



Original Article

Preparation and Characterization of Natural Fish Bone as an Implant Material

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ARTICLE INFO

Article history

Receive: 2022-06-29

Received in revised: 2022-08-22

Accepted: 2022-09-07

Manuscript ID: JMCS-2208-1682

Checked for Plagiarism: Yes

Language Editor:

Dr. Fatimah Ramezani

Editor who approved publication:

Dr. Majid Hajifaraji

DOI:10.26655/JMCHMSCI.2023.3.23

KEYWORDS

Cyprinus carpio L. Fish

Natural fish bone

Calcium phosphate

Fillers

ABSTRACT

Background: The globe currently faces two major issues: a deficit of new resources and an excessive amount of trash creation. To solve these issues, the extra waste might be used as a supply in the manufacturing process. The industrial sector is focusing on this kind of waste reclamation.

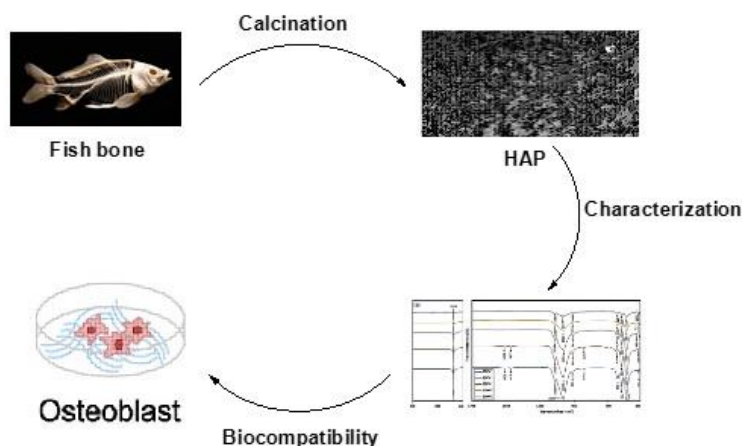
Aim: To prepare natural fish bone based bioactive material, natural fish bones are considered potential options for artificial bone due to their high bioactivity and biocompatibility.

Methods: To synthesize a composite material, natural fish bones were used from sources of raw materials. The X-ray diffraction, Fourier-transform infrared spectroscopy, and electron dispersive X-ray analysis were used to assess the synthetic material.

Results: The results showed that phosphate and calcium were presented in the synthesized powder. The average particle size of the calcined fish bone was 300 -500 nm.

Conclusion: Because of their possible biological and mechanical properties, materials made from waste natural resource materials might be appropriate for use as fillers for different dental implants.

GRAPHICAL ABSTRACT



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Introduction

Water, type I collagen, carbonated hydroxyapatite, and non-collagenous proteins make up the hierarchical structure of bone. Typically, the amount of minerals of bone tissue varies based on type and the mineral content ranges from 50 to 74 weight percent [1]. The common carp species are among the important fishes cultured in many parts of the world [2]. Their cultivation dates back to the 5th century B.C. in China, and it eventually spread to the other continents [3]. It is characterized by its resistance to adverse environmental conditions, such as a wide heat range and the ability to endure a 4.5 mg/l fall in oxygen levels [4]. They can survive in a variety of fresh and brackish-water conditions. For many years, carp production has been the principal aquaculture production in many countries in Asia, Africa, Europe, and Latin America, accounting for the greatest quantitative sector of worldwide fish farming [5].

Humans eat fish because of the nutrients and vitamins it contains. The fish-bone wastes are not utilized for any purpose. The environmental issues such as organic odor have been generated by fish-bone waste. Calcium phosphate, collagen fiber, calcium carbonate, and hydroxyapatite are the primary constituents of fish bones. As a result, employing natural calcium sources like fish bones to produce hydroxyapatite may be a viable option [6].

Natural hydroxyapatite has been synthesized from Brazilian river fish bones such as pintado (*Pseudoplatystomacorruscans*), ja (*Paulicealutkeni*), and cachar (*Pseudoplatystomafasciatum*) [7]. The duration of the process, the fish bone was calcined at 900 °C to achieve an ideal natural hydroxyapatite, fish bone has a low production cost, inexpensive [8], and has the potential to be a calcium donor.

Poly (methylmethacrylate) (PMMA) bone cement, calcium phosphate cement (CPC), and glass-based bone cement (GBC) are some of the most common methods used in the bone treatment procedure.

As a result, hydroxyapatite was considered to have critical importance in bone therapy over the traditional approach [9]. Hydroxyapatite (HA) is

a substance that can be found naturally in humans either from bone or teeth, with calcium and phosphorus being the primary elements [10]. If the ratio is closer to 1.67, HAP is more stable and that the bioactivity is inversely correlated with the ratio. The degree of crystallinity influences the reactivity [11]. The structure of hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) in bone is often comparable to that of composites made of natural minerals [12].

Hydroxyapatite is formed by the direct precipitation of calcium and phosphate ions from either the bone or the direct precipitation of calcium and phosphate ions. Natural-source hydroxyapatites have a higher metabolic activity and a more dynamic reactivity to the environment than synthesized hydroxyapatites [13]. The issues with hydroxyapatite powder created by synthetic technique include the necessity for additional chemicals to boost mechanical strength and various chemicals used in the process to stabilize the structures and keep them in place in the human body. To generate HA, this procedure is highly difficult and requires a large starting cost. Furthermore, the natural HA is less expensive and less difficult than the synthetic HA. Furthermore, this technique is time-consuming and difficult to control the generated hydroxyapatite sample [14].

The dispersion state, geometrical shape, surface quality of the filler particles, and the particle size, all affect the properties of filled polymers. The extender fillers simply increase the bulk capacity and lower the production price, whereas reinforcing fillers improve the mechanical qualities particularly the tensile strength, in addition to add the bulk [15]. Every year, the plastic industry uses several million metric tons of extender fillers and reinforcement fillers [16]. With the introduction of enhanced compounding agents that allow the use of increased amount of filler content, the usage of these additive's in plastics is likely to increase [17]. It has been predicted that fillers of up to 75 parts per hundred (pph) could be incorporated that would lead to a decrease the use of petroleum-based plastics. With increasing the volume fraction,

filler particles form aggregates or agglomerates, which is dependent on their surface.

The derived calcined natural fish bone (NFB) powder can be applied in various medicinal and industrial settings, thereby increasing the economic worth of *Cyprinus carpio L.* fish bone. In this study, we utilized *Cyprinus carpio L.* fish bone from a fresh marine to prepare bone powder and performed its physiochemical characterization.

Materials and Methods

Preparation of bio-waste materials

NFB was boiled in distilled water for 1 hour and rinsed with a high-pressure water jet to get rid of all the organic and protein debris. The untreated waste was then crushed and ground into powders with a mortar and pestle before being dried for an entire night at 40 °C in the oven. These dried powders were further broken down in a planetary mill (SFM-1(QM-3SP2)) fitted with alumina balls of various sizes at a speed of 4,000 rpm for four hours, and the powder was mesh sieve through a 100 mm sieve.

Calcination process

About 10 g of sieved bone powders was placed on an open alumina-based crucible throughout the calcination process. These samples were at the rate of 10 °C/min before allowing them to gradually cool till the room temperature in a furnace. Because it is inexpensive and simple, the calcination process is considered an intelligent way to generate hydroxyapatite powder.

Characterization of natural bio-waste materials

Fourier transform infrared spectroscopy (FT-IR)

A mixture of 1-2 mg fish bone powder and 200 mg spectroscopic grade potassium bromide was used. Spectra from the 400 cm⁻¹ to 4000 cm⁻¹ areas were registered by using an ASHIMADZO (Japan).

X-Ray diffraction

The crystalline structure and phase composition of the uncalcined raw powders were determined by using XRD (XRD-6000, SHIMADZU). CuK α radiation ($k = 0.154056$ nm) was employed, with a voltage of 40 kV and a current of 30 mA, and data was stored over a period of two hours, from 10 to 50 [18].

Scanning electron microscopy

The NFB microstructure was examined by using a scanning electron microscope (SEM-FEIQuanta model, the Netherlands) at a voltage of 12.5 kV before and after the calcination procedure. The chemical compositions of the raw powders before and after the calcination process were determined by using energy dispersive spectroscopy (EDS).

Results and Discussion

The calcined NFB powder is used to breakdown organic molecules at high temperatures. The calcination process was carried out at various temperatures of 400, 500, 700, and 900 °C for 2 hours (Figure 1).

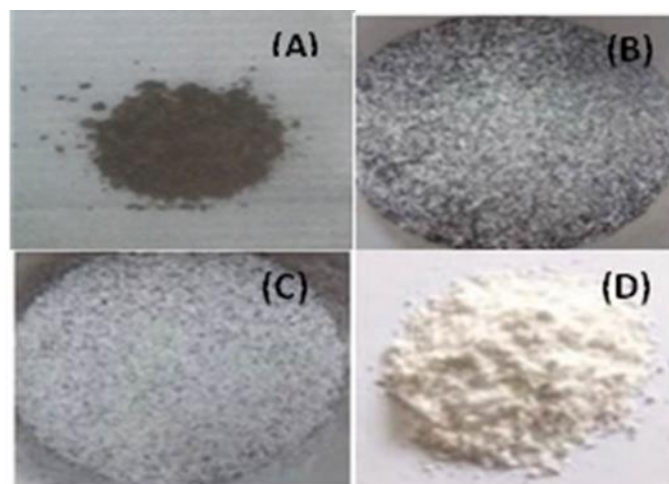


Figure 1: The calcined NFB at various temperatures; a) 400 °C, b) 500 °C, c) 700 °C and d) 900 °C

In Figure 1, the result showed that a black powder produced at a temperature of 400 °C. When the temperature was raised to 500 °C, the color changed to dark grey, and 700 °C, the color changed to light grey [19]. A white powder was obtained at 900 °C. This white powder showed that the calcination procedure has removed the organic components from NFB [20].

Fourier transform infrared spectroscopic analysis

The purpose of this test intended to obtain the accurate information references on the sources of vibration of the phosphate, carbonate, and amide groups. Figure 2 displays various temperatures of the FT-IR spectrum of NFB. A single distinctive peak of HA was seen in the FT-IR spectra of NFB powder calcined between 400 °C and 900 °C, which is consistent with previously published data [21-23]. The spectra 600, 632, 872, 961, 961, 1026, 1078, 1411, 1455, 2035, 2156 cm^{-1} , and a wide band detected between 3200–3500 cm^{-1}

and are in close accord with the HA reference spectrum [24]. At lower temperatures (400 °C), phosphate (PO_4^{3-}) group peak at 1088 cm^{-1} was absent and only arose at temperatures over 700 °C. This might be owing to the raw NFB being purified from of all the organic content.

A crucial quality of calcined NFB is thermal stability. The greater temperatures may cause calcium phosphate to develop in the bovine bone, demonstrating that the synthesized bone is not thermally stable. On the other hand, calcination-related phase transformations were not seen in this investigation, even at high temperatures (500 °C to 900 °C). The peaks between 1025 and 1087 cm^{-1} and 961 cm^{-1} are then assigned to PO_3 . Moreover, due to the existence of the hydroxyl group, all of the FT-IR spectrum samples showed a peak at 633 cm^{-1} and a wide peak at 3200-3500 cm^{-1} . The spectra of calcined NFB shows the significant peaks at 1412 and 1456 cm^{-1} , which are due to CO_3 .

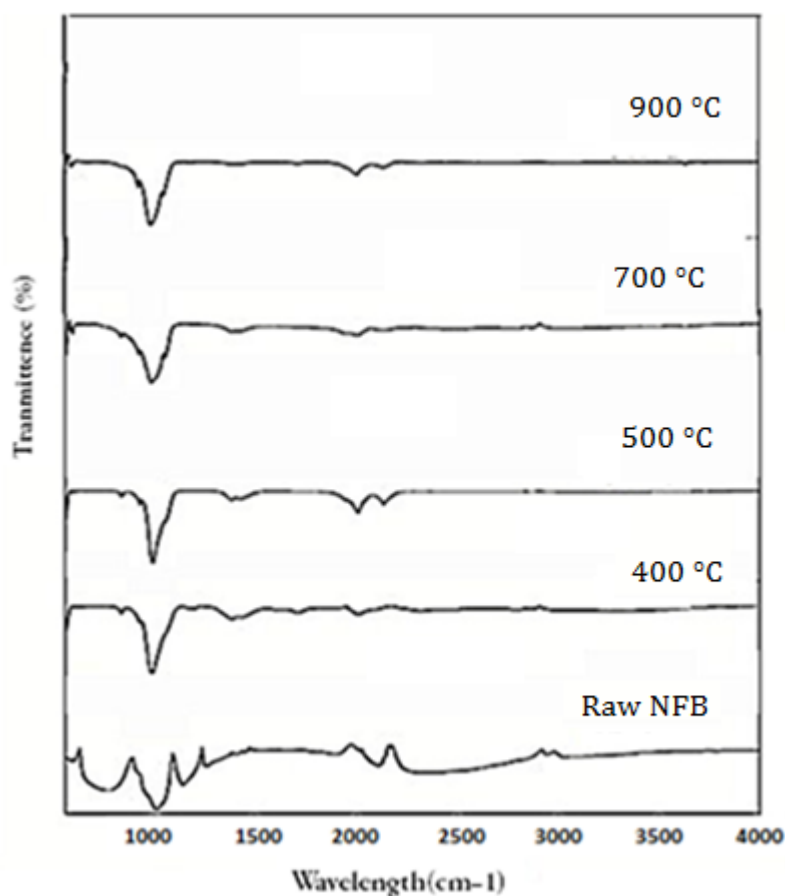


Figure 2: The FTIR spectra of calcined NFB a) Raw NFB, b) 500 °C, c) 700 °C, and d) 900 °C

X-Ray diffraction analysis of cyprinus carpio L. Fish Bone

The finding of X-Ray diffraction analysis indicated the phase and clarity of calcined NFB crystals. The XRD pattern of raw NFB and processed bone at different temperatures are both indicated in [Figure 3](#). The current XRD data indicate that when calcined in air up to 900 °C, the stability in the bone matrix was not compromised, because the HA chemical makeup was not found to be altered and the pure HA was detected. The NFB powder crystalline structure when calcined between 700 and 900 °C was compared with that of the structure HA (JCPDS-09-0432/1996). Typically, the peak's intensity rises with the increase in temperature, but its width decrease [25]. This finding indicated that HA obtained at various temperatures in the current study has striking similarity to the standard HA in terms of purity and stability. At 900 °C, it was concluded that the relative intensity of calcined bone was exactly the same as conventional HA's. However, dehydroxylation of the HA phase would result in a little peak displacement in the XRD trace, according to Wang and Chaki.

Field emission-scanning electron microscope analysis

The surface shape and crystal size of the calcined NFB powder were investigated by using FE-SEM [26]. The FE-SEM images of raw and calcined NFB powder at different temperatures are depicted in [Figure 4a, b, c, and d](#), respectively. Fine particles with a crystalline size of 5–10 nm, 10-15 nm in width, and more than a few micrometers in length made up the microcrystal of calcined NFB [27, 28]. Due to the presence of organic materials, the microstructures of raw NFB seemed clustered, as illustrated in [Figure 4a](#). The development of nanoparticles in the treated NFB at 500 °C with crystal sizes ranging from 80 to 300 nm was clearly seen in [Figure 4b](#). The structure of the treated bone was observed in [Figure 4c and d](#) as the temperature is raised from 700 °C to 900 °C. NFB was sintered at 700 °C and 900 °C, and the crystal sizes comprised 0.3-1.0 μm and 0.5-2.0 μm , respectively. It was theorized that the crystal size rises with temperature based on the surface shape. The higher temperatures had a tendency to cause crystallization and the particles agglomeration [29-32].

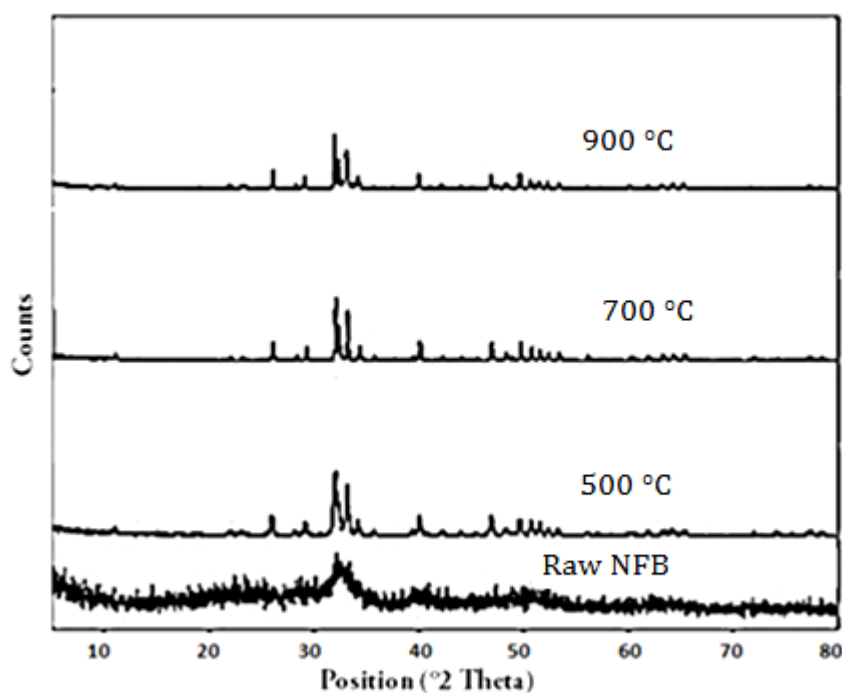


Figure 3: The XRD results of calcined NFB at different temperatures; a) Raw NFB b) 500 °C, c) 700 °C, and d) 900 °C

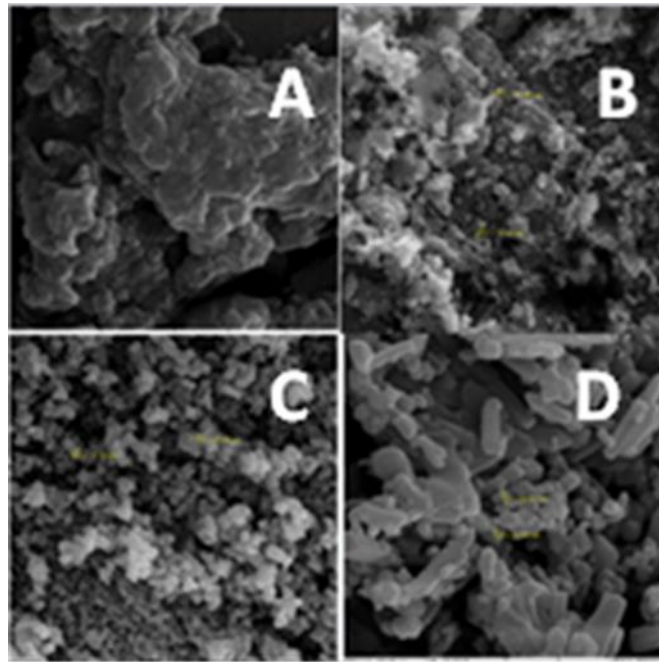


Figure 4: Calcined NFB powder were studied under FE-SEM at various temperatures; a) 400 °C, b) 500 °C, c) 700 °C, and d) 900 °C

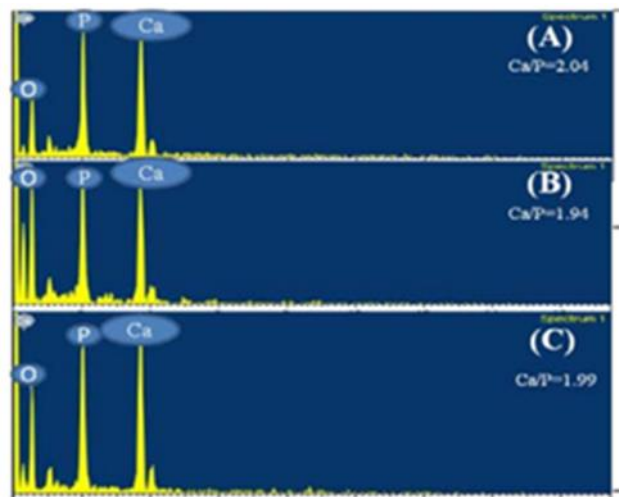


Figure 5: The EDX results at different temperatures; a) 500 °C, b) 700 °C, and c) 900 °C

Electron dispersive X-ray analysis

Figure 5a, b, and c displays the elemental analysis at 500 °C, 700 °C, and 900 °C, respectively. The ratio for treated NFB was calculated as 2.04, 1.94, and 1.99, respectively, to the temperatures. These findings are in contrast with the previous literature. It may be assumed that the Ca/P weight ratio is independent of the calcination temperature since a various temperatures, there was no noticeable difference in the Ca/P weight ratio of the calcined bone [33].

Conclusion

Recently, there has been a lot of interest in the development of novel composites derived from biological waste and byproducts. The amorphous nature of the material was confirmed by the lack of significant peaks in the XRD pattern. The calcined bone powder was successfully synthesized from NFB within the parameters of the current experiment by using a basic calcination treatment. The potential biological and mechanical properties were assessed by using the FT-IR, XRD, and EDX characterization of the chemical, physical, and morphological

properties of the calcined NFB formulation, warrants its use as a filler for different implants.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

The author declared that they have no conflict of interest.

References

[1]. Hajirezaee S., Abed-Elmdoust A., Alekhina, N., Chupradit S., Mustafa Y.F., Metabolite profiling of the post-ovulatory oocytes of the common carp, *Cyprinus carpio*: A 1H NMR-based metabolomics approach, *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*, 2021, **40**:100917 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]

[2]. Davies S.J., Gouveia A., Comparison of yttrium and chromic oxides as inert dietary markers for the estimation of apparent digestibility coefficients in mirror carp *Cyprinus carpio* fed on diets containing soybean-, maize-and fish-derived proteins, *Aquaculture Nutrition*, 2006, **12**:451 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]

[3]. Abell R., Thieme M.L., Revenga C., Bryer M., Kottelat M., Bogutskaya N., Coad B., Mandrak N., Balderas S.C., Bussing W., Stiassny M.L., Freshwater ecoregions of the world: a new map of biogeographic units for freshwater biodiversity conservation, *BioScience*, 2008, **58**:403 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]

[4]. Barai A.K., Chandra K.J., Majumder S Population ecology and infestation of monogenetic trematodes in juvenile carp fishes of Mymensingh, *Journal of the Bangladesh Agricultural University*, 2005, **3**:121 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]

[5]. Buchmann K., Slotved H.C., Dana D., Epidemiology of gill parasite infections in *Cyprinus carpio* in Indonesia and possible control methods, *Aquaculture*, 1993, **118**:9 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]

[6]. Pallela R., Venkatesan J., Kim S.K., Polymer assisted isolation of hydroxyapatite from *Thunnus obesus* bone, *Ceramics International*, 2011, **37**:3489 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]

[7]. Coelho T.M., Nogueira E.S., Weinand W.R., Lima W.M., Steimacher A., Medina A.N., Baesso M.L., Bento A.C., Thermal properties of natural nanostructured hydroxyapatite extracted from fish bone waste, *Journal of applied physics*, 2006, **101**:084701 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]

[8]. Chakraborty R., Roy Chowdhury D., Fish bone derived natural hydroxyapatite-supported copper acid catalyst: Taguchi optimization of semibatch oleic acid esterification, *Chemical engineering journal*, 2013, **215**:491 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]

[9]. Hammood A.S., Hassan S.S., Alkhafagy M.T., Comparison of natural and nano-synthetically-produced hydroxyapatite powder, *Jom*, 2019, **71**:272 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]

[10]. Qiang F., Nai Z., Wenhai H., Deping W., Liying Z., Haifeng L, Preparation and characterization of novel bioactive bone cement: Glass based nanoscale hydroxyapatite bone cement, *Materials in Medicine*, 2004, **15**:1333 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]

[11]. Sharifi S., Lotfipour F., Ghavimi M.A., Dizaj, S.M., Shahi S., Yazdani, J., Hydroxyapatite-gelatin and calcium carbonate-gelatin nanocomposite scaffolds: Production, physicochemical characterization and comparison of their bioactivity in simulated body fluid, *Eurasian Chemical Communications*, 2021 **3**:70 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]

[12]. Gur D., Leshem B., Oron D., Weiner S., Addadi L., The Structural Basis for Enhanced Silver Reflectance in Koi Fish Scale and Skin, *Journal of the American Chemical Society*, 2014, **136**:17236 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]

- [13]. Juang H.Y., Hon M.H., Effect of calcination on sintering of hydroxyapatite, *Biomaterials*, 1996, **17**:2059 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [14]. Venkatesan J., Lowe B., Manivasagan P., Kang K.H., Chalisserry E.P., Anil S., Kim D.G., Kim S.K., Isolation and characterization of nano-hydroxyapatite from salmon fish bone, *Materials*, 2015, **8**:5426 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [15]. Venkatesan J., Kim S.K., Effect of temperature on Isolation and characterization of Hydroxyapatite from Tuna (*Thynnusobesus*) Bone, *Materials*, 2010, **3**:4761 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [16]. K. Abass A., Naji Jassim Al Sieadi W., A.K.A.M. Al-Sammarraie 'Investigation of the electrical, compositional, and magnetic features of hybrid lead oxide nanocomposites', *Eurasian Chemical Communications*, 2022, **4**:1044 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [17]. Nejres A.M., Ali H.K., Behnam S.P., Mustafa Y.F., Potential effect of ammonium chloride on the optical physical properties of polyvinyl alcohol, *Systematic Reviews in Pharmacy*, 2020, **11**:726 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [18]. Katz H.S., Milewski J.V., Eds., Van Nostrand Reinhold, New York, 1987, 467 pp. Handbook of fillers for plastics, [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [19]. Ayoub H.A., Khairy M., Rashwan F.A., Abdel-Hafez H.F., Synthesis of calcium silicate hydrate from chicken eggshells and combined joint effect with nervous system insecticides, *Asian Journal of Green Chemistry*, 2022, **6**:103 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [20]. MIYAH K., Loekitowati P., Yuliasari N., "Sintesis dan Karakterisasi Hidroksiapatit dari Cangkang Telur Ayam dan Pengaru Penambahan Alumina Terhadap Sifat Mekanik Hidroksiapatit," Skripsi Jurusan Kimia, FMIPA. Universitas Sriwijaya, 2018 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [21]. Venkatesan J., Kim S.K., Effect of temperature on isolation and characterization of hydroxyapatite from tuna (*Thunnus obesus*) bone, *Materials*, 2010, **3**:4761 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [22]. Joschek S., Nies B., Krotz R., Göpferich A., Chemical and physicochemical characterization of porous hydroxyapatite ceramics made of natural bone, *Biomaterials*, 2000, **21**:1645 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [23]. Walters M.A., Leung Y.C., Blumenthal N.C., Konsker K.A., LeGeros R.Z., A Raman and infrared spectroscopic investigation of biological hydroxyapatite, *Journal of inorganic biochemistry*, 1990, **39**:193 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [24]. Koutsopoulos S., Synthesis and characterization of hydroxyapatite crystals: a review study on the analytical methods, *Journal of Biomedical Materials Research: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials*, 2002, **62**:600 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [25]. Ooi C.Y., Hamdi M., Ramesh S., Properties of hydroxyapatite produced by annealing of bovine bone, *Ceramics international*, 2007, **33**:1171 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [26]. Ozawa M., Suzuki S., Microstructural development of natural hydroxyapatite originated from fish-bone waste through heat treatment, *Journal of the American Ceramic Society*, 2002, **85**:1315 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [27]. Zaid Almarbd Z., Mutter Abbass N., 'Synthesis and characterization of TiO₂, Ag₂O, and graphene oxide nanoparticles with polystyrene as a nonocomposites and some of their applications', *Eurasian Chemical Communications*, 2022, **4**:1033 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [28]. Glimcher M.J., Molecular biology of mineralized tissues with particular reference to bone, *Reviews of Modern Physics*, 1959, **31**:359 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [29]. Barakat N.A., Khil M.S., Omran A.M., Sheikh F.A., Kim H.Y., Extraction of pure natural hydroxyapatite from the bovine bones bio waste by three different methods, *Journal of materials processing technology*, 2009, **209**:3408 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [30]. Han Y., Li S., Wang X., Jia L., He J., Preparation of hydroxyapatite rod-like crystals by protein

precursor method, *Materials research bulletin*, 2007, **42**:1169 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]

[31].Mustafa Y.F., Classical Approaches and Their Creative Advances in the Synthesis of Coumarins: A Brief Review, *Journal of Medicinal and Chemical Sciences*, 2021, **4**:612 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]

[32].Lara-Ochoa S., Ortega-Lara W., Guerrero-Beltrán C.E., Hydroxyapatite nanoparticles in

drug delivery: Physicochemistry and applications, *Pharmaceutics*, 2021, **13**:1642 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]

[33].Hajinasiri R., Esmaeili Jadidi M., Synthesis of ZnO nanoparticles via flaxseed aqueous extract, *Quarterly Journal of Iranian Chemical Communication*, 2022, **2**:101 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]

HOW TO CITE THIS ARTICLE

Ali Mohammed Korsheed, Ghassan Abdul-Hamid Naji. Bastian Lubis. Preparation and Characterization of Natural Fish Bone as an Implant Material. *J. Med. Chem. Sci.*, 2023, 6(3) 668-676

<https://doi.org/10.26655/JMCHMSCI.2023.3.23>

URL: http://www.jmchemsci.com/article_158421.html