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Research Paper

Hydroxyapatite Nano-Particles Extracted from Miscellaneous Bestial Resources: Study of Crystal Structure and Microstructure

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ABSTRACT

In this work, hydroxyapatite nano-particles were successfully synthesized via the usage of available fish, hen and bovine bones in waste foods. The crystal structure, microstructure, and functional groups of the synthesized nanostructured bioceramics were studied using X-ray diffraction (XRD), field emission scanning electron microscopy (FESEM), and fourier transform infrared spectroscopy (FTIR). It was shown that all the used natural resources provide well crystallized crystal structures of hydroxyapatite with a narrow distribution microstructure in the range of 24.4-93.4 nm. As well, it was found that the hydroxyapatite extracted from the fish bone has smaller particle size than those of the hen and bovine. Additionally, all the synthesized productions possess the same functional groups, they can be employed as promising candidates for the bone filler materials in biomedical engineering.

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1. Introduction

In the great family of bioceramic materials, the family of calcium phosphate materials are of very promising candidates for medical applications [1]. Different calcium phosphate-based bioceramics such as Hydroxyapatite (HA), Tetracalcium Phosphate (TTCP), Tricalcium Phosphate (TCP), Amorphous Calcium Phosphate (ACP) have been developed since they possess a wide range of applications for drug delivery [2], scaffolds for tissue regeneration [3], bone grafts [4], and coatings on the surface of implants [5]. Among all, hydroxyapatite (HA: $Ca_{10}(PO_4)_6(OH)_2$) has attained tremendous interest of researchers because of its prominent thermodynamical stability, biocompatibility and also exceptional similarity to the mineral part of the bones of human beings [6]. The mineral part reinforces polymeric matrix (collagen) of the bone, improving its stiffness and toughness [7]. As a matter of fact, HA has sparked remarkable scientific attentions due to its mentioned potential applications. The evaluation of previous works indicates that there are a lot of research works on hydroxyapatite but mostly the used hydroxyapatites have been synthesized through chemical approaches. As a matter of fact, there is a lack of information on bestial synthesized hydroxyapatites which has been concentrated in this research. The literature review reveals that the natural compositions and biomimetic resources have been concentrated for producing hydroxyapatite [6]. The extracted hydroxyapatite from the natural sources is proposed to be more compatible with the human tissues, because of good physicochemical similarity to the mineral part of the human bone but the desirable characteristics of produced hydroxyapatites are still arguable. The comprehensive study of the synthesized hydroxyapatite can efficiently provide a platform to address this necessity. Noteworthy, because food wastes are of the most important related resources, the employment of natural resources gives rise to environmental and economic benefits. In addition, the mechanical, chemical and biological properties of hydroxyapatite, mainly depend on its particle size and morphology, stoichiometry, phase crystallinity and purity. Specially, the particle size concerns have driven research activities toward the synthesis of HA via miscellaneous techniques. In other words, since different synthetizing methods provide different characteristics of productions, a wide variety of properties can be achieved for the production of hydroxyapatite [6]. Many studies have been carried out by different researchers to optimize the particle size of extracted hydroxyapatites from the natural resources [7-10]. Ruksudjarit et al. synthesized hydroxyapatite from bovine bone while the final particle size had been significantly reduced [8]. Some researchers have used ball- mill after extraction, but it should be noted that this method is time consuming and also increases the probability of contamination [11]. So, besides the benefits of hydroxyapatite extraction from natural sources [12-13], reduction of its particle size is still in challenge [6]. Hereby, yet little attention has been taken to the use of bestial resources within a facile calcination procedure to produce hydroxyapatite powder. Motivated by the described background, in this work we prepared hydroxyapatite powders through the heat treatment of fish, hen and bovine bones, while no comparative study has been observed in literature review of hydroxyapatite extraction from natural sources. This investigation also confirmed that the synthesis of high quality and uniform hydroxyapatite materials via the employment of different bestial resources is feasible. Consequently, XRD, FESEM and FTIR analyses were employed to get more insight about the crystallographic information and microstructure of the extracted hydroxyapatite particles.

2. Experimental

2.1. Sample preparation

Since the mineral part of bone is composed of hydroxyapatite, the bones of fish (F), hen (H) and bovine (B) were used for hydroxyapatite extraction and subsequent comparative study. Hereby, the bones were immersed in boiling water for 1 h, separately, to remove extra tissues. After cleaning, the bones were heated at 400 °C for 1 h to burn out and remove the organic part of bones (mainly collagen). It is already well known that at the approximate temperature of 600 °C the organic compounds of bones are removed completely. So, the ash like hydroxyapatites were calcined at 750 °C for 1 h in air atmosphere [13-14]. All the heating and cooling steps were carried out at the constant rate of 5 °C/min.

2.2. Characterization

The X-ray diffraction analysis (XRD: Philips X'Pert-MPD, Netherland) was used to identify the phases of the synthesized powders. Correspondingly, X-ray was generated via the use of Cu-Kα lamp with the wavelength of 1.54060 Å in the 2 theta range of 10-80°.

The particle size and morphology of samples were studied via secondary electron mode of field emission scanning electron microscope (FESEM: TESCAN MIRA3, Czech Republic). In order to inhibit electron charge accumulation during the FESEM observations, the samples were coated by gold element using a sputtering equipment.

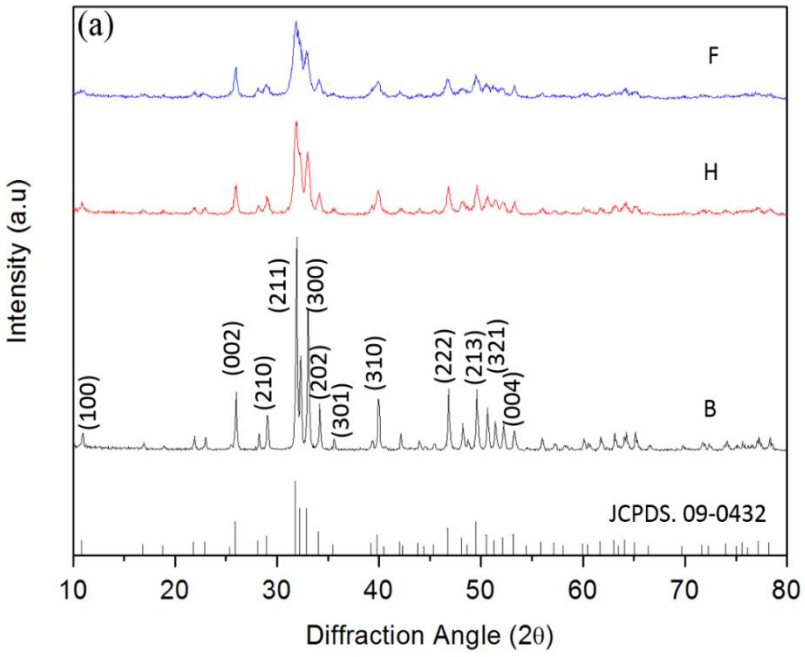
The chemical structures and functional groups of hydroxyapatite compounds, extracted from the bones of fish, hen and bovine were monitored using fourier transform infrared spectroscopy (FTIR: SHIMADZU, 8400S, Japan) in a dry KBr matrix in the range of 400-4000 cm^{-1}.

3. Results and Discussion

3.1. Crystal structure and XRD Studies

The XRD spectra of the synthesized powders extracted from the central part of femur bone of different animals of hen (H), bovine (B) and bones of fish (F) have been indicated in Fig. 1.

Interestingly, it is seen that the use of bones of the different animals gives rise to the synthesis of hydroxyapatite materials with very similar crystal structures (see Fig. 1a). As well, the strongest diffraction peaks of F, H and B spectra originate from (100), (002), (210), (211), (300), (202), (301), (310), (222), (213), (321) and (004) diffraction planes which it can be implied that the spectra are properly consistent with the JCPDS No. 009-0432. It is observed that the most intense peak of the spectra has occurred at the approximate 2θ of 31.8. As a matter of fact, the utilization of the mentioned bones provides different full-width at half maximum (FWHM). In order to assess the broadening of XRD spectra, the peaks existing between 2θ of 31 to 35, have been shown in Fig. 1b. Obviously, it is found that the use of bovine and fish bones results in the formation of the narrowest and broadest peaks, respectively which induces that the use of fish bones bring smallest particle size of HA. Accordingly, the lattice parameters, distance of (211) planes, diffraction angle, FWHM, and height of the XRD peaks related to the productions have been presented in Table 1. The average crystallite sizes of powders (D) were estimated employing the Scherrer equation, $D= 0.9\lambda/\beta\cos\theta$ [15-17]. It should be noted that λ is the used X-ray wavelength (0.154 nm) and θ and β are the angle of diffraction and FWHM of the considered peaks, respectively. Accordingly, the crystallite sizes of hydroxyapatites synthesized by fish, hen and bovine bones have been calculated 12.5, 35.1 and 94.3 nm, respectively. As well, the minimum height of XRD spectra belongs to the consumption of fish bones which means that the small particles of the produced HA, somehow more behave like an amorphous material. By contrast, the HA powders produced from bovine possesses more crystallinity. Interestingly, it can be realized that any extra phase attributing to impurities cannot be traced in the XRD spectra.



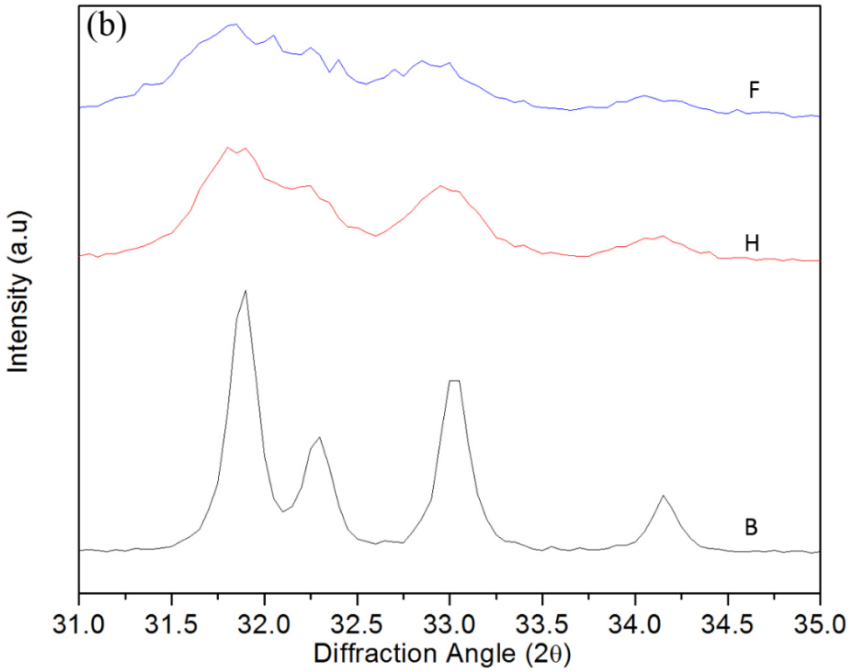


Fig. 1. XRD spectra of the synthesized powders via the employment of fish, hen and bovine bones in the angle ranges (2θ) of (a) 10-80° and (b) 31-35°.

Table 1. Diffraction angle, FWHM and Height of the XRD peaks related to different samples.

Sample	**a (**Å)	**b (**Å)	**c (**Å)	**V (**Å^3)	**d (** Å **)**	θ (2θ)	β (2θ)	Height (cts)	D (nm)
F	9.421	9	6.889	505.894	2.82	31.7359	0.72	290.65	12.5
H	9.418	9	6.883	505.298	2.81	31.8174	0.2952	366.92	35.1
B	9.43	9	6.881	505.75	2.81	31.8862	0.1476	1357.66	94.3

Through the employment of Maud software, it can be easily found that the consumption of miscellaneous bestial resources gives rise to the formation of different sizes of unit cells [18-20]. Accordingly, with the use of fish, hen and bovine bones as the source of hydroxyapatite, the volume of the produced unit cells will be 505.894, 505.298 and 505.750 Å^3, respectively. Interestingly, it can be concluded that although the volumes of HA unit cells are different but the dimensions of lattices in Y direction (b) are exactly same

3.2. FESEM Analysis

FESEM microstructure images of the synthesized powders via the employment of the considered bones are shown in Fig. 2. The particle size distribution is rather homogeneous and any indication of abnormal growth of particles cannot be seen in the microstructures. Almost all the particles possess quasi-spherical morphology with relatively smooth surfaces. Additionally, any impurity with specific size or color is not seen in the microstructure that is significantly consistent with the XRD spectra. It can be easily induced that the average particle size of powders which is 24.4 nm is attributed to the fish bone, while those related to hen and bovine bones are 34.3 and 93.4 nm, respectively. The estimated sizes are surprisingly in a good agreement with the calculated crystallite sizes of powders, explained earlier. It is clear that load bearing bones will have better bone formation rates. Since bovine bones are subjected to a greater mechanical loads than fish bones, the size of larger hydroxyapatite particles seems reasonable.

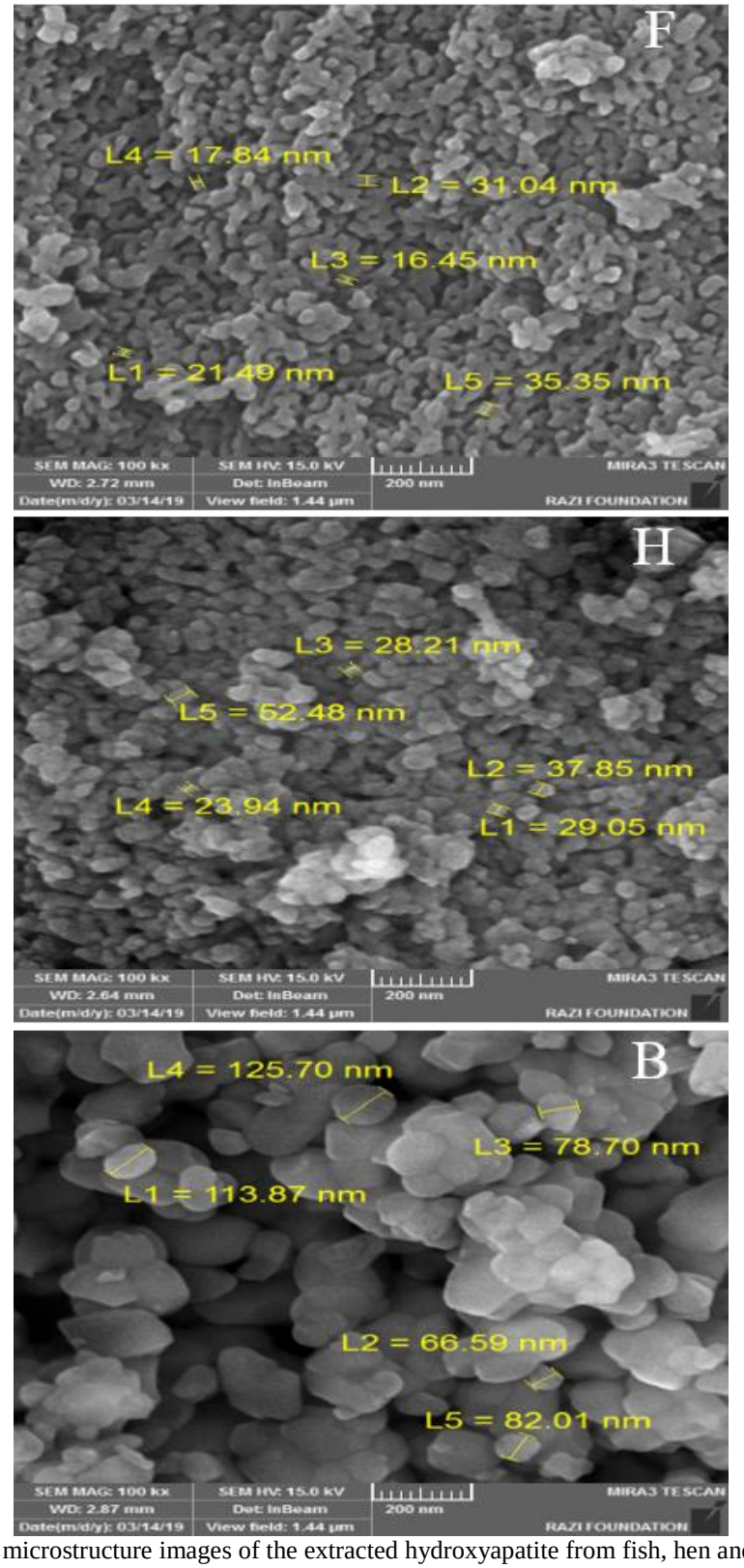


Fig. 2. FESEM microstructure images of the extracted hydroxyapatite from fish, hen and bovine bones.

Roohani-Esfahani et al. stated that the size and morphology of nano-particles of calcium phosphate in the coating layer of scaffold, significantly affect the mechanical and biological properties [21]. Also, according to the report of Dorozhkin, higher cell viability and better proliferation of different types of human cells have been observed in smaller sizes of calcium orthophosphates crystals [22]. So, according to the microstructure related results, it is evident that hydroxyapatite nano-particle, extracted from fish bones, would have better bio-functionalization *in-vivo*, than those of hen and bovine bones.

3.3. Functional groups study using FTIR

Fig. 3 indicates the FTIR spectra of the extracted hydroxyapatites from the bones of fish, hen and bovine. It can be easily concluded that the considered spectra are almost same and there is no significant difference between them. Accordingly, there is a relatively sharp band at 3572 cm^{-1}, which is related to the stretching vibration of the structural OH^- functional group. Also, another band due to vibration mode of the structural OH^- functional groups is observable at 630 cm^{-1}. The other bands at 473, 570, 600, 961, 1046 and 1087 cm^{-1}, are attributed to PO_4^{3-} functional groups. Moreover, the literature review reveals that both of the bands around 1500 cm^{-1} and also 860 cm^{-1} originate from CO_3^{2-} groups [23].

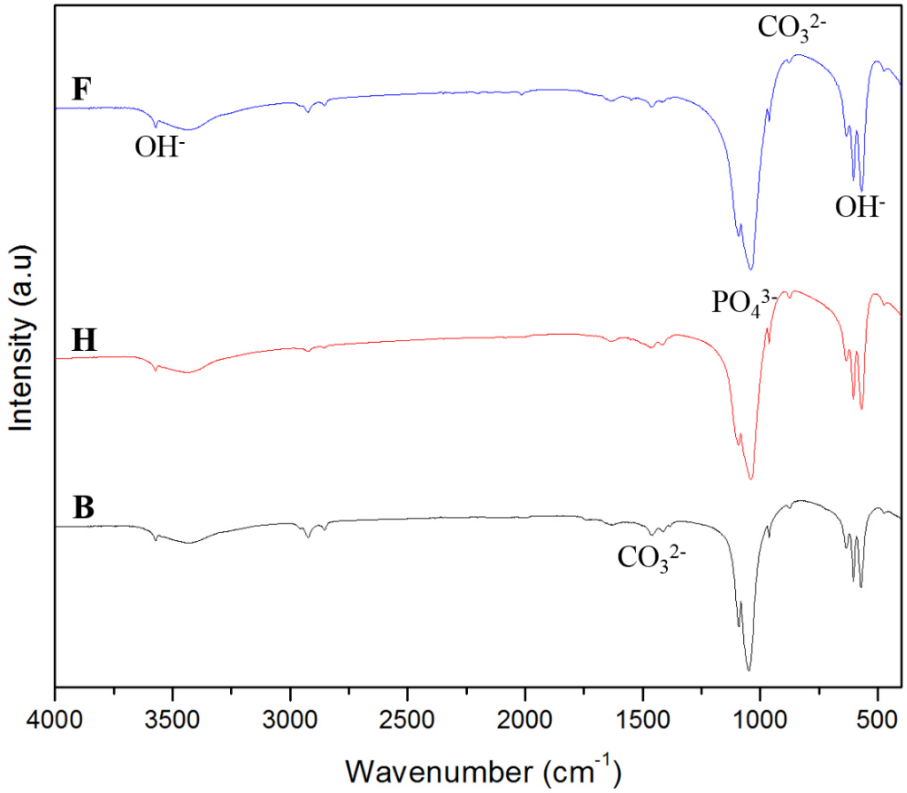


Fig. 3. FTIR spectra of the extracted hydroxyapatite from fish, hen and bovine bones.

Accordingly, it can be easily concluded that the hydroxyapatite powders extracted from the bones of fish, hen and bovine possess resemble functional groups. So the obtained nano hydroxyapatite particles can be used for some biomedical applications such as dentistry, cancer treatment and bone repair [24, 25], regarding their particle size as an important factor [26]. So, many researcher have tried to reduce the particle size of extracted hydroxyapatite from natural sources. Ruksudjarit et al. have used vibro-milling technique to reduce the particles size of extracted hydroxyapatite form bovine bone, they stated that using this technique, the particle size have reduced to smaller than 100 nm [27]. While at the present study by using another natural source, and without any milling technique, hydroxyapatite with particle size smaller than 100 nm have been extracted successfully.

Conclusions

This investigation interestingly showed that the production of hydroxyapatite can be successfully achieved from the food waste (the bones of fish, hen and bovine) while the properties of produced hydroxyapatites will be meaningfully different. The XRD and FESEM characterizations revealed that the use of fish bones, bring prominent method for nano structure hydroxyapatite synthetize for biomedical applications. The FTIR spectra proved that although the crystal structures and the morphology of the produced HA are somehow different, but the surface functional groups are surprisingly similar.

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