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RESEARCH ARTICLE

SIMPLE EXTRACTION OF THE HYDROXY-APATITE FROM THE EGYPTIAN NILE TILAPIA (*OREOCHROMIS NILOTICAS*) FISH SCALES

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ABSTRACT

This study aims to recycle the fish scales to extract the hydroxyl-apatite (HApT) compound via a simple and fast chemical treatment of the Egyptian Nile Tilapia (*Oreochromis niloticas*) fish scales. The method depends on both the NaOH and/or the HCl solutions to dissolve the fish scales then precipitate the HApT 30-32% of the start. The product was characterized by the elemental analysis, Fourier Transform Infrared Spectroscopy (FTIR), X-rays Diffraction (XRD), Field Emission Scanning Microscopy (FESEM), Energy Dispersive X-rays spectroscopy (EDX), and Thermo-Gravimetric Analysis (TGA). The results of the FTIR and XRD have revealed the main peaks of an amorphous/crystalline mixed HApT phase that was almost thermally stable after elimination of the entrapped water of preparation and/or the residual organic portion. The particles have exhibited soft homogeneous morphology rather than specifically shaped particles with a 2.25 Ca/P ratio as indicated by the results of the SEM and EDX analysis. The particle size distribution (PSD) analysis has indicated a range of particle size ~250 – 2500 nm with 50% of sample volume < 1 µm. It can be concluded from the results that the studied method is an efficient and economic approach for the HApT extraction as well as the fish scales recycling.

KEYWORDS

Biocompatible; Egyptian Nile Tilapia; Hydroxy apatite; Separation and Purification; Sustainable Resources

1. INTRODUCTION

Marin's wastes are good renewable sustainable resources when recycled to get some of their precious components to be applied as low-cost biocompatible substrates for miscellaneous applications. Nile tilapia (*Oreochromis niloticus*) is one of the most common freshwater fish species with increasing production because of their attractive marketing. Fish processing discards large amounts of bio-wastes such as skins, scales, and bones that are harmful to the ecosystem because they act as bacterial incubators and produce harmful gases such as the methane CH4 (Mkhize, 2023). Recycling of such wastes is an environmental demand. Some studies showed that the biomaterials derived from the fish scales are biologically better than the chemically synthesized and promising for use as a bone scaffold or as regenerative materials (Mansour, 2021). Nile Tilapia scales could be used for the biomedical applications to extract the collagen (El-Rashidy, 2015).

Fish scales are bio-composites of highly ordered type I collagen fibers and the hydroxy-apatite $Ca_{10}(OH)_2(PO_4)_6$ with fatty acids, vitamins, antioxidant and trace elements (Muhammad, 2016). A powdered mixture of fish scales of the *abramis brama* (freshwater bream), *carassius carassius* (crucian carp), and *sander lucioperca* (pikeperch) have contained a 36.5 wt % inorganic components, primarily hydroxy-apatite (HApT) and magnesium whitlockite. Heat treatment of this fish scale powder at 800–1000 °C resulted in sintered grains (< 100 nm at 800 °C, < 200 nm at 900 °C, and 100–1000 nm at 1000 °C) for ceramics applications. Fish scales have been also applied for the production of collagen, HApT, guanine, animal feed, fertilizers, food, cosmetics, adsorbents, biomaterials, etc (Safronova, 2022; Razali et al., 2020). Scales of *Sardinella longiceps* and titanium had been studied for the isolation of the HApT. Hydroxyapatite could be doped with bactericidal silver nitrate and a biopolymer, bio-adsorbable chitosan for the controlled release, targeted delivery, and corrosion resistance (Ashwitha et al., 2020; Pon-On, 2016). Chitosan/HApT particles were extracted from tuna fish heads (Ma et al., 2021). The HApT nanoparticles and chitosan substrates were studied for the adsorption of the β-carotene/lycopene from the tomato extract (Kongsri, 2013).

Hydroxyapatite (HApT) had been used as an adsorbent for ions or molecules with a photocatalytic performance and was used for the depollution applications (Wang, 2023). The HApT obtained from the marine waste byproducts, as fish scales possess excellent ability to promote the teeth and bone cells growth and propagation. Hydroxy-apatite has a similar chemical composition with the natural bones and known as a bone mineral used for a rapid bone repair being a thermodynamically stable at the physiological pH. Natural sources also include mammalian, aquatic species, plants and algae, and minerals. The source of the natural waste and extraction procedure affect the critical properties of the HApT such as Ca/P ratio, crystalline and phase assembly, particle size, and morphology (Mohd Puád, 2019).

Most of the separation methods of fish scale constituents depend on their solubilization using heating and treating with enzymes, acids, alkalis, or organic solvents with consuming of time, washing liquids, and high energy. They are multistage as washing, drying, and thermal treatment are the mandatory stages. Hydroxyapatite could be prepared via de-proteinization, microwave irradiation, alkaline heat treatment, and thermal decomposition methods. i.e. calcination at 1000 °C from fish scales after cleaning, de-proteinization by washing with 1 N HCl, thoroughly washing several times with distilled water, treatment with 1 N NaOH solution, filtration, washing with distilled water, and drying at 60 °C in a

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hot air oven for some hours (Mkhize, 2023; Safronova, 2022).

The HApT has been synthesized using two different types of fish scales, carpa (CA) and pink perch (PP) by calcinations, by wet chemical precipitation method from the Red Snapper fish (*Lutjanus campechanus*) Scales and by ionic liquid treatment (1-butyl-3-methylimidazolium acetate) (Kodali, 2022; Ulfyana, 2018; Muhammad, 2016). Thermal decomposition was a preferred technique to extract HApT from Tilapia fish scales (Majhool, 2019; Hamzah, 2019). Hydroxy-apatite was synthesized from the black tilapia scales by the calcination method (Yudhit et al., 2019). Black tilapia (*Oreochromis mossambicus*) fish scales were used as a source of natural HApT by thermal technique followed by ball milling 48 hours and spray dry to produce powder at about 1.86 µm. The spray-drying method produced different sizes in the range 2.18 - 6.36 µm (Ismail, 2019). The Tilapia scales also were pyrolyzed at different temperatures (450–600 °C) and the pyrolysis produced biochars, which are carbon/HApT nanocomposites. The presence of the organic residues on the carbon could potentially block the calcium sites on the HAP and lower the dissolution efficiency used as P fertilizers (Sittitut, 2022). This study aims to extract, separate and purify the hydroxyl-apatite compound via recycling of the fish scales of Egyptian Nile Tilapia (*Oreochromis niloticas*) using a chemical dissolution/precipitation approach.

2. Materials and Methods

2.1 The Extraction Procedure

About 300 g of the Tilapia fish scales were washed by tab water containing a little soap then washed again by water only then by distilled water. The washed fish scales were oven dried at 120 °C for 4 h then ground in a grinder to obtain as a fine powder. Twenty-five grams of the powder were suspended in 1 L of a 0.75 M NaOH with magnetic stirring at 60 °C – 70 °C for 5 min then the stirring was stopped and the suspension was left for settling. The clear brown (beaker 1) solution was decanted and the solid portion was washed thoroughly by distilled water using another beaker for a repeated settling/decantation. The washed solid portion was transferred to a third beaker containing 1 L of a 1.3 M HCl with magnetic stirring at 60 °C – 70 °C for 30 min tell complete dissolution forming a clear solution (beaker 2). The stirring was stopped and the solution was left to attain the room temperature. After that, the solution stirring was re-started with adding a 50 – 60 mL NaOH (5 M) drop wise until a dens white precipitate form (beaker 3). The stirring was stopped and the precipitate was separated by filtration and oven-dried at 120 °C for 4 h then ground. Scheme 1 presents the rout of extraction.

2.2 Characterization

Samples of the fish scales and the obtained precipitate were subjected to the elemental analysis and Fourier transform infrared spectroscopy (FTIR) analysis. Freeze dried samples were individually mixed at a 1:5 ratio with vacuum dried potassium bromide (KBr) and pressed into pellets by a hydraulic press. The infrared spectra were obtained in the range between 4000 and 400 cm^{-1} with the KBr disc method using an infrared spectrometer (JASCO FT/IR 4100, TOKYO, JAPAN).

The X-ray diffraction patterns were obtained using Cu Kα radiation from a rotating anode generator (XPERT, PRO, PANalytical, Netherland). The PAN analytical X-Ray Diffraction equipment model X'Pert PRO with Secondary Mono-chromator, Cu-radiation (λ = 1.542 Å) at 45 KV, 35 mA and scanning speed 0.04°/sec. The diffraction peaks between 2θ =2° and 60°, corresponding spacing (d, Å) and relative intensities (I/I°) were obtained. The diffraction charts and relative intensities are obtained and compared with ICDD files.

The particles' surface morphology and their Ca/P ratio as well as elemental analysis were analysed using the field emission scanning electron microscope (FESEM) (Quanta FEG 250) model attached with Energy dispersive X-rays spectrometry (EDX). Thermal stability up to 800 °C was assessed for the samples by thermal analyzer (TGA-50H) using Platinum cell in liquid N_2 atmosphere with a flow rate 50 mL min^{-1} and heating rate 10 °C min^{-1}. The particle size distribution (PSD) was analyzed to obtain the Gaussian distribution for the solid state HApT sample.

3. Results and Discussion

3.1 Physico-Chemical Characterization of Fish Scales And The Extracted Hapt

3.1.1 The Chemical Composition and Functional Groups of Samples Under Study

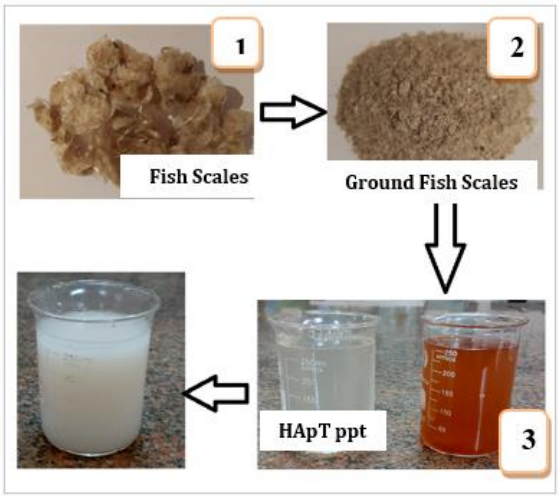
The Carbon-hydrogen-nitrogen (C/H/N) as well as the EDX elemental analysis results in Tables 1 and 2 indicate the transformation of the fish scales into the extracted HApT via the described procedure. About 89.5% of the total C/H/N content has been destroyed as it was decreased from 37.14% in the fish scales to 3.89% in the extracted HApT. The total C/N/O in the scales was also decreased from 70.07% to 53.89% in the HApT as shown from the EDX results. Additionally, the phosphorus and calcium contents have increased respectively by 3.75% and 10% with increased Ca/P ratio from 2.08 to ≈ 2.25 of the fish scales and HApT powders, respectively. Sodium (Na) and Si traces present in the HApT may be remained from the reagents used for the extraction. It can be said that the extracted HApT contains an organic carbonaceous content in the range 3.89% – 8.89% (chitosan/either amino or fatty acids /vitamins…etc). Some studies have been mentioned that calcination produced crystalline HApT with Ca/P ratio of 2.036 for camel and 2.131 for horse, both of which are higher than that seen for stoichiometric HApT. The presence of CaO can increase the Ca/P ratio. Elemental analysis showed that besides calcium and phosphorus, traces elements such as Na, Mg, Sr, Fe, Al, and Zn were present in the calcined camel and horse bone. The presence of trace elements in the HAp can enhance and accelerate the growth of the bones. Most biological apatite is non-stoichiometric owing to the presence of the trace elements that replace the Ca in the apatite lattice (Mohd Puád, 2019).

Table 1: Carbon-Hydrogen-Nitrogen (C/H/N) Elemental Analysis Results of The Fish Scales and Extracted Hapt

	C, %	H, %	N, %
Fish scales	24.6	3.95	8.59
Extracted HApT	1.51	1.09	1.29

Table 2: The EDX Elemental Analysis Results of The Fish Scales and Extracted Hapt

Element	Weight %		Atomic %		Net Int.		Error %	
	Fish scales	HApT	Fish scales	HApT	Fish scales	HApT	Fish scales	HApT
C K	30.54	6.90	42.97	11.90	62.44	8.86	10.63	17.20
N K	8.99	1.99	10.85	2.95	5.49	1.13	25.25	79.87
O K	30.54	45.00	32.26	58.29	56.69	83.19	12.65	11.96
Na K	–	1.80	–	1.63	–	9.58	–	23.37
Mg K	0.43	0.57	0.30	0.49	5.79	5.76	42.10	45.52
Si K	–	0.48	–	0.35	–	7.65	–	35.07
P K	9.57	13.32	5.22	8.91	173.69	190.64	4.13	4.50
Ca K	19.93	29.93	8.4	15.48	244.6	299.9	2.87	2.84



Scheme 1

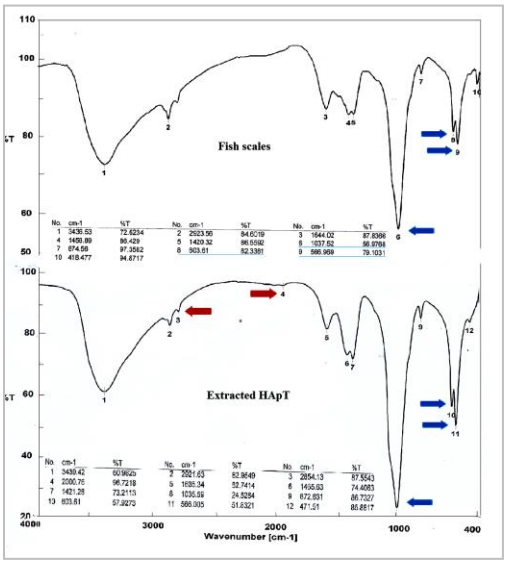


Figure 1: FT-IR Spectra of the Fish scales and Extracted HApT

The higher Ca/P ratio obtained can be attributed to the presence of carbonate ions substituted phosphate in the apatite structure (β-type carbonate hydroxyl-apatite) confirmed by the FT-IR spectra (Figure 1). The functional groups of fish scales and HApT were determined in FT-IR spectrum in the range 4000–400 cm^{-1} that confirms the chemical composition. The intensity difference in bands at 874.56 cm^{-1} (fish scales) and 872.63 cm^{-1} (HApT) may refer to the bending mode β-type carbonate hydroxyl-apatite. This is added to the bands at 1465.63 and 1421.28 cm^{-1} being due to the substitution of PO_4^{3-} by CO_3^{2-} due to absorbed carbon dioxide. The FT-IR bands in both samples at in the 3500–3000 cm^{-1} regions are characterizes the —OH stretching of H_2O. The bands at 1035.59 cm^{-1} and between 603.61 and 566 cm^{-1} are due to the PO_4^{3-} stretching and bending modes, respectively (Majhool, 2019). The band at 2000 cm^{-1} may be due to overtone of the 1035.59 cm^{-1} band (Mohd Puád, 2019).

3.1.2 Crystal Structure and Morphology of Samples Under Study

The XRD diffracto-grams in Figure 2 and EDX spectrum (Figure 3) along with data in Table 3 indicate a well-defined HApT phase with characteristic peaks higher and sharper in comparison with the fish scales pattern according to the reference cards. The phase composition description of fish scale powder may contain: mixture of amorphous hydroxyl-apatite ($Ca_{10}(PO_4)_6(OH)_2$) or hydroxyl-apatite-dental ($Ca_{4.7}H_{0.46}Mg_{0.05}Na_{0.1}O_{12.51}P_{1.61}$) and/or magnesium whitlockite ($Ca_{10.115}Mg_{0.385}(PO_4)_7$) or $Ca_{9.5}Mg(PO_4)_7$ (Safronova, 2022; Ashwitha et al., 2020; Pon- On, 2016). Similar results have been obtained by the calcination of the fish scales at 600 ^{0}C for 1-5 h (Mkhize, 2023).

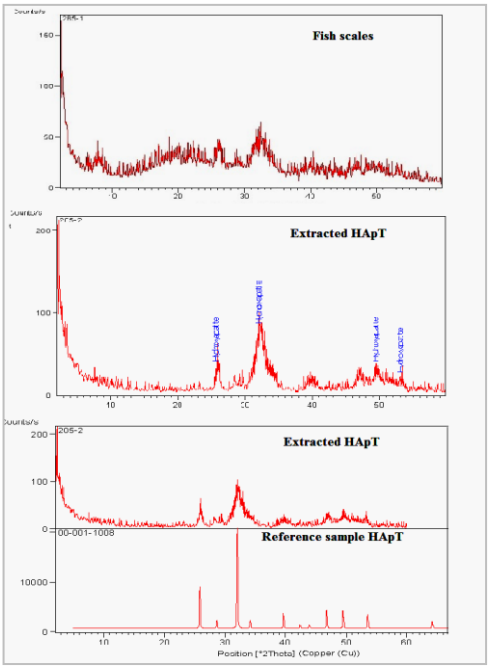


Figure 2: X-ray diffraction (XRD) patterns of the Fish scales and Extracted HApT

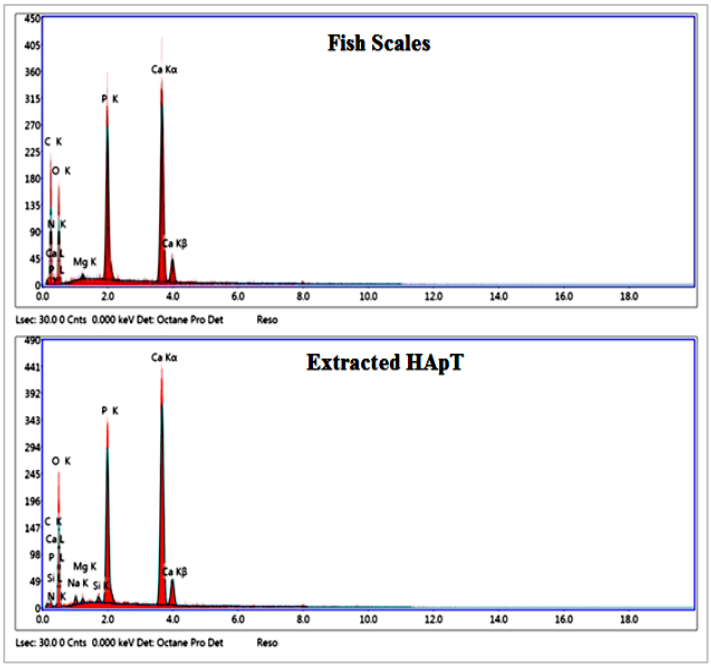


Figure 3: The EDX spectrum of Fish scales and the Extracted HApT

Table 3: X-ray dDiffraction (XRD) Analysis Results of The Fish Scales and Extracted Hapt

Peak Number	Pos. [°2θ]	*d*-spacing	Height [cts]	FWHM Left [°2θ]	Rel. Int. [%]
	Fish Scales				
1	26.0462	3.41832	9.81	0.8640	100.00
	Extracted HApT				
1	25.9434	3.43448	14.72	0.3542	37.91
2	32.2364	2.77695	38.82	0.9446	100.00
3	49.7676	1.83217	10.43	0.7085	26.87
4	53.3086	1.71852	6.64	0.7085	17.10
Ref. Code	Mineral Name		Chemical Formula		Semi Quant [%]
00-001-1008	Hydroxyapatite		$Ca_{10}(OH)_2(PO_4)_6$		100

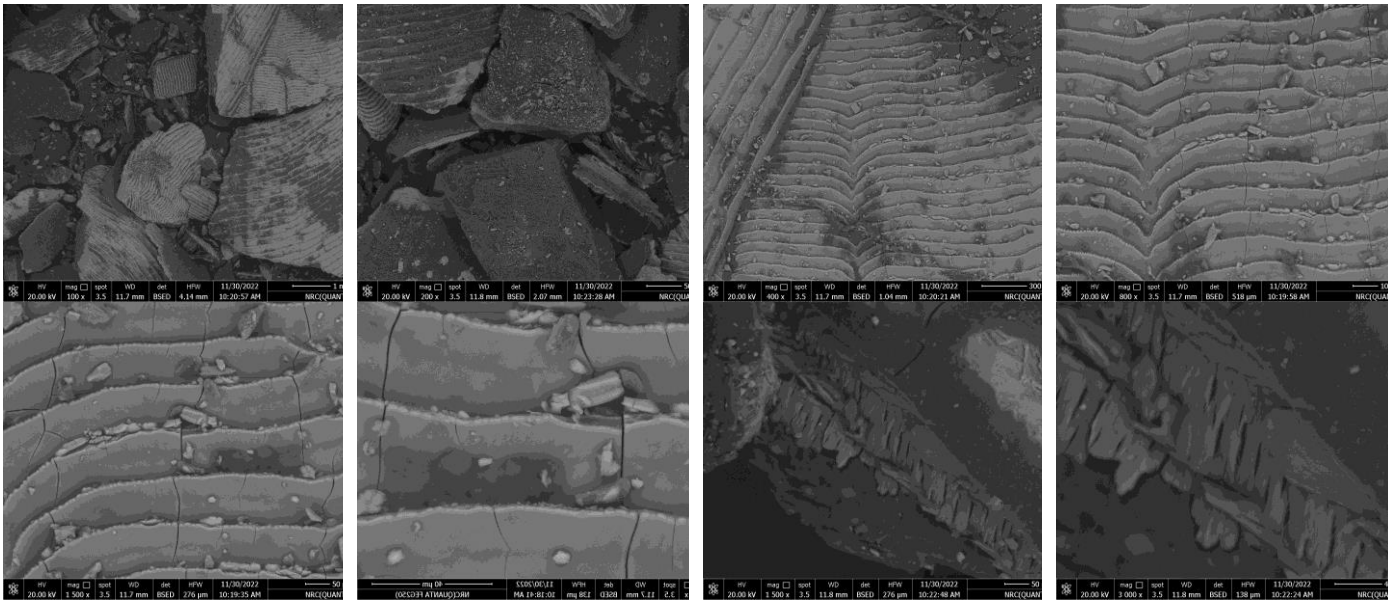


Figure 4a: Scanning Electron Micrographs (SEM) of the oven-dried fish scale ground powder

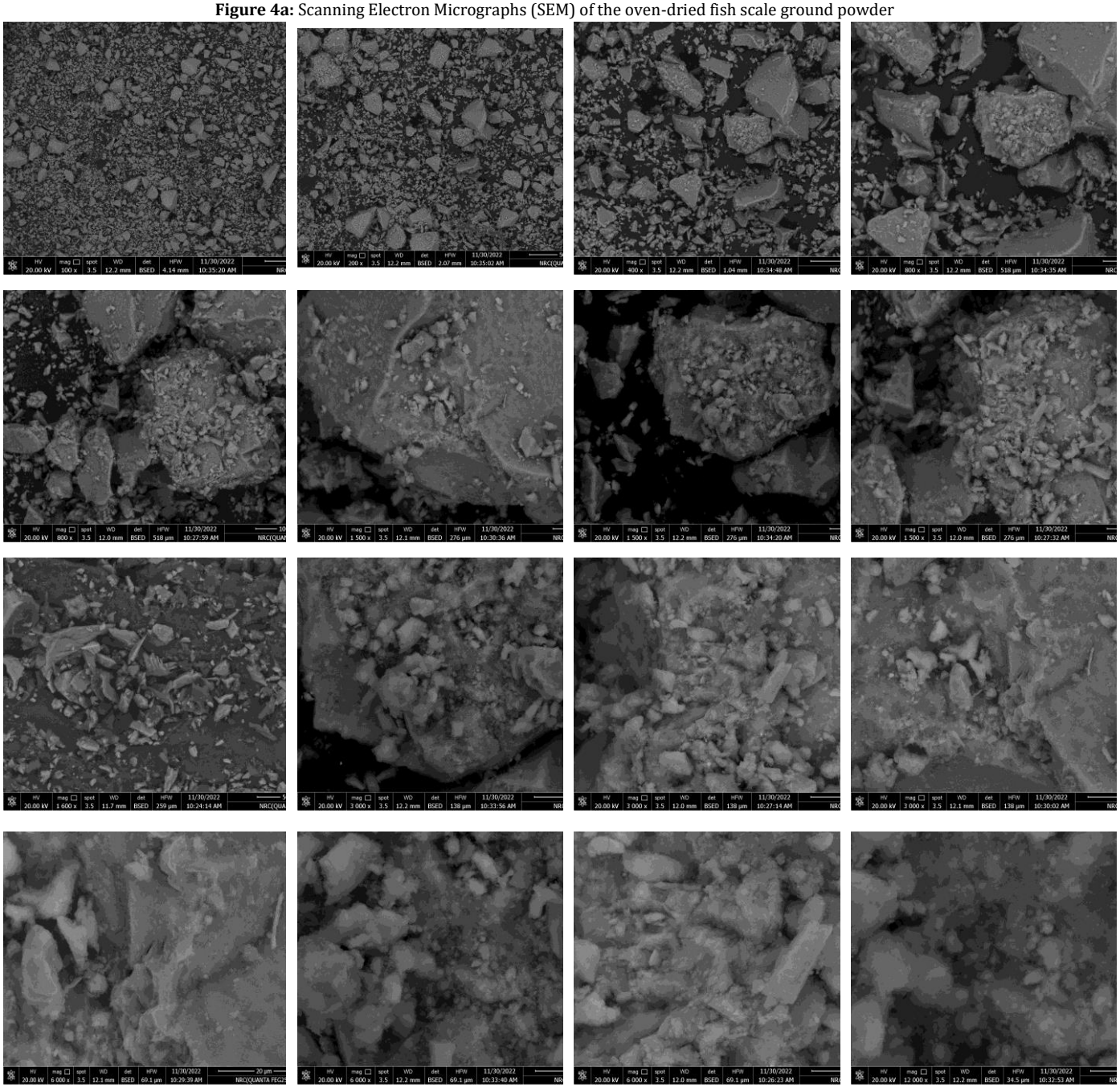


Figure 4b: Scanning Electron Micrographs (SEM) of the oven-dried HApT powder extracted from the fish scales

The SEM images arranged in Fig. 4a are showing that the magnification of fish scales powder be composed of flat-plate like morphology of in the form of cells in the range 1 mm at 100x to 50µm at 1500x magnification. The un-smooth edges and broken particles that appear in the images may be resulted from grinding of the dried fish scales. The HApT (Fig. 4b) appeared in a micro-scale dense agglomerated soft morphology with higher surface roughness (1 mm at 100x – 10µm at 12000x).

Thermo-grams of the studied fish scales and extracted HApT are presented in Fig 5 and reveal their thermal stability tell 800 °C. The TGA curve of the fish scales powder shows a total weight loss by 64.03% through three endothermic decomposition steps at 77.92 °C, 323.96 °C, and 438.01 °C. This can indicate a combination of complex chemical reactions taking place in the organic component of fish scales leaving the inorganic residual upon heating at temperature >500 °C. Mainly, they are the dehydration and decomposition of organic compounds.

The HApT powder has exhibited a 16.87% total weight loss most of them before 500 °C, which can be attributed to the dehydration of preparation H_2O and/or thermally degraded organic remains not removed during the extraction procedure (Safronova, 2022). The phase transformation of the hexagonal structure of crystalline HApT can decompose into tricalcium phosphate ($Ca_3(PO_4)_2$, TCP) at temperatures > 1000 °C. There are three forms of TCP: β-calcium phosphate (β-TCP, rhombohedral structure), α-calcium phosphate (α-TCP, monoclinic structure) and α′ -calcium phosphate (α′ -TCP, hexagonal structure). The β-TCP is stable up to 1200 ◦ C, and converted to α and α′ -TCP at temperatures > 1430 °C. The phase transformation of HApT to β-TCP, α-TCP, and other phases inhibits its mechanical properties. Previous studies had been indicated that the thermal analysis (DSC and TGA) of tilapia bone in the 20 – 800 °C range with a heating rate of 10 °C /min showed a weight loss of 66% and maximum degradation of moisture and collagen or protein of fish bone at 741.6 ◦ C (Khamkongkaeo, 2021). The particle size distribution (PSD) analysis in Fig. 6 has indicated a range of particle size ~250 – 2500 nm with 50% of sample volume < 1 µm.

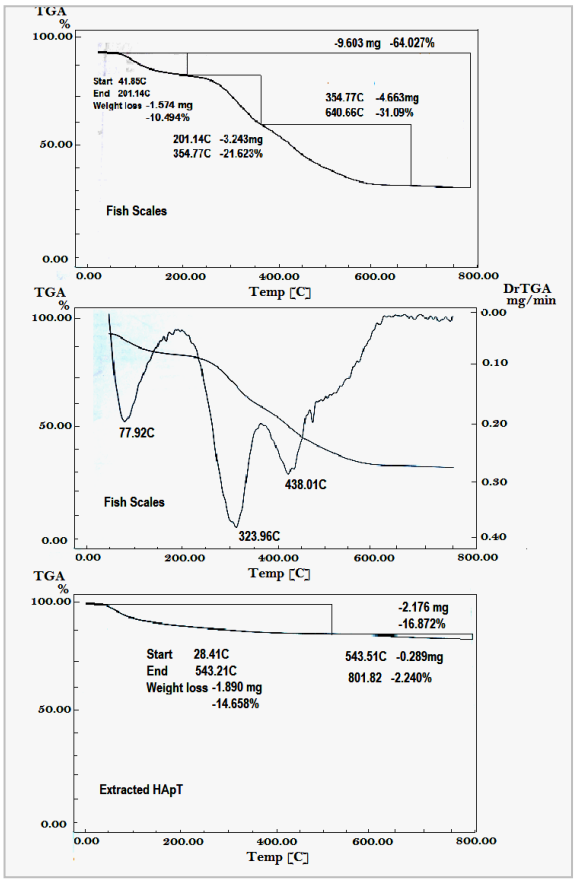


Figure 5: Thermo-Gravimetric analysis results of oven-dried the fish scales and HApT powder extracted

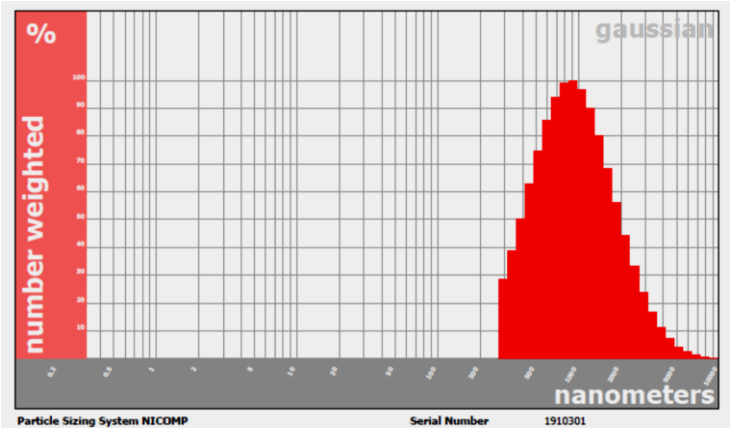


Figure 6: Particle size distribution analysis results of the HApT powder

4. Conclusion

This study presents an economic simple and fast extraction procedure to obtain the hydroxy-apatite (HApT) via the chemical recycling of the Egyptian Nile Tilapia (*Oreochromis niloticas*) fish scales. Characterization of the product by the elemental analysis, FTIR, XRD, SEM, EDX, and TGA has revealed the main peaks of amorphous/crystalline mixed HApT phase ($Ca_{10}(PO_4)_6(OH)_2$) or hydroxyl-apatite-dental ($Ca_{4.7}H_{0.46}Mg_{0.05}Na_{0.1}O_{12.51}P_{1.61}$) and/or magnesium whitlockite ($Ca_{10.115}Mg_{0.385}(PO_4)_7$). The particles showed a soft homogeneous morphology rather than specifically shaped particles with a 2.25 Ca/P ratio. The results ensure the efficiency of studied method for HApT extraction as well as fish scales recycling.

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Conflict of Interest

The author declares that there is no conflict of interest.

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