from the El Porvenir animal feedlot in Trujillo, Peru, and utilized as a source to make hydroxyapatite. Ten liters of distilled water were purchased and used throughout the procedure. To remove contaminants, distilled water was used to clean the raw bovine bones. The bone samples were then deproteinized by boiling them for 3 hours, rinsed once more with distilled water, and dried for 8 hours at 105 °C. They were then heated to 400 °C on a gas burner in order to burn off all organic material. Finally, the bones were calcined at 900 °C with a ramp rate of 5 °C/min in an electric oven, held for 2 hours, and then allowed to cool in the oven. The resulting samples were crushed with mortar and then sieved through 50 (300 μm), 100 (150 μm), 200 (75 μm) and 270 (53 μm) mesh.

D. Characterization

The XRD test was performed on the hydroxyapatite sample after the sintering process. A Rigaku diffractometer, model Miniflex 600 and a scanning speed of 2 theta of 2°/min was used.

The thermogravimetry test was performed on the bovine bone sample and the hydroxyapatite sample using Setsys Evolution TGA-DTA / DSC equipment.

The materials' microstructure was examined using a Tescan Vega 3 XMU SEM running at 10 kV. The microscope is fitted with an Oxford EDS detector for elemental analysis. The samples were prepared by sputtering gold at a low deposition rate onto their surface. Each sample was observed at 5000x and 7500x.

E. Powder compaction

With 5% water, 22 g of hydroxyapatite powder was prepared. It was put into a steel mold and compressed uniaxially with a Humboldt HM 3000 Press at 1 MPa and 1 mm/s. For experiments on compressive strength and porosity assessment, cube-shaped specimens measuring 25 mm on each side were obtained.

F. Sintering

The specimens were sintered in a muffle-type electric furnace at a temperature of 1000 °C for 2 hours at a heating rate of 5 °C/min.

G. Porosity measurements

The porosity of the sintered samples was determined by (1) [1,10].

$$\text{Porosity (\%)} = [1 - \text{Weight}/(\text{Volume} \times \text{Density})] \times 100 \quad (1)$$

The density of hydroxyapatite used was 3.16 g/cm³ [1].

H. Compressive strength

Using a Humboldt HM 3000 testing apparatus with a 50 kN load cell, the compressive strength of the pressed cubes was measured.

III. RESULTS AND DISCUSSION

A. XRD analysis of sintered HA samples

X-ray diffraction was performed to confirm the presence and crystallinity of hydroxyapatite in the sample obtained, as shown in Fig. 1, the test was performed on the hydroxyapatite sample sintered at 1000 °C.

The distinctive peaks of hydroxyapatite are seen in Fig. 1, demonstrating its presence and purity. The extremely sharp, narrow, intense, and well-defined peaks signify a higher degree of crystallinity in the compound's phases.

In the X-ray diffraction patterns, the presence of peaks characteristic of hydroxyapatite is observed. The main and highest intensity peak of hydroxyapatite is found at $2\theta = 31.70^\circ$, corresponding to the (211) plane, it is accompanied by three nearby peaks; the one at $2\theta = 32.20^\circ$, corresponding to the (112) plane, of lower intensity of the previous peak; the one at $2\theta = 32.90^\circ$, corresponding to the (300) plane of almost equal intensity, but higher than the previous one and a peak at $2\theta = 34.22^\circ$, smaller than the previous three, corresponding to the (202) plane. This set of four peaks and their respective relative intensities are indicative of pure hydroxyapatite of high crystallinity, as are the lower intensity peaks that are also present (ICCD-JCPDS data sheet # 9-0432).

This experiment proves that, as stated in [1], hydroxyapatite retains its purity phase up to a maximum sintering temperature of 1000 °C without disintegrating into biphasic hydroxyapatite.

B. Thermogravimetric analysis

Fig. 2 shows the graph of the TGA performed on two samples, bovine bone powder and sintered hydroxyapatite, obtained from the same bone.

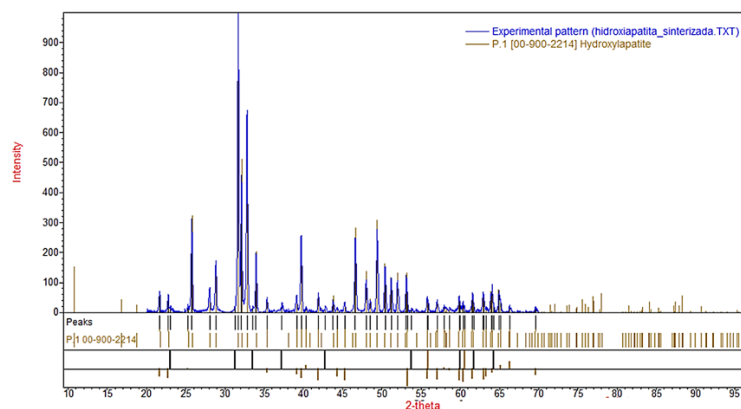


Fig. 1 X-ray diffraction patterns of the hydroxyapatite sample.

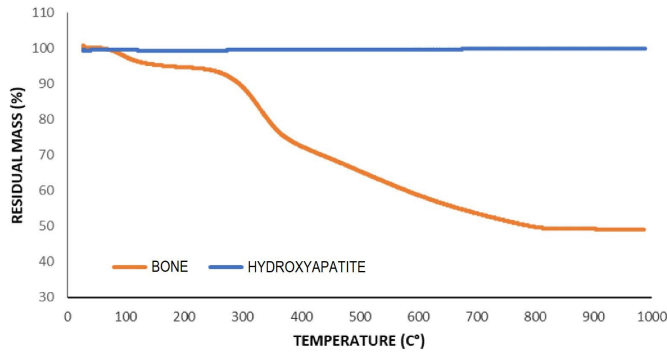


Fig. 2 TGA of bovine bone powder and hydroxyapatite powder.

The three stages of thermal decomposition can be seen in the orange-colored curve, which represents bovine bone powder. The first stage occurs between 100 – 200 °C, the second between 200 – 400 °C, and the third between 400 – 800 °C.

The first stage, occurring between 100 – 200 °C, results in a 10% mass loss due to desorption of water present in the bovine bone powder.

The second stage, occurring between 200 – 400 °C, results in a 22.22% mass loss attributed to the combustion of organic components (such as fats and collagen) in the bovine bone powder.

The third stage, occurring between 400 – 800 °C, results in 28.57% mass loss, which can be caused by residual organic matter and the decomposition of carbonate groups.

According to the results obtained, in the range of 200 – 400 °C, the incineration of all the organic components present in the bovine bone sample takes place. Reference [4] also indicates that from 250 °C onwards, the combustion of these components occurs. Due to this, a color change of the bone sample is observed, going from a brownish-yellow color to a gray powder that is attributed to the presence of ashes generated in the organic incineration process.

The blue line represents the hydroxyapatite obtained from the bovine bone powder, as shown in Fig. 2; as this material does not present mass loss, the curve is stable and remains constant throughout the thermogravimetric process.

C. SEM microstructure

Fig. 3 shows SEM micrographs of the hydroxyapatite sample sintered at 1000 °C at high magnification.

The grain boundaries can be seen in the densely packed surface morphology in Fig. 3. It confirms what is indicated in Reference [9], where they state that the phase transition starts at a temperature of 900 °C, where the β -TCP phase transforms to hydroxyapatite and there is a clearer formation of the grain.

According to Reference [11], the interconnection between the grain structures that are nearest to one another often defines the commencement of a definite crystalline grain structure of hydroxyapatite.

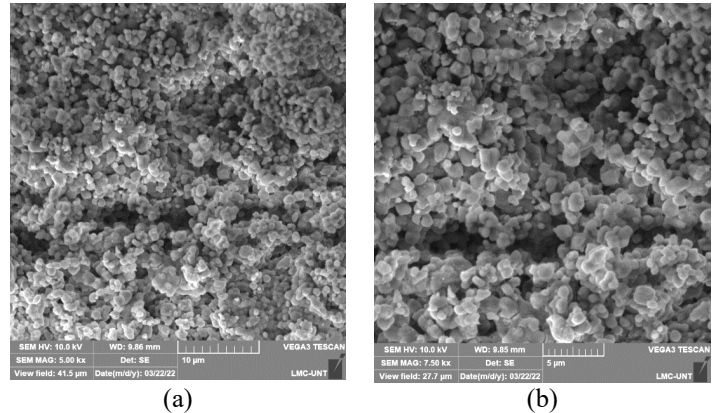


Fig. 3 SEM micrograph of hydroxyapatite sintered at 1000 °C. a) Micrograph at 5000x. b) Micrograph at 7500x.

D. EDS analysis

Fig. 4 presents the results of the EDS investigation into the elemental makeup of sintered hydroxyapatite. With traces of trace elements like magnesium (Mg) and sodium (Na), the three elements calcium (Ca), phosphorus (P), and oxygen (O) makeup most of the sintered samples. The elemental makeup of the samples, according to Reference [8], is strikingly close to the chemical makeup of real bone.

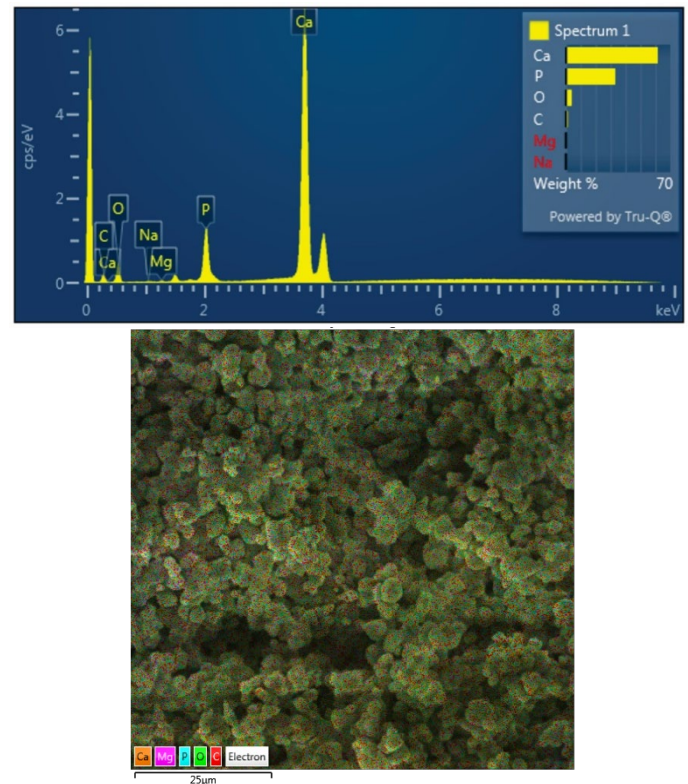


Fig. 4 EDS analysis of sintered hydroxyapatite at 1000°C.

TABLE I
ELEMENTAL COMPOSITION OF HYDROXYAPATITE SAMPLE

ELEMENT	Wt%
C	1.31
O	3.29
Na	0.02
Mg	0.07
P	36.23
Ca	59.08
Total	100.00

The elemental composition and weight percentage of the hydroxyapatite sample subjected to EDS analysis are shown in Table I. Calculated for the proportion of sample weight, the Ca/P ratio was 1.63. The predicted value for pure stoichiometric hydroxyapatite is 1.67, and the Ca/P ratio found in this experiment deviates somewhat from that value [12]. One of the variables that could explain this variation is the sintering temperature, which has an impact on the kind and composition of calcium-based compounds that would show up in the final hydroxyapatite bioceramic.

E. Compressive strength

The results of the compressive strength test of hydroxyapatite sintered at 1000 °C are shown in Fig. 5. The graph shows the variation of strength with respect to hydroxyapatite grain size.

The compression test was the type of test used on these materials. During this test, the specimen is subjected to compressive stresses in a direction parallel to the load application and more significant tensile stresses in a direction perpendicular to the load application. The specimen fails because of the stresses being greater than the material's strength.

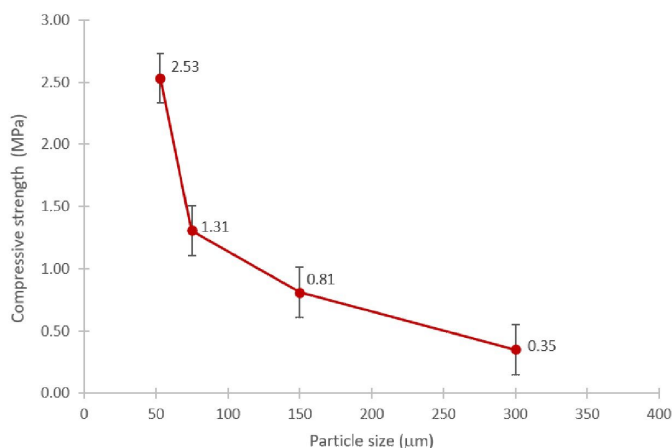


Fig. 5 Compressive strength test results of hydroxyapatite sintered at 1000 °C

Figure 5 demonstrates the relationship between compressive strength and particle size, demonstrating an 86% increase in strength from a minimum compressive strength of 0.35 MPa for a particle size of 300 µm to a maximum strength of 2.53 MPa for a 53 µm particle. The difference in compressive strength results from the fact that better compaction occurs with smaller particle sizes because there are fewer spaces between them (defects), which results in a better arrangement and a stronger material.

In their study, Reference [1] discovered that sintered hydroxyapatite samples with a particle size of 300 µm had compressive strengths that varied from 0.30 to 0.69 MPa. They found a compressive strength of 0.35 MPa with the same particle size. When comparing their results with the present study, it can be said that the strength grew as the particle size decreased, reaching a maximum value of 2.53 MPa at 53 µm.

It is confirmed that these results are better than those of Reference [7], who obtained a maximum compressive strength of 0.44 MPa for a hydroxyapatite sample sintered at 1200 °C. This improvement is attributed to the stability that hydroxyapatite exhibits at the sintering temperature of 1000 °C and that, at higher temperatures, it exhibits degradation.

F. Porosity

Fig. 6 shows the results of how the percent porosity of hydroxyapatite varies as a function of particle size.

Because larger particles leave more empty spaces in the formed specimens, the porosity of hydroxyapatite increases primarily as the particle size increases. For example, the porosity of hydroxyapatite was 44.89% at a particle size of 300 µm and 44.24% at a particle size of 53 µm.

A higher compaction pressure (1 MPa) was used in this study, resulting in lower porosity due to improved particle ordering. This is compared to Reference [1], whose research obtained a porosity percentage of 44.89% for a particle size of 300 µm sintered at 1000 °C at a compaction pressure of 500 Pa.

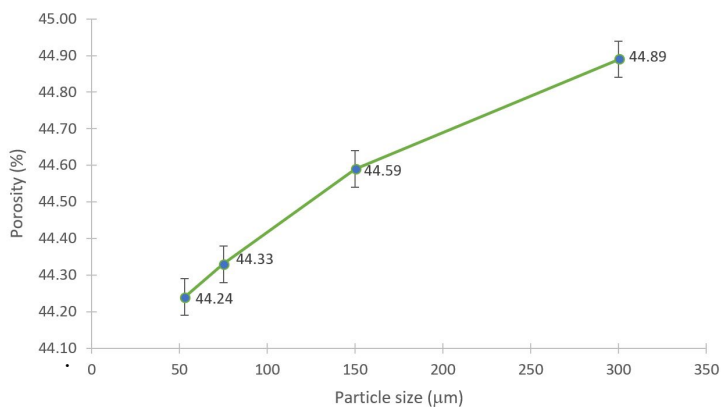


Fig. 6 Porosity results of hydroxyapatite sintered at 1000 °C

It may be inferred from Figs. 5 and 6 that porosity and compressive strength are closely related. The compressive strength of hydroxyapatite increases as the percentage of porosity decreases. The results are consistent with studies by other authors in References [1] and [10], which claim that the porosity of hydroxyapatite affects its mechanical properties and that a decrease in the porosity of the samples results in an increase in those properties. It was found that there is a relationship between the two by comparing the compressive strength results with the porosity results, as the compressive strength increases, the porosity decreases.

IV. CONCLUSIONS

The impact of particle size on the compressive strength and porosity of hydroxyapatite obtained from bovine bones sintered at 1000 °C was assessed. Results showed that a particle size of 53 µm had the highest compressive strength value of 2.5 MPa and a particle size of 300 µm had the highest value of 44.89% porosity.

The biological source synthesis technique was used to produce hydroxyapatite from the bones of cattle.

To assess the sample's purity and crystallinity, the XRD test was employed. The hydroxyapatite-specific peaks were seen in the diffraction patterns, and because they are very clearly defined, sharp, intense, and narrow, they suggest that the phases are more crystalline.

The TGA determined the hydroxyapatite's thermal stability, and in the bone sample under investigation, three stages of thermal decomposition were visible: the first stage, which takes place between 100 and 200 °C, is when water that is present in the powdered bovine bone is desorbed; the second, between 200 and 400 °C, is when the organic material is burned; and the third, between 400 and 800 °C, is when the carbonate groups are broken down.

The hydroxyapatite was found by SEM to have a surface that was densely packed, with grain boundaries visible in the morphology, as well as closer interconnectivity between the grain structure. In hydroxyapatite, this typically denotes the start of a distinct crystalline grain structure. According to the EDS test results, the three elements calcium (Ca), phosphorus (P), and oxygen (O) make up the majority of the sintered samples, with traces of other elements including sodium (Na) and magnesium (Mg). In terms of weight %, the sample's computed Ca/P ratio was 1.63, which deviates somewhat from the 1.67 theoretical value predicted for pure stoichiometric hydroxyapatite.

It was found that the compressive strength and porosity of hydroxyapatite made from bovine bone are considerably influenced by granulometry. Additionally, since the results demonstrate that the compressive strength obtained was higher (2.53 MPa) at a lower porosity (44.24%), it is possible to conclude that the compressive strength of hydroxyapatite depends on porosity.

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