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Extraction of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ from Thread fish Bio-waste and characterization of phase purity by ISO 13779-3:2018

Mohammad Ali Ghane ^{1*}

1. Standard administration of Hormozgan, Bandar Abbas, Iran

Abstract

Standards are essential in product manufacturing because, in addition to helping ensure the quality, safety, and efficiency of products and processes, they are also effective in identifying products. The present work demonstrates a low-cost route to produce a biocompatible calcium hydroxyapatite material with the formula $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ from native Persian Gulf fish bones (a natural source) through a simple heat treatment process. The crystallinity and phase purity of the produced powder were determined using X-ray diffraction (XRD), which did not show any new phases. Therefore, the analysis of the heat-treated powder also confirmed its phase purity and crystallinity. By examining the diffraction pattern of the product and comparing it with the data provided in ISO 13779-3:2018, the results of the phase purity were well confirmed. This study demonstrates the practical application of the developed standards in important research and emphasizes the role of standards and their assistance in new material identification

Keywords Hydroxyapatite, Standard, Crystallinity

Introduction

Calcium hydroxyapatite, HA ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$), is thermodynamically the most stable calcium phosphate under physiological conditions in the human body and has a very similar composition to bone mineral [1]. The crystal structure of hydroxyapatite was first precisely characterized following Roentgen's X-ray discovery in 1895 [2]. It can integrate with bone causing no local or systemic toxicity, inflammation, or other negative responses [3]. It has also some applications as a sorbent [4], catalyst [5], tissue engineering [6], and biosensors [7]. Furthermore, due to its reactive surfaces and porous structure, it can serve as an effective adsorbent for heavy metals

and organic compounds [8]. HA can be obtained either through synthetic methods or by extraction from natural resources. Synthetic methods include processes such as precipitation [9], mechano-chemical synthesis [10], and sol-gel methods [11].

HA extracted from natural sources is available in large quantities and can be considered as an environmentally friendly, sustainable, and economical material to be used in biomedical applications, such as the production of various kinds of prostheses, repairing cracked or broken bones, coating of metallic biomaterials and production of implant materials [12]. Thus, it has a positive contribution to the economy, environment, and general health.



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* Correspondence:
ali.ghane@gmail.com

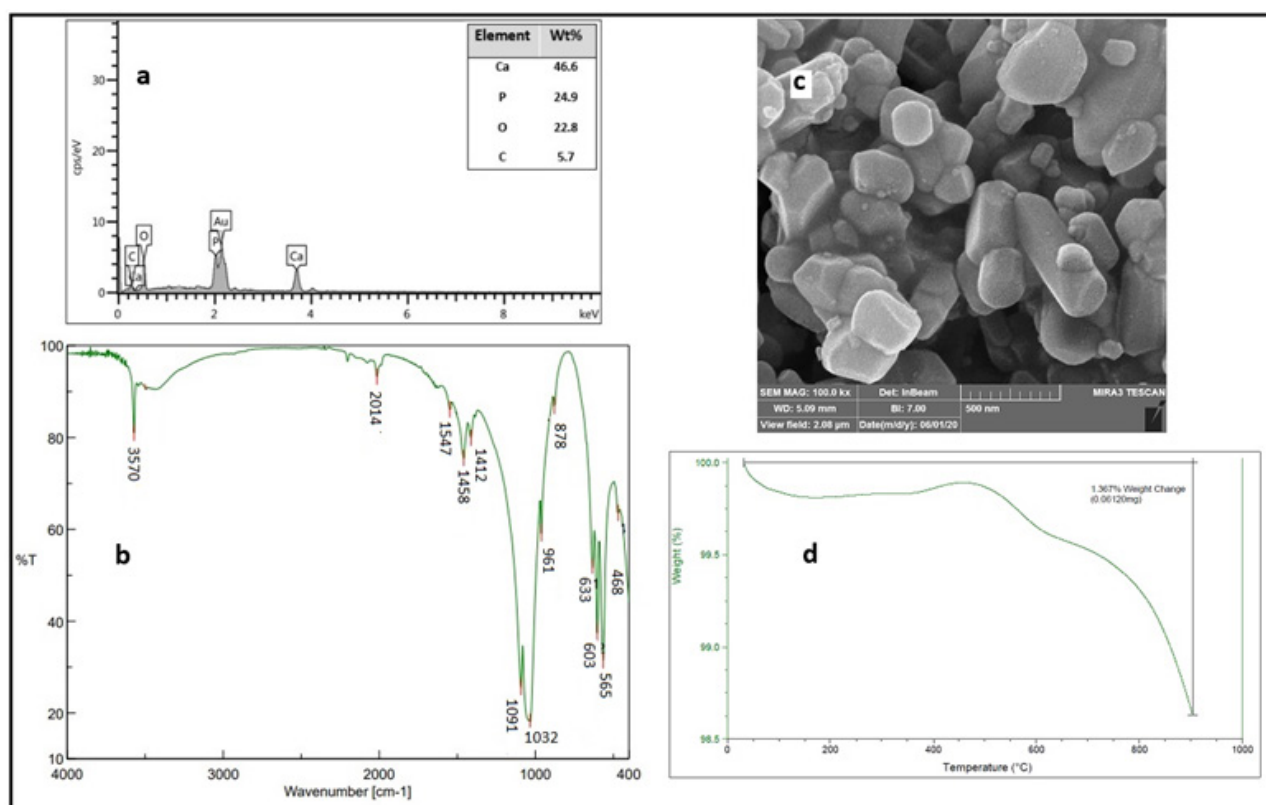


Fig 1. EDAX analysis (a), FT-IR spectra (b), FE-SEM image (at magnification of 500 nm) (c), and thermogravimetric analysis (d) of HA

Natural hydroxyapatite can be extracted from biological sources or wastes, such as mammalian bones [13], marine or aquatic sources [14], shell sources [15], plants [16], and mineral sources [17]. There are large amounts of fish waste in the form of scales and bones in the fisheries industry around the world. Recovery of these fish scales and bones allows for the extraction of HA and reduction in solid wastes. The morphology and particle size of HA are strongly dependent on its source and its preparation method [18].

In this study, we first produced HA by thermal processing from bio-waste of tuna species *Thunnus tonggol*, which is abundant, cheap, and available in the Persian Gulf. Then, it was characterized using FESEM, EDAX, TG and FT-IR. The phase purity and crystallinity of the produced powder were investigated using X-ray diffraction (XRD), and the analytical results were compared with the data provided in ISO 13779-3:2018 [19]. It should be noted that the XRD chemical analysis appendix of this standard is used for reference and does not imply that the apatite produced in this study is intended for surgical implants or medical device applications.

2. Materials and methods

2.1. Materials

In this research, no specific chemicals, particularly acids or bases, were used in the product extraction steps.

2.2. Preparation of hydroxyapatite from Thread fish scales

The Indian Thread fish bone wastes were collected from the Tuna Fish Cannery at the Persian Gulf shore. They were boiled for 1 h and washed using a strong water jet to eliminate meat, salts, blood, and other dirty substances. Then, they were dried in the air in front of sunlight for several days. A mechanical grinder cuts the waste into small pieces and grinds it to a fine powder. The powder was heated gradually to 600 °C in an electric oven with a rate of 10 deg/min in air and then heated to 850 °C for 2.5 h, to remove its organic components. The residue was milled in a planetary ball mill with a steel chamber and zirconia balls (Retsch PM100, Germany) with a rotational speed of 100 rpm for 90 minutes to obtain narrow dispersed particles [20].

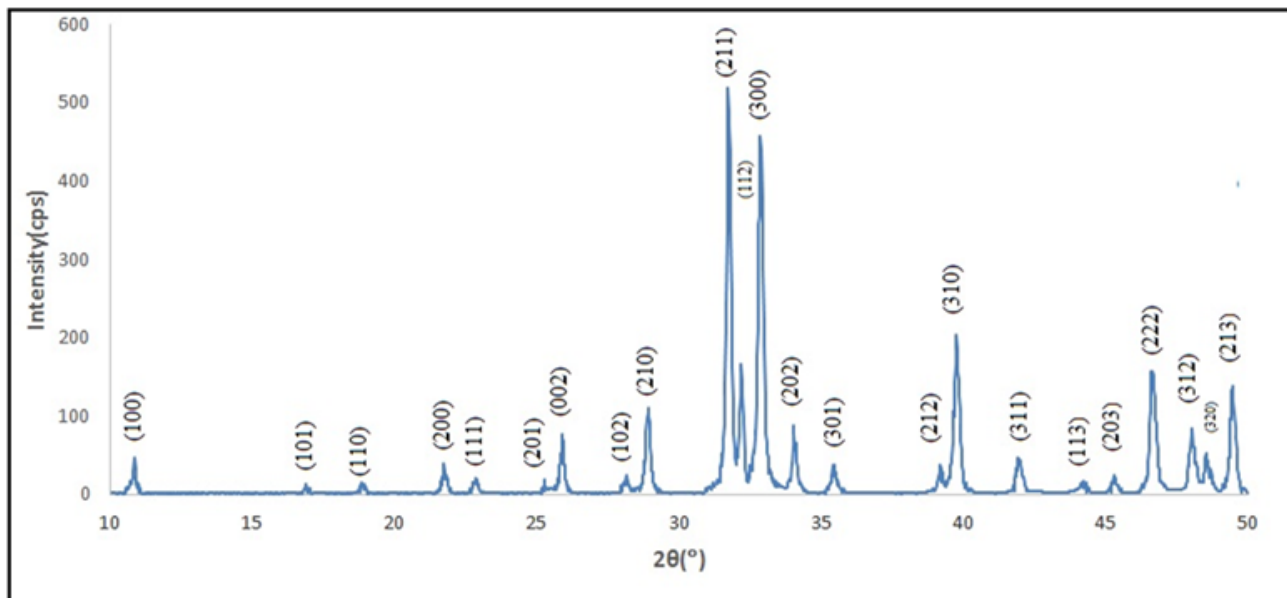


Fig 2. XRD pattern of HA nano powder extracted at 850°C for 2.5h.

2.3. Characterization of HA

Phase identification and crystallinity of HA were determined by X-ray diffraction method (XRD) (Bruker D8 Advance, Germany, with Cu K α radiation, $\lambda = 0.15406$ nm). Scanning was done between 20 and 80° with 0.1 step size. Then the unit cell parameters were calculated by using software (UnitCell, UK). HA crystallite sizes were calculated from measurements of the half-width of the 211 reflection plane in the XRD pattern using the Scherrer equation.

FT-IR spectroscopy characterized functional groups in the range of 400–4000 cm⁻¹ (Thermo Nicolet 6700). FE-SEM and EDAX analysis determined the morphologies, sizes, and elemental compositions of nanoparticles (HITACHI S4500, Tokyo). Weight losses were measured using TGA (SDT Q600 V20.9, USA) under nitrogen atmosphere at a heating rate of 10 °C/min from 30 to 900 °C.

3. Results and Discussion

3.1 Preparation and characterization of HA

HA was extracted from natural resources via literature methods [21]. EDAX analysis (Fig.1a) showed the existence of C, O, P, and Ca in the sample, which agrees with the suggested formula for HA having some carbonate impurity. The presence of small amounts of carbonate in biological HA is quite regular. Substitution of carbonate ions in place of hydroxyl and phosphate ions results in A-type and B-type carbonated apatite, respectively [22]. FT-IR spectra (Fig. 1b) showed the existence of HA vibrational bands. The peaks at 3570 and 632 cm⁻¹ are due to stretching and bending vibrations of the hydroxyl group,

respectively. The peak at 2014 cm⁻¹ is a difference band in water, and the peak at 1548 cm⁻¹ is due to the bending vibration of water. Peaks at 1091, 1032, 961, 603, 565, and 468 cm⁻¹ are due to vibrations of phosphate ions. Peaks at 1458, 1412, and 878 cm⁻¹ are due to vibrations of carbonate impurity. Peaks are in agreement with the reference hydroxyapatite [23].

FE-SEM image (Fig. 1c) showed that HA is in the form of spherical with sizes ranging from 26 to 122 nm (mean 64 nm). Fig. 1d shows the thermogravimetric analysis for HA. The mass loss (1.36%) shows that after 850 °C HA is the only phase remained in the residue. Therefore, this indicates a good thermal stability of the sample [24]. To emphasize the crystal structure of the obtained nano-hydroxyapatite (HA) powder, X-ray diffraction (XRD) measurements were conducted. XRD pattern showed the presence of pure HA (Figure 2) [25]. It showed that the prepared HA is crystallized in a hexagonal structure (P 63/m, $a=b=9.423$ Å, $c=6.88$ Å; $\alpha=\beta=90$, $\gamma=120$) (JCPDS Card No. 00-09-0432) [26]. The sharp, narrow, and well-defined peaks indicate the high purity and crystallinity of the product at a specific temperature of 850°C. Additionally, no extraneous peaks are observed in the diffraction pattern of the nano-hydroxyapatite (HA) powder. It is important to note that the X-ray diffraction method only approximates the crystal size distribution.

Thus, variations in peak width reflect changes in crystal size distribution. The degree of peak broadening is a key parameter that plays a significant role in determining the structural characteristics of the product, including its degree of crystallinity. Various methods exist for analyz-

ing diffraction patterns and calculating crystallinity, the simplest being the Scherrer's equation. The percentage of crystallinity and the average crystal size, computed using software at a temperature of 850°C and a heating duration of 2.5h, were found to be 67% and 63.5nm, respectively. The results demonstrate that the degree of crystallinity is influenced by factors such as crystal grain size, indicating that products with higher crystallinity possess larger average crystal sizes.

The average crystallite size was calculated using Debye-Scherrer's equation, $D (\text{\AA}) = K\lambda / (\beta \cos\theta)$, where D is the average crystallite size in $\text{\AA}$, K is the shape factor (0.9), λ is the wavelength of X-ray Cu $K\alpha$ radiation (1.5406 $\text{\AA}$), θ is the Bragg angle, and β is the full width at half maximum FWHM [27]. The strongest peak (211 reflection plane at $2\theta=31.7$) was used to determine the crystallite size.

The XRD pattern of HA is shown in Fig. 1. The observed positions of diffraction lines (2θ and corresponding $d_{2\theta}$) and their relative intensities (I_{rel}) are listed in Table 1.

In this study, the absence of β and α -tricalcium phosphate (TCP) has been confirmed. Based on ISO 13779-3:2018, the d-spacing lines at 2.880 and 2.905 $\text{\AA}$ are for the β -form and the α -form, respectively. The d-spacing can be described as the distance between planes of atoms that give rise to diffraction peaks. Each peak in a diffractogram results from a corresponding d-spacing [28].

The absence of Calcium oxide (CaO) was also tested using X-ray diffraction. The absence of a line at 2.405 $\text{\AA}$ in the pattern (in Fig. 1) confirmed the purity of HA by ISO 13779-3:2018. On the other hand, according to the same standard, the absence of calcium oxide in the apatite structure is confirmed when no color is observed after mixing apatite powder in phenolphthalein-ethanol solution. This test was also performed on the mixture according to the standard, and the results proved the absence of calcium oxide impurities.

The absence of the tetra-calcium phosphate)TTCP(is tested using X-ray diffraction by ISO 13779-3:2018. The obtained result showed that the line at 3.00 $\text{\AA}$ was absent in the X-ray diffraction pattern, confirming the absence of TTCP in the HA extracted.

4. Conclusion

This study shows that the XRD pattern of HA reveals a highly crystalline phase by ISO 13779-3:2018. The results did not indicate the presence of any extraneous phases corresponding to TCP, TTCP, CaCO_3 or CaO. However, the calculated cell values of all HA samples were close to the JCPDS standard, although slight differences were observed among them. These slight variations in the axes of HA may be attributed to the trace-level substitution of

Table 1. The reflecting miller planes (hkl), Positions of diffraction lines (2θ , and corresponding $d_{2\theta}$) and their relative intensities (I_{rel}) are listed in the XRD pattern of HA nano powder extracted at 850°C for 2.5h.

$2\theta(^{\circ})$	Standard [9]	Intensity (cps)	d-spacing ($\text{\AA}$)	hkl
10.84	10.85	47.61	8.15737	100
16.86	16.87	12.96	5.26649	101
18.82	18.84	15.21	4.7144	110
21.72	21.75	39.69	4.08663	200
22.84	22.84	21.16	3.88615	111
25.26	25.29	18	3.58201	201
25.88	25.86	77.44	3.43902	2
28.14	28.11	25	3.16565	102
28.92	28.92	110.25	3.08326	210
31.72	31.77	519.84	2.81821	211
32.18	32.18	166.45	2.77981	112
32.86	32.9	457.96	2.72209	300
34.02	34.04	88.36	2.63409	202
35.44	35.44	38.44	2.53151	301
39.2	39.18	36.81	2.29685	212
39.76	39.793	204.49	2.26608	310
41.92	41.98	46.24	2.15246	311
43.84	43.84	15.21	2.06135	113
45.36	45.31	20.25	1.99821	203
46.62	46.68	156.25	1.94552	222
48.06	48.06	84.64	1.89213	312
48.54	48.58	53.29	1.84131	320
49.48	49.45	139.24	1.80787	213

carbonate. The previous results suggest that the crystallographic parameters and phase purity of HA depend on the reaction ingredients and preparation methodology.

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Conflict of interest statement

The authors declare no conflict of interest.

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Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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