Vol. **131** (2017) *ACTA PHYSICA POLONICA A* No. 3

Special Issue of the 6th International Congress & Exhibition (APMAS2016), Maslak, Istanbul, Turkey, June 1–3, 2016

Physical Characterization of Turbot (*Psetta Maxima*) Originated Natural Hydroxyapatite

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Nowadays hydroxyapatite is one of the most popular biomaterials, which is used in various medical and dental applications areas as graft material. Bovine bone is the biggest source for natural hydroxyapatite production, but its production can lead to very dangerous disease, like mad cow disease, without high degree calcination. Hydroxyapatite produced from marine sources is much safer and easier to produce than bovine hydroxyapatite. Here in this study natural hydroxyapatite and related phases were produced from a local source turbot (*Psetta maxima*). Beside the main bony internal structure, there are koshers (cycloid scale) on its skin. Koshers are bulky bumps, looking like flat, small and rounded structures. Internal bones and those bulky bumps were cleaned from flesh with chemicals and calcined at 850 °C for 4 hours. After calcinations, especially those bulky bumps, were formed into mesoporous structures with very light bluish color. Those mesoporous structures can be used as natural mesoporous hydroxyapatite structures for bone grafting purposes. The internal bones have also formed hydroxyapatite. Scanning electron microscope and X-ray diffraction studies were performed. I this study it is found that the bones of turbot consist of hydroxyapatite and TCP related phases. The aim of this study is to produce natural hydroxyapatite structures from turbot scale with low carbon footprint, without harming the environment and without using complex chemicals.

DOI: 10.12693/APhysPolA.131.397

PACS/topics: 81.05.–t, 81.05.Mh, 87.85.J

1. Introduction

Applications of bulk hydroxyapatite (HA) are limited for large bone defects, because of its inferior mechanical properties. Increase of traffic accidents and increase of life span makes the value of this biomaterial very strategic. HA is usually produced from synthetic sources (i.e. from reagent chemicals) using sol-gel precipitation, hydrothermally, by electrodeposition, by biomimetic deposition and by multiple emulsion techniques [1]. However, those techniques are time consuming and cannot bear easily some strategic trace elements of bone structure such as Sr, Zn, Cu, Si, Mg and many others.

There are some other advanced production methods (i.e. mechano-chemical processing, chemical processing) which enable production of nano-HA structures from natural calcitic-aragonitic sources (mussel shells, sea urchins shells, sea snail shells, land snail shells and cuttlefish bones) [2]. There are also some studies getting HA from fish bones. High calcination temperature (800–850 °C) prevents transmission of prion diseases from different sources like sheep (i.e. Scrapie), bovine (i.e. bovine spongiform encephalopathy – BSE) and human (i.e. Creutzfeldt-Jakob disease – CJD) [3] and from many

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other sources. Instead of calcination method bovine and human bones are usually treated with diluted HCI acid, demineralization and freeze-drying processes [3].

If the standard safety procedures for this production method are not carefully followed, there is always a risk for cross contamination of those dangerous prion diseases, which were discussed above.

The aim of this study is economical production of natural HA (otherwise those bone remains are disposed to regular city trash without paying any attention) from a big flat fish (turbot) bones with limited carbon footprint.

2. Material and methods

Fish bones of turbot were obtained from a local fish market (Besiktas Fish Market in Istanbul) (Fig. 1a). Beside the general skeleton (i.e. spine), the bulky bumps on its skin were also separately collected. All collected parts were cleaned with a sharp knife from flesh residues. Bones and small bulky bumps were treated with reagent grade NaOH and then cleaned with distilled water. The cleaned and dried parts were calcined for 4 hours at 850 °C (Fig. 1b). The calcined rib bones and bulky bumps have a very light blue color (Fig. 1c). Yubao et al. had reported that trace element manganese Mg can color the HA structure to blue [4]. The bulky bumps have formed small wonderful mesoporous structures. The main skeleton and bulky bumps were subjected separately to X-ray diffraction (XRD) and scanning electron microscopy (SEM) studies.

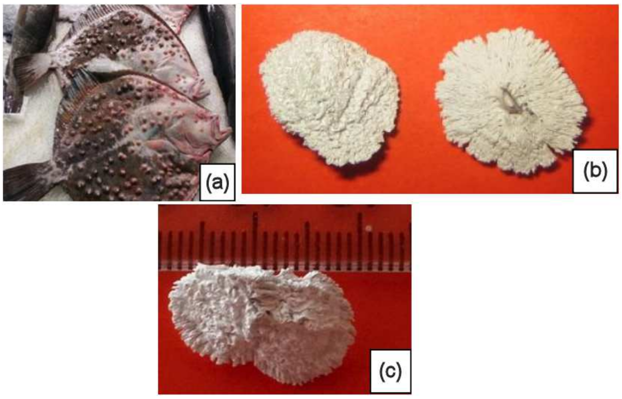

Fig. 1. Turbot (*Psetta maxima*). (a) Sold on the market bench, (b) calcined bulky bumps, (c) bumps with millimeter scale.

3. Results and discussion

In this study HA bioceramic material was produced from fish bones. A previous literature study of Coelho et al. must be taken into consideration. In that study different fish species were calcinated at 900 °C for various time periods (4–12 h) [5]. In the secondary study samples were ball milled from 0 to 32 h and nanostructures were produced with sizes from 80 to 24 nm. The produced samples were pressed in the shape of disks at less than 350 MPa pressure in a high temperature setup at up to 1000 °C. Here in this study the size of crystallites has dropped from 80 nm to nearly 22 nm when the time of milling was increased from 0 to 16 hours and agglomerates have formed at up to 32 hours [6].

In this study, the fish bones were obtained from turbot. Turbot is widely distributed almost in all European coastal waters in the Northern Atlantic from Norway to Morocco, including the Baltic, Mediterranean and Black Seas [7]. It is also an important commercial fish [8]. Turbot is a very large broad bodied left-eyed flat fish (Fig. 1a), which belongs to family Scophthalmidae [9].

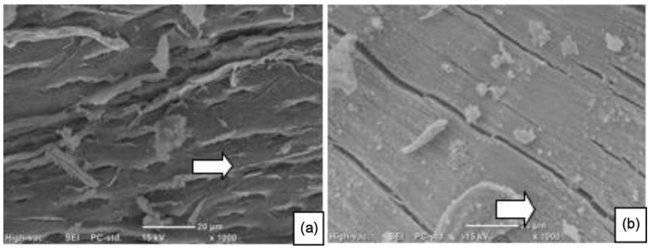

Fig. 2. SEM images (a) ×1000, taken from ribs, seen as long spines, (b) ×1000, taken from upper part of a piece of a vertebral column.

In sample in Fig. 2a HA drifted structures were formed (shown with a big white arrow). In Fig. 2b the upper part of the vertebral column was formed of stretched structures. Some small independent HA particles (from ~ 1 μm up to ~ 50 μm, marked with a white arrow) which were detached from the main structure, were also observed (Fig. 2b).

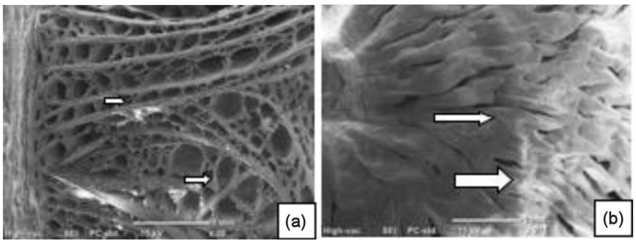

Fig. 3. SEM images of (a) ×30 cross-section of vertebral column, (b) ×27 a bulky bump.

in Fig. 3a some half rounded (some of them have an ellipsoid appearance) porous structures have appeared (from ~ 0.33 μm, shown with a big white arrow, up to ~ 0.16 μm, shown with a small white arrow). In Fig. 3b some dense structures (shown with a long white arrow) which were stretched by about $\sim 2 - 2.5$ mm, can be seen.

The major phase of uncalcinated raw bone parts (Fig. 4a) (lower a) is calcium phosphate (V) hydroxide (JCPDS card number 98-007-9408). Calcium phosphate (V) hydroxide is hydroxyapatite because it can be considered as $3Ca_3(PO_4)_2$+$Ca(OH)_2$. The major phase of

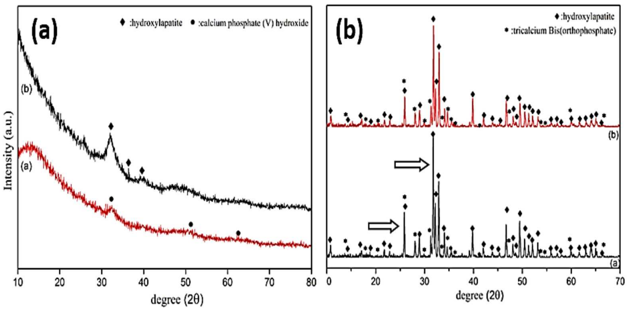

Fig. 4. X-ray diffraction of the raw bony parts and raw bulky bumps of turbot. (a) fish bone (b) bulky bumps.

uncalcined bulky bumps (upper b) is HA (JCPDS card number 98-003-5597), which is 100% HA.

The major phase for calcined turbot's bulky bumps was found to be 92.3% HA (JCPDS card number 98-009-0247) and the minor phase was found to be 7.7% Tricalcium bis (orthophosphate) (JCPDS card number 98-008 - 2984). Tricalcium bis (orthophosphate) was also detected by Gunduz et al. as a minor phase in chemical agitation method (at 400 and 800 °C heat application) from Atlantic Cowrie (*Cypraea cervus Linnaeus*) shells [10]. Tricalcium bis (orthophosphate) is a synonym of calcium phosphate tribasics [11]. Tricalcium bis (phosphate) is the IUPAC (International Union of Pure and Applied Chemistry) name of tricalcium phosphate (TCP). There are also some sources which are describing a phase with the name of tricalcium diorthophosphate beta as one of the polymorphs of TCP (tricalcium phosphate) beta, other names are $CaPO_4$ tribasic beta or tricalcium bis (orthophosphate) beta [12]. Dorozhkin had also this information given in his 2015-dated report as: "b-tricalcium phosphate [b-$Ca_3(PO_4)_2$". The IUPAC [13–14] name is tricalcium diorthophosphate beta, known as $CaPO_4$ tribasic β or tricalcium bis(orthophosphate) beta – is one of the polymorphs of TCP [14]. When Fig. 4b is investigated, it is clearly seen that bulky bumps and fish bone parts consist from HA and tricalcium bis (orthophosphate). In bulky bumps the intensities of some peaks (shown with white arrows) are sharper and denser than those from fish bones.

4. Conclusions

In this study the production of HA bioceramics from turbot was investigated. Proposed process is a cheap alternative way of production of HA 92.3% with TCP 7.7%, without using any harmful chemicals. Due to low TCP content, this bioceramics will resorb at a much lower speed. It sounds a bit unusual that the disposed bony parts of turbot can be a useful resource for bioceramic production with very low carbon footprint. Besides, any production based on fish bone will be much safer, because it will not bear any prion diseases like the bovine bones do, especially with freeze drying method (high temperature calcination is safe) [3]. Natural HA is always the best resource for human bone tissue corrections for orthopedic, dental and cosmetic surgeries.

Acknowledgments

This study has been supported by Marmara University (BAPKO Project Number: FEN-B-080415-0117).

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