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Biphasic Calcium Phosphate Derived from a Sardine By-Product

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Abstract

In this work, phosphate-based compound used in biomedicine was extracted from scales of Moroccan sardines (Sardinapilchardus). This is the first time that a by-product of Moroccan fish is used for the extraction of these kinds of materials. The scales were subject to a simple calcination process. The scales formed a mixture of hydroxyapatite ($Ca10(PO_4)_6(OH)_2$, HAP) and β-tri-calcium phosphate (β-$Ca_3(PO_4)_2$, β-TCP), with a higher content of β-TCP obtained at high temperature. This bi-phasic biomaterial has an added value, as it is used as a bioceramic; in reality HAP has good biocompatibility whereas β-TCP has good resorbability.

Keywords: HAp/β-TCP; Scales; Calcination method; FTIR; XRD

Introduction

Various samples of bio wastes are being discarded around the globe every day and nowadays, management of wastes have been major problems in big cities. So, extracting products which have high value is a way of addressing this problem whereas valorizing such wastes. One of these bio wastes such as fish scales are used to synthesis hydroxyapatite (HAP) [1-4]. In fact, there are various types of calcium phosphate salts, which are present in fish scales due to their extreme biological response in physiological environment [4]. During evolution, scale formation process shows the same mechanism as in the formation of teeth and bone [4].

Sardine fish are plenty in Morocco and are widely used as a source of food. Sardine wastes (scales, skin and bone) are discarded by local fish markets and industrial enterprises. This waste is hazardous to the ecosystem and poses a risk to the environment and health. To avoid this risk, this is the first time we have employeda cost-effective method of producing a new bio-based manufacturing process of apatitic calcium phosphate (Hydroxyapatite (HAP)/β-tricalcium phosphate (β-TCP)) from Moroccan sardine scales for various applications; commercial and biomedical.

Materials and Methods

Sample preparation

Figure 1 shows schematic flow diagram demonstrating various steps in preparation of apatitic calcium phosphate powder from the fish scales. The sample preparation is begin with washed the sardine scales with hot water in order to remove all types of proteins and other organic impurities. Then, the scales were washed with distilled water to remove sodium chloride and dried overnight at 40 °C. After that, the crushing process was taken place to reduce the size of scales into powder form in 0.2mm of range size. Last step, the raw powder of sardine scales then continues to calcination process.

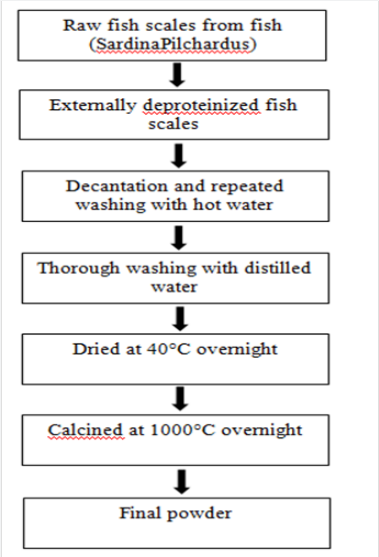

Figure 1: Schematics for biphasic powder from sardine scales.

Calcination process

The apatitic bi-phasic powder was produce after the raw powder of fish scales were undergoing a calcination stage. Calcination process is categorized as intelligent way in produce the HAP/β-TCP powder due to low cost and uncomplicated method. The raw sample powder was heated overnight in a furnace at 1000 °C in air. As soon as the calcination temperature has been reached, the sample will maintain cooled naturally in the furnace.

Sample powder characterization

The characterization of sample powder after calcination process was analyzed by X-ray Diffraction (XRD) to identify the mineralogy of sample powder and by Infrared Spectroscopy (IR) to analyze the molecular structure. X-ray diffraction analysis was realized by means of a SEIFERT XRD 3000 P using CuK radiation (wavelength λ=1.54.10-10m; tension V=45kV, intensity I=35mA) and a monochromator eliminating Kβ radiation. The analyze was carried out using the classical θ-2θ configuration, with 2θ angle steps of 0.02° and counting times of 19s per step.

Identification of the phases was realized by comparing the experimental XRD pattern to standards complied by the International Centre for Diffraction Data (ICDD) using the cards 00-009-0432 for hexagonal HAp structure and for β-TCP using JCPDS file n° 09-0169. The weight percentages of phase were calculated, from XRD pattern, using Full Proflogiciel. For infrared absorption analysis, 1mg of the powered samples was carefully mixed with 300mg of KBr and palletized under vacuum. The pallet was analyzed using a Perkin Elmer 1600 FTIR spectrophotometer.

Results and Discussion

General descriptions

The raw sardine scales powder was undergo to calcination process in order to produce the calcium phosphate apatite powder. The raw powder is subjected to high-temperature at 1000 °C to lead to the formation of biphasic HAP/β-TCP [5,6]. The calcium phosphate apatite powder is observed as soft blue color.

X-ray diffraction (xrd) analysis

The crystalline phase analysis of apatitic powder from sardine scales is realized by X-ray diffraction study. The figure below exhibits the XRD pattern for sardine scales that have been treated at 1000 °C. Identification of the phases was carried out by comparing the experimental XRD pattern to standards complied by the International Centre for Diffraction Data (ICDD) using the cards 00-009-0432 for hexagonal HAP structure and for β-TCP using JCPDS file n° 09-0169.

Sharp peak intensity and well resolved peaks in XRD pattern of obtained powder proves complete crystallization of the powder. Too, it can be seen that a second phase as well as HAp is detected in this sample, which peaks at 31.02° corresponding to β-TCP is marked with a β in this Figure 2.

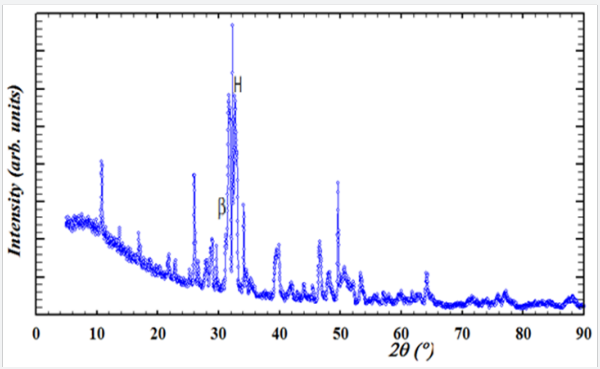

Figure 2: XRD pattern for sardine scales calcined at 1000 °C.

The letter H indicates peak belonging to HAP.

The letter β indicates peak belonging to β-TCP.

Consequently, the scales formed a mixture of hydroxyapatite and β-tri-calcium phosphate, with a higher content of β-TCP (54.2% of HAP and 45.8% of β-TCP (wt%)). This bi-phasic apatite has a high added value, as it is employed as a bioceramic. Indeed, HAP has good biocompatibility while β-TCP has good resorbability despite being less biocompatible. The presence of this compound was previously reported in apatitic calcium phosphates of marine origin [7,8].

Infrared spectroscopy (ir) analysis

The formation of apatite phase in sardine scale derived powder is confirmed by FTIR analysis. In the spectra, signals due to phosphate ions (PO43-, P-O and O-P-O) from both HAp and β-TCP were detected. It is possible to see peaks in the region of (500-1100cm^{-1}) (HAp) and at 1122cm^{-1} (β-TCP). The major peaks at ≈1046cm^{-1} and ≈1095cm^{-1} could be identified as symmetric n3 vibration of PO43- group and is the most intensified peak among the phosphate vibration modes. The other bands at 960-968 cm^{-1} and at 568-602cm^{-1} are due to n^{1} and n4 symmetric P-O stretching vibrations of the PO43- group respectively. The band between 565 cm^{-1} and 601cm^{-1} belongs to n4 vibration mode of phosphate group (Figure 3).

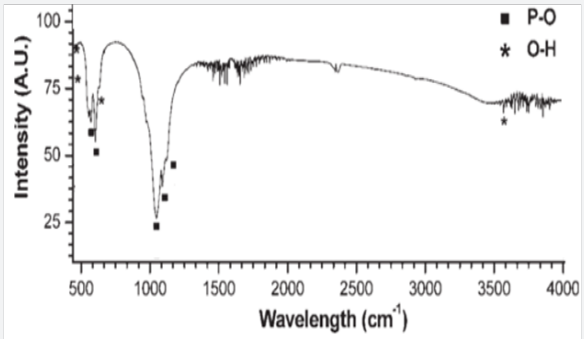

Figure 3: FTIR curve of obtained sardine scales calcined at 1000 °C.

Conclusion

In this present study, HAP/β-TCP powder has been successfully extracted from sardine scales by calcination process. It is an uncomplicated and low cost process. This study

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shows how it is possible to valorize by-products of the food industries, obtaining high added value products which can be used in biomedicine and other potential applications.

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