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Physico-chemical and biological properties of hydroxyapatite extracted from chicken beaks

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ABSTRACT

In this investigation, we have extracted biological hydroxyapatite (HA) containing magnesium, sodium, aluminium, zirconium and silicon ions from the chicken beaks. Raw chicken beaks were calcined at different temperatures after washing with boiled water and organic solvents. The calcined biological HA was characterized by X-ray diffraction (XRD), Fourier transforms infrared (FTIR), Field emission electron microscopy (FESEM), X-ray fluorescence (XRF) spectroscopy and Thermogravimetric analysis (TGA). The viability of Saos-2 cells treated with extracts of biological HA was higher than cells on tissue culture plates (TCPs) and synthetic HA, suggesting a good cytocompatibility of biological HA. Our research has successfully shown that the chicken beaks are a cheap source of biological HA.

1. Introduction

Recently, isolation of hydroxyapatite (HA), a well-known bioceramic from natural sources is receiving escalating attention due to its resemblance with the natural hard tissues. HA has been isolated from various natural resources like bovine bone [1], pig bones [2], fish bones [3], chicken bones [4], chicken eggshells [5], sea shells [6], mussel shells [7] and plants [8]. Chicken beaks (CBs) are waste materials that are considered as a source of many environmental problems. The use of CBs to generate HA will help to overcome the pollution problem and further to convert it into the highly valuable product. In the current investigation, CBs have been employed as a cheap source for the isolation of biological HA through calcination. The process of transforming CBs into HA is environmentally friendly and at the same time, the process will serve as a step towards reducing the cost of biomaterials.

2. Materials and methods

Waste CBs were cut into small pieces and boiled in deionized water for 90 min, then soaked inside an acetone-ether mixture (3:2) for 24 h [9]. The CBs were dried overnight at 80 °C, ground into small pieces followed by calcination at 600 °C, 800 °C, 1000 °C and 1200 °C for 2 h (5 °C/min). The calcined powder was characterized by using XRD, FTIR, FESEM, XRF and TGA techniques. Lattice parameters were calculated by using unit cell software [10].

The *in vitro* cytotoxicity of calcined materials was evaluated by indirect contact MTT assay using human osteosarcoma cell line (Saos-2, ATCC) as per the procedure defined elsewhere [3]. Samples (n = 4) were cultured for 24 h. Cells cultured in only cell culture media were used as control. Statistical analysis was achieved by employing Student's *t*-test method, with $P < .05$ being considered statistically significant.

3. Results and discussion

The XRD patterns were in good agreement with the standard crystalline HA (JCPDS No 09–432) (Fig. 1(a)), raw CBs and CBs calcined at 600 °C showed presence of poorly crystalline HA structure with wider peaks, which were attributed to the poorly crystalline material, incomplete removal of extracellular matrix and fibrous proteins present in the CBs. The intensity of the peaks increased and the peaks became narrower as the calcination temperature was raised to 800 °C and above, indicating the increase in the crystallinity and crystallite size of the biological HA Table 1. A small peak detected at 37.49° was attributed to the (2 0 0) plane of CaO (JCDPS 48-1467), which could be due to partial decomposition of HA [11]. The absence of β-TCP in the calcined CBs material can be considered a good indication of the thermal stability of the iso-

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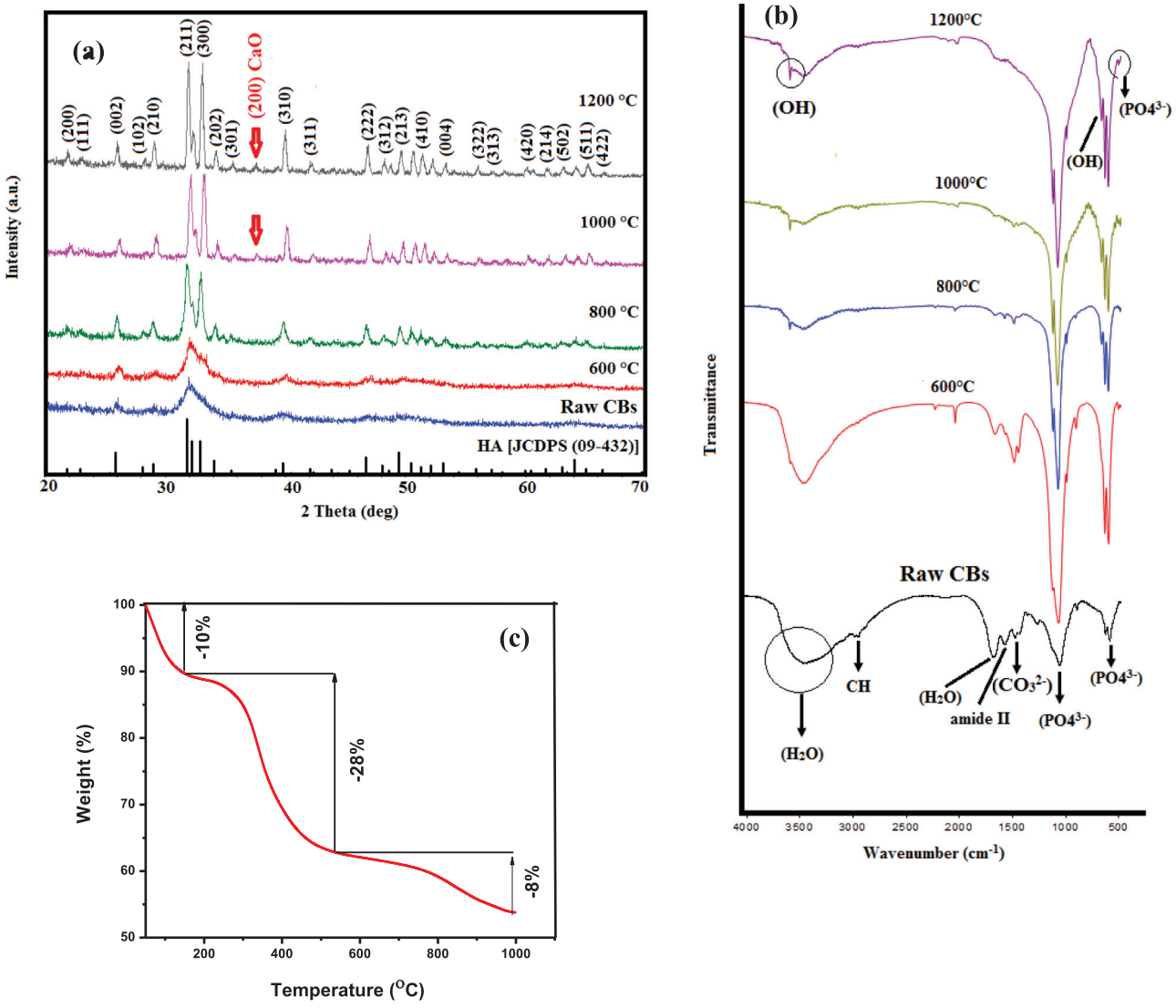

Fig. 1. (a) XRD patterns (b) FTIR spectra of CBs calcined at different temperatures and (c) TGA analysis of CBs.

Table 1
Cell parameters of standard HA and calcined CBs materials.

Sample ID	Lattice Parameter				Crystallite size			*Xc* (%)
	a (Å)	*c* (Å)	*c*/*a*	*v* (Å)^3	D_{nm} (002)	D_{nm} (300)	D_{nm} (310)	
Standard HA	9.418	6.884	0.73	528	–	–	–	–
CB-800 °C	9.417	6.857	0.73	527	24	22	18	69
CB-1000 °C	9.449	6.826	0.72	528	25	29	33	87
CB-1200 °C	9.388	6.854	0.72	523	35	36	38	90

lated HA. Previous literature reports revealed that biological HA isolated from bovine bone is thermally stable up to 1000 °C and above this temperature HA undergoes decomposition into CaO and β-TCP [1]. It was also reported that HA isolated from Pig bones was thermally stable up to 700 °C [12]. In another study, it was shown that HA isolated from sword and tuna fish bones were thermally stable up to 950 °C [3]. Our results showed that HA isolated from CBs was highly crystalline and thermally stable up to 1000 °C. Lattice parameters of calcined samples are summarized in Table 1. Slight variations in lattice parameters of the HA extracted from CBs could be attributed to the dehydroxylation which is very common for the samples calcined at high temperatures [4]. Furthermore, small weight loss was observed above 800 °C in TGA analysis, which could be due to the partial dehydroxylation of HA [13] Fig. 1(c).

FTIR spectra of the biological HA showed the characteristic bands of the HA structure Fig. 1(b), the absorption peak detected at 475 cm^{-1} was assigned to υ2 bending mode of PO_4^{3-} and the absorption peaks located at 571 cm^{-1} and 602 cm^{-1} were due to υ4 vibration mode of PO_4^{3-} group. Similarly, the absorption band in the range 985–1095 cm^{-1} was attributed to the υ3 vibration mode of PO_4^{3-} group. In addition, hydroxyl groups were detected at 633 cm^{-1} and 3562 cm^{-1} and were endorsed to bending and stretching mode of OH group respectively. In general, the intensity and sharpness of the peaks due to the phosphate and hydroxyl group increased with increase in the calcination temperature, indi-