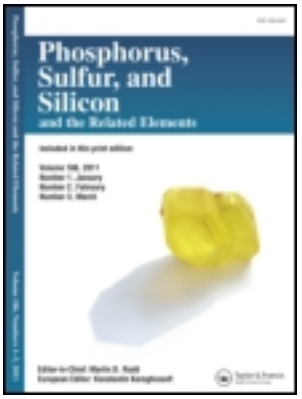

Phosphorus, Sulfur, and Silicon and the Related Elements

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gpss20

Chicken Bone as a Bioresource for the Bioceramic (Hydroxyapatite)

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Available online: 04 Jan 2012

To cite this article: R. Rajesh, A. Hariharasubramanian & Y. Dominic Ravichandran (2012): Chicken Bone as a Bioresource for the Bioceramic (Hydroxyapatite), Phosphorus, Sulfur, and Silicon and the Related Elements, 187:8, 914-925

To link to this article: http://dx.doi.org/10.1080/10426507.2011.650806

Phosphorus, Sulfur, and Silicon, 187:914–925, 2012

ISSN: 1042-6507 print / 1563-5325 online
DOI: 10.1080/10426507.2011.650806

CHICKEN BONE AS A BIORESOURCE FOR THE BIOCERAMIC (HYDROXYAPATITE)

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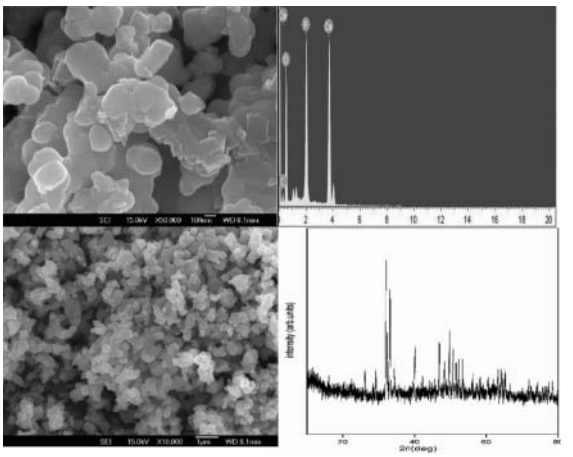
GRAPHICAL ABSTRACT



Natural hydroxyapatite (HAP) is isolated from waste chicken bone by thermal calcinations at different temperatures in the range of 200 °C to 1000 °C. The isolated HAP has been characterized using thermo gravimetric analysis (TG) and differential thermal analysis (DTA), Fourier Transformed Infrared spectroscopy (FTIR), X-ray diffraction (XRD), field emission electron microscope (FE-SEM), and energy dispersive X-ray (EDX). The XRD results showed that the enhanced crystallinity of HAP phase by thermal calcination above 600 °C and the crystal size has been found to increase with increasing temperature of thermal calcinations due to agglomeration. Value addition for the waste chicken bone is given by the isolation of useful bioceramics (HAP) and the optimum temperature for the thermal calcination is found to be 600 °C. The isolated HAP has been characterized as carbonated HAP of B type with the hexagonal structure.

Received 1 November 2011; accepted 11 December 2011.

The authors gratefully acknowledge the management of the VIT University, Vellore, India for the facilities offered to carry out this piece of work. The authors also acknowledge the powder XRD facility at SAS, VIT jointly funded by VIT and FIIST, DST India.

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These results will not only make the chicken bone as an important bioresource for the HAP but will also reduce the environmental pollution caused by dumping of the waste chicken bone.

Keywords Calcium phosphate; hydroxyapatite; chicken bone; calcination; FTIR; XRD

INTRODUCTION

The natural bone consists of 70% inorganic minerals, 20% organic material, and 10% water. The organic material is mostly made of type I collagen while the inorganic mineral consists of carbonated hydroxyapatite (HAP).[1] The biologically active calcium phosphate ceramic HAP [$Ca_{10}(PO_4)_6(OH)_2$] is biocompatible and this property has been found to play a vital role in various fields including drug delivery,[2] orthopedics, dentistry, maxillofacial,[3] bone, and wound tissue engineering.[4] Furthermore, it also finds applications in nonmedicinal fields such as gas sensor[5] and is used for the removal of nitrate from water.[6] Even though there are many reports available on synthetic methods for the preparation of HAP including sol-gel method,[7] polymer assisted synthesis,[8] mechanochemical method,[9] hydrothermal process,[10] and wet chemical method,[11] the synthetic parameters pH, temperature, reaction concentration, and mixing concentration have made the synthesis of HAP complicated. Moreover, the synthetic methods are more expensive and the HAP synthesized is found to exhibit lower biocompatibility, osteoconduction, and bioresorption. Above all, they do not have adequate biological properties compared to the natural carbonated HAP.[12,13] Recently, isolation of natural carbonated HAP has been found to be inexpensive and less complicated. One of the commonly used methods of isolation of natural HAP is thermal calcination.[12] The carbonate containing HAP has been found to be easily resorbed by living cells and also to posses higher solubility than carbonate free HAP. Further, it has also been found to lead to faster bone regeneration. The natural HAP can be A-type (carbonate group substituted by OH^-) or B-type (carbonate group substituted by PO_4^{3-}) and sometimes both types can coexist. Among them the B-type HAP has been found to resemble the biological apatite.[14–16]

There are a number of reports on the isolation of biocompatible HAP from natural sources including bovine bone,[13,17] pig bone,[12] fish bone,[14] bovine teeth,[18] human teeth, and pig teeth.[19] So far, there has been no report on the isolation of natural HAP from the chicken bone dumped as a waste leading to environmental problems. Hence, the value addition to the chicken bone by the isolation of HAP through thermal calcinations with the optimum temperature is reported. Moreover an attempt has also been made to introduce the chicken bone as an important source for HAP.

RESULTS AND DISCUSSION

General Observation

The color changes observed from the raw chicken bone when calcined at different temperatures are given in Table 1. The color of the raw chicken bone was observed as light yellow. The yellow color was found to change from light yellow to tanish, brown, light brown, and off-white when subjected to calcinations at 200 °C, 300 °C, 400 °C, and 500 °C. However, the color of the samples obtained from calcination between 600 °C and 1000 °C was white. This suggests the complete removal of organic matrix when the sample has been calcined between 600 °C and 1000 °C. The yield of the isolated HAP when calcined at

Table 1 Effect of calcination on color and residue of calcined chicken bone

Sample no.	Temperature (°C)	Time (h)	Weight		Residue (%)	Color
			Before calcination (g)	After calcination (g)		
1	Raw bone	—	—	—	—	Yellow
2	200	20	3.0005	2.7049	90.15	Tanish
3	300	20	3.0006	2.0679	68.92	Brown
4	400	20	3.0020	2.0671	68.86	Light brown
5	500	20	3.0014	1.9362	64.51	Off-white
6	600	20	3.0048	1.9053	63.41	White
7	700	20	3.0000	1.8086	60.29	White
8	800	20	3.0000	1.8080	60.27	White
9	900	20	3.0000	1.8058	60.19	White
10	1000	20	3.0000	1.8048	60.16	White

different temperatures 600 °C, 700 °C, 800 °C, 900 °C, and 1000 °C were 63.41%, 60.29%, 60.27%, 60.19%, and 60.16% respectively, indicating the maximum yield to at 600 °C.

Thermal Analysis of Chicken Bone

The thermo gravimetric analysis (TG) and differential thermal analysis (DTA) served as an important tool in identifying the temperature at which organic portion was removed from raw chicken bone. The TG and DTA analysis for raw chicken bone is shown in Figure 1. The weight loss observed in the temperature range of 30 °C to 150 °C can be attributed

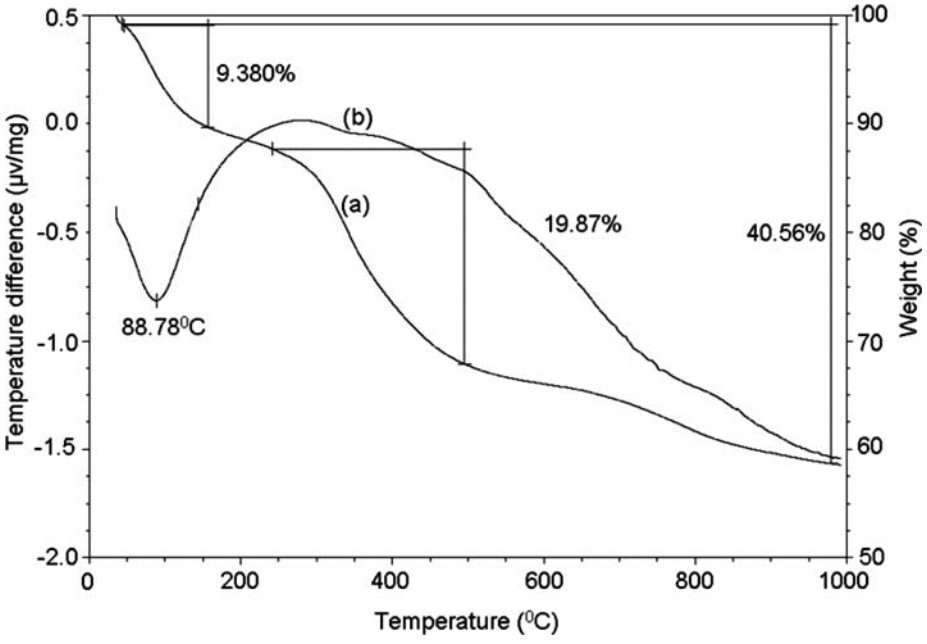

Figure 1 TGA (a) and DTA (b) curve for raw chicken bone from 30 °C to 1000 °C.

to the removal of incorporated water. This is supported by the endothermic peak observed at 88.78 °C in DTA curve. Further, there was a continuous loss of weight from 250 °C to 500 °C due to the removal of organic portion such as collagen and protein.[17,19,20] No significant weight loss has been observed during the calcinations above 600 °C confirming the complete removal of organic substances. Moreover, HAPs have been found to exhibit the characteristic mineral phase when the calcination temperature of the bone was above 600 °C. Furthermore, the weight loss during thermal calcinations in the temperature range of 30 °C to 500 °C of raw chicken bone was observed to be due to the removal of water (9.380%) and organic portion (19.87%).[17] The total weight loss in the TG/DTA studies till 1000 °C was found to be 40.56%.

FTIR Analysis

The Fourier Transformed Infrared (FTIR) spectrum is an important tool for identifying functional groups. It has been extensively used to identify groups including phosphate, carbonate, and hydroxyl in mineral research.[19] The FTIR spectra of raw chicken bone and HAP isolated through calcinations at various temperatures (200 °C–1000 °C) are shown in Figure 2.

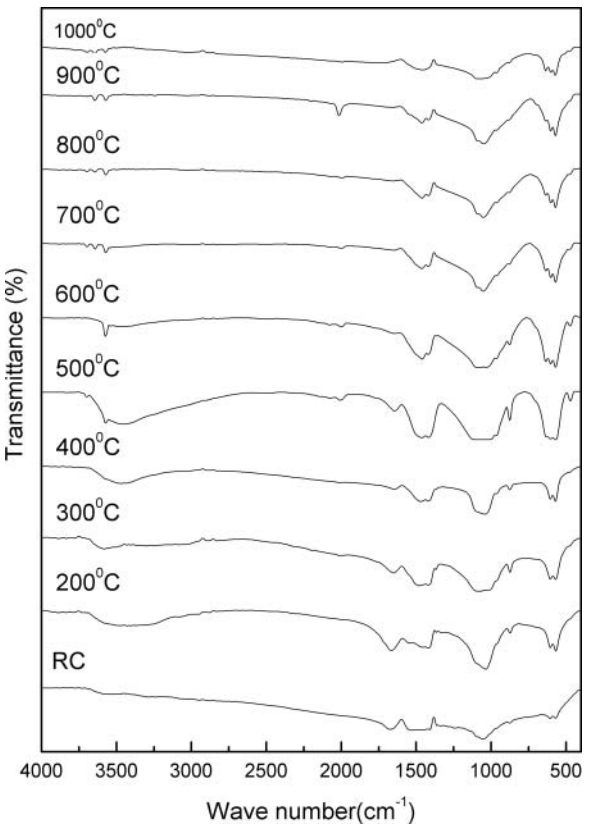

Figure 2 FTIR spectrum for raw chicken bone and calcined from 200 °C to 1000 °C.

The HAPs isolated from the chicken bone by thermal calcinations below 600 °C have not shown the characteristic bands of the HAP but the thermal calcinations of the chicken bone in the range of 600 °C to 1000 °C have exhibited the characteristic IR bands in the range of 4000 to 400 cm^{-1}. The samples which underwent thermal calcinations in the range of 200 °C to 500 °C showed characteristic band at ~1650 cm^{-1} due to the amide I band of the collagen[21,22] but with the increase in the temperature of calcinations above 600 °C the amide peak disappeared. This may be attributed to the complete removal of organic matrix present in the chicken bone at 600 °C.[17] Moreover, the appearance of an additional peak at 962 cm^{-1} due to the symmetrical stretching of phosphate (PO_4^{3-}) in the bones calcinated above 600 °C indicates the presence of HAP free from organic matter.

The four characteristic bands 1092–1049 cm^{-1} due to the symmetrical triply degenerate O—P—O bending (ν_3 PO_4^{3-}), the 962 cm^{-1}symmetrical nondegenerate P—O stretching (ν_1 PO_4^{3-}), the band at 570–569 cm^{-1}, 603–601 cm^{-1} triply degenerate antisymmetric and harmonic O—P—O bending (ν_4 PO_4^{3-}), and 472–470 cm^{-1} antisymmetric doubly degenerate P—O stretching (ν_2 PO_4^{3-}) for the PO_4^{3-} were observed in all bones calcined in the temperature range of 600 °C to 1000 °C. Further, the bands between 2200–1997 cm^{-1} due to the overtones and combination of the ν_3 and ν_1 PO_4^{3-} were also observed but the intensity of the ν_2 was found to decrease with the increase in the temperature of calcinations. With the increase of the calcination temperature, the relative intensities of the OH— vibration bands decreased, indicating the occurrence and development of dehydroxylation.[14,17,23–25]

The well-defined peaks at 634, 603–601, and 570–569 cm^{-1} indicate the crystalline nature of isolated HAP obtained by the thermal calcinations above 600 °C.[26] The samples calcined below 500 °C showed the broad band around 3570 cm^{-1}, which may be due to the association of organic moiety, Whereas the sample by thermal calcinations at 600 °C showed sharp and intense characteristic peaks of HAP at 3572 and 634 cm^{-1}.[25] The thermal calcinations of chicken bones in the range of 700 °C to 1000 °C show a decrease inOH— band at 3572 cm^{-1} with increasing temperature. This is attributed to the dehydroxylation of the OH— in the HAP.[17,25] The peak at 3643 cm^{-1} was assigned to OH— of $Ca(OH)_2$ and the adherent water.[20,25] This may be due to the decomposition of HAP with increasing temperature. The $Ca(OH)_2$ obtained by the decomposition of HAP with increasing temperature give out CaO which readily react with atmospheric water during handling and sampling.[25] Moreover, this is also supported by the energy dispersive X-ray (EDX) result and the Ca/P ratio.[20]

The HAPs isolated by the thermal calcinations of chicken bone in the temperature range of 600 °C to 900 °C also showed IR bands between 1462–1460 cm^{-1} and 1417–1415 cm^{-1} due to asymmetric stretching or ν_3 of carbonate. This is attributed to the type B carbonate groups. The high intensity band at 1417–1415 cm^{-1}, 1461–1453 cm^{-1} compared to the weak band at 875–872 cm^{-1} confirm the HAP derived from the chicken bone is B type.[17,23,26]

XRD Analysis

X-ray powder diffraction (XRD) is an analytical technique used for identification of phase and unit cell dimensions. The XRD pattern of raw chicken bone and the samples obtained by thermal calcinations in the range of 200 °C to 1000 °C was recorded and is shown in Figure 3. The crystalline nature and purity of isolated HAP have been confirmed from XRD analysis. The XRD results suggest that the stability of HAP present in the bone matrix was not disrupted when isolated from chicken bone by calcination in air up to 1000 °C. The XRD values of the isolated HAP from chicken bone by thermal calcinations

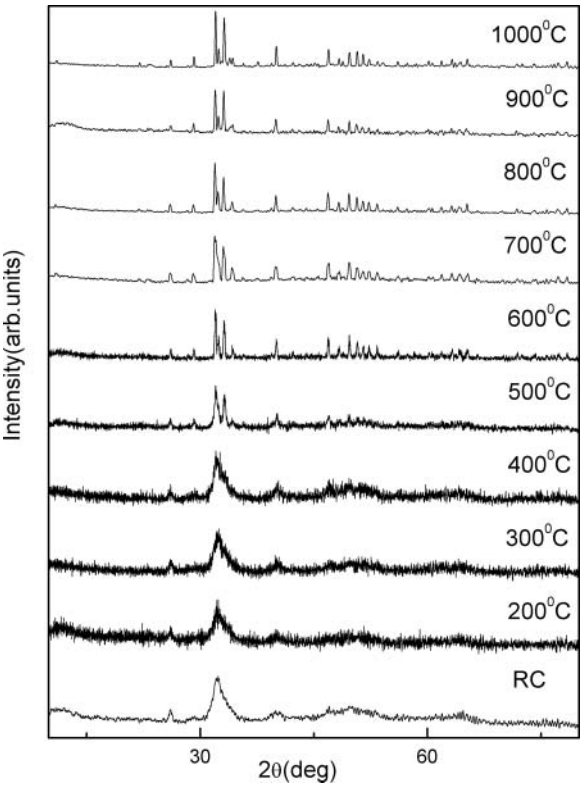

Figure 3 XRD pattern for chicken bone calcined from 200 °C to 1000 °C.

in the range of 600 °C to 1000 °C were found to be in good conformity with that of the standard HAP (JCPDS-09-0432/1996). The intensity of the peak increased and width of the peak decreased with increasing temperature. This could be attributed to the increasing crystalline nature and crystal size. The samples from thermal calcinations below 500 °C showed wider peak of low intensity indicating the incomplete removal of organic matter present in the bone matrix. However, thermal calcinations above 600 °C showed intense and sharp peaks indicating the crystalline nature and complete removal of organic matter. This is in good agreement with the reported literature.[17,19]

The interplanar distance d and angle 2θ of isolated HAP by thermal calcinations of the chicken bone in the range of 600 °C to 1000 °C were compared with standard JCPDS-09-0432/1996 value and are shown in Table 2. These values were found to be in close conformity with the standard values of the HAP. The minor variations in the angle (2θ) of isolated HAP with standard HAP is attributed to the dehydroxylation of the hydroxyl group which is very common at higher temperature calcinations.[16,19]

The lattice parameter (a and c) and unit cell volume (v) of the isolated HAP by thermal calcinations of chicken bone in the range of 600 °C to 1000 °C were calculated using (002) and (300) plane d-spacing values and shown in Table 3. The calculated values were found to match well with that of the standard values (JCPDS-09-0432/1996) for the hexagonal structure with the lattice parameters $a = b \neq c$.[1] Hence, the HAPs isolated by thermal calcinations in the range of 600 °C to 1000 °C were assigned hexagonal structure.

Table 2 The d-spacing and 2θ angle of HAP obtained by thermal calcination and compared with standard JCPDS value

	d-spacing (Å)						Position (2θ)					
hkl	JCPDS	600 °C	700 °C	800 °C	900 °C	1000 °C	JCPDS	600 °C	700 °C	800 °C	900 °C	1000 °C
002	3.440	3.410	3.417	3.417	3.410	3.410	25.90	26.10	26.00	26.06	26.11	26.11
211	2.814	2.795	2.795	2.799	2.795	2.790	31.80	31.99	31.99	31.93	31.99	32.04
112	2.778	2.754	2.785	2.763	2.758	2.758	32.22	32.48	32.04	32.30	32.43	32.43
300	2.720	2.701	2.709	2.705	2.701	2.696	32.93	33.13	32.97	33.08	33.13	33.19
202	2.631	2.627	2.617	2.617	2.613	2.642	34.08	34.28	34.17	34.22	34.23	33.90
213	1.814	1.935	1.932	1.937	1.934	1.932	46.75	46.91	46.9	46.86	46.92	46.97

JCPDS—Joint Committee on Powder Diffraction Standards.

Table 3 Lattice parameter and unit cell value for HAP calcined between 600 °C and 1000 °C

	JCPDS	600 °C	Error (%)	700 °C	Error (%)	800 °C	Error (%)	900 °C	Error (%)	1000 °C	Error (%)
a (Å)	9.418	9.3576	0.64	9.3842	0.36	9.3721	0.49	9.3572	0.65	9.3419	0.81
c (Å)	6.884	6.8202	0.92	6.834	0.73	6.8340	0.73	6.8200	0.93	6.8200	0.93
v (Å)	528.8	517.18	2.19	519.83	1.32	519.83	1.69	517.12	2.21	514.43	2.72

a and *c*—lattice parameters.
v—unit cell volume.

FE-SEM Analysis for Calcined Chicken Bone

The crystalline nature and surface morphology of HAP isolated from chicken bone at 600 °C, 800 °C, and 1000 °C were studied using field emission electron microscope (FE-SEM) and are shown in Figure 4 (*a*, *b*, and *c*). The morphology of the samples obtained by thermal calcinations of the chicken bone at 600 °C, 800 °C, and 1000 °C showed a porous network after the removal of organic materials from the bone.[17,26] These FE-SEM images confirm the derived HAP has flake like structure. The crystalline size has been measured and found to be 100–200 nm, 300–400 nm, and 500–700 nm for HAP isolated

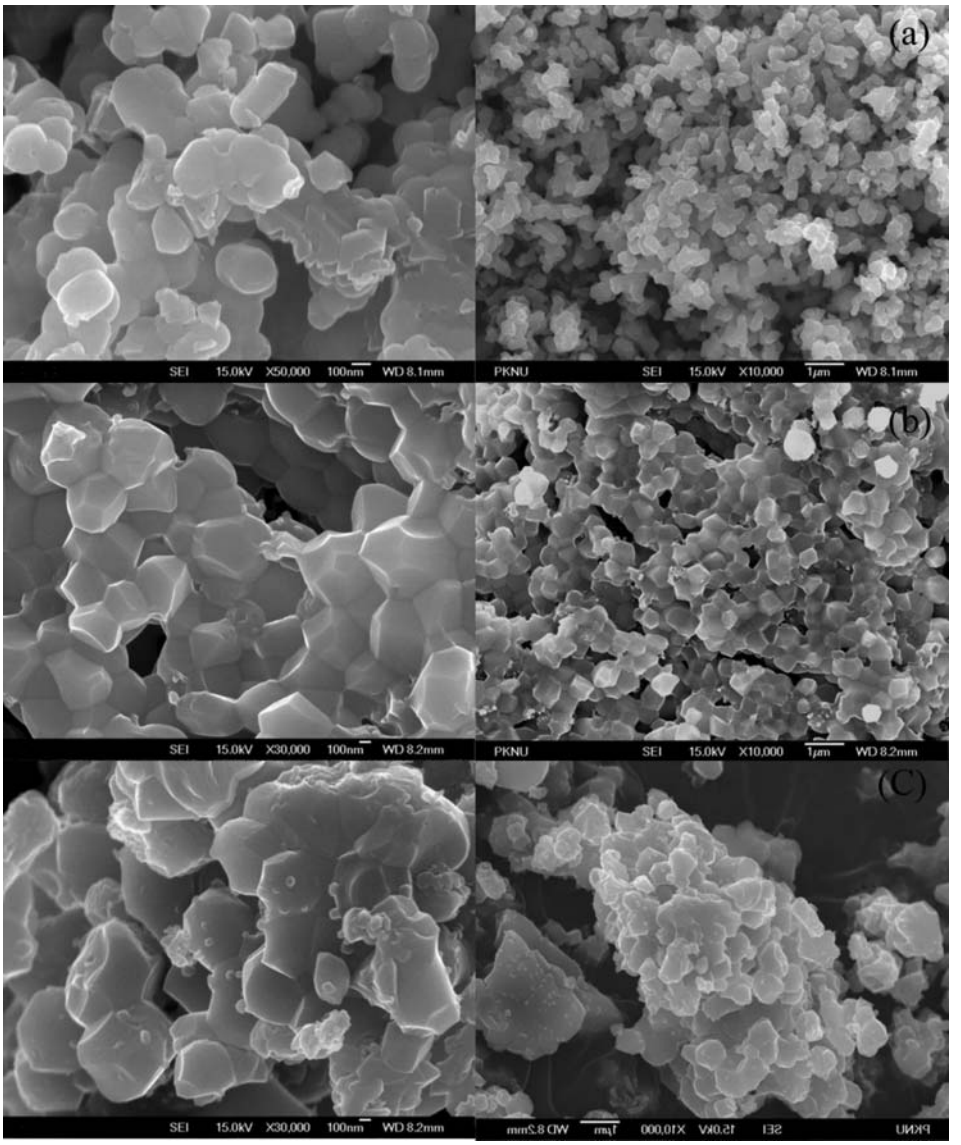

Figure 4 FE-SEM picture for chicken bone calcined at (a) 600 °C, (b) 800 °C, and (C) 1000 °C.

from chicken bone by thermal calcinations at 600 °C, 800 °C, and 1000 °C respectively. Fine small crystals found for the sample by thermal calcinations at 600 °C were found to have agglomeration with the increase in the calcinations temperature.[19]

Energy Dispersive X-ray Analysis

EDX analysis was used for the elemental analysis and determination of chemical compositions of the sample. The EDX result for the isolated HAP at 600 °C, 800 °C, and 1000 °C is shown in Figure 5 (*a*, *b*, and *c*) and the Ca/P ratio with weight percentage of Ca and P is given in Table 4. These results for the isolated HAP with higher amount of Ca, P with small amounts of other elements like Na and Mg match well with the reported values.[17,19] The Ca/P ratio for the isolated HAP is slightly higher than that of stoichiometric HAP value of 1.67. This could be attributed to the CaO formed during calcinations.[20] Among the HAPs isolated, the one isolated at 600 °C showed the Ca/P ratio to be in close proximity to the stoichiometric value indicating the optimum temperature of thermal calcinations.

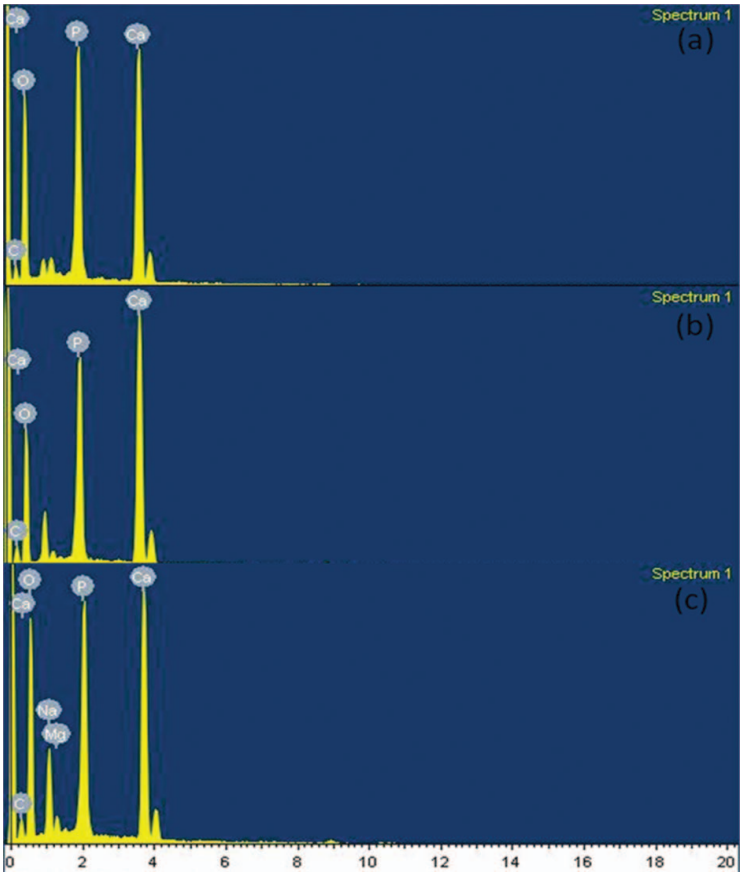

Figure 5 EDX analysis for chicken bone calcined at (a) 600 °C, (b) 800 °C, and (c) 1000 °C.

Table 4 Weight percentages and Ca/P ratio for HAP calcined at 600 °C, 800 °C, and 1000 °C

Temperature (°C)	Weight of calcium (g)	Weight of phosphorous (g)	Ca/P ratio
600	28.02	14.82	1.89
700	33.12	13.79	2.4
800	26.03	12.64	2.05

EXPERIMENTAL PROCEDURE

The chicken bones were purchased from a local slaughterhouse and washed with sodium hydroxide (AR grade) solution followed by rinsing in distilled water to remove skin and meat present on the surface of the bones. Then, the bones were dried at 100 °C and grained into small pieces. **Pretreated dried chicken (3 g) bone was placed in open silica crucible and calcined in a previously heated electric furnace (SUNSIM, India) at different temperatures ranging from 200 °C to 1000 °C with 20 h holding time.

The thermal stability of HAP derived from chicken bone was studied using TG/DTA (SDTQ 600 TA Instrument, USA). The TG/DTA of the chicken bones was recorded from 30 °C to 1000 °C at a constant heating rate of 10 °C/min in the stream of nitrogen. The vibrational frequency of HAP obtained by calcination of chicken bone at various temperatures was studied by FTIR (Jasco FTIR4100, Japan). The transmission FTIR spectrum has been recorded over the range of 400 cm^{-1} to 4000 cm^{-1} using KBr pellet. The phase and crystalline nature of calcined bones were analyzed by XRD (Bruker, D8 Advance X-ray Diffraction (XRD) spectrophotometer, German). It was carried out at room temperature using CuKα as the radiation source in the wavelength 1.504 Å, over the angle range 10°–80°, step size 0.02°, and scan speed 0.5°/min. The resultant XRD values were then compared with the standard JCPDS cards available in the system software. The morphology and the effect of temperature on agglomeration of particles and crystalline nature of the derived HAP were examined by FE-SEM (JSM-6700F, JEOL, Japan) equipped with an in-situ EDX spectrophotometer.

CONCLUSIONS

In the present study, value addition to the waste chicken bone was offered by the isolation of HAP. This not only gives value addition to the waste chicken bone but also reduces the environmental problems. HAP isolated between 600 °C to 1000 °C was found to be free from organic matter. Hence, the optimum temperature range for the thermal calcinations was found to be in the range of 600 °C to 700 °C. Furthermore, the chicken bone could serve as a useful bioresource for the HAP with various applications including bone engineering.

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