HYDROXYAPATITE FROM FISH SCALE AND ITS APPLICATIONS

[Article ID: SIMM0221]

S. Mahalakshmi, A. Kamalii*, Dr. Cheryl Antony, Dr.B. Ahilan

Dr. M.G.R Fisheries College and Research Institute, Ponneri, Tamil Nadu (601 204), India

INTRODUCTION:

In many developing nations, aquaculture plays a significant role in producing food and generating high income however, the waste generated creates a major environmental problem. Natural waste products are great resources for extracting and recovering valuable substances. Around the world, 18-30 million tonnes of fish waste are produced each year. High-value proteins, unsaturated essential fatty acids, vitamins and antioxidants, minerals or trace metals, and physiologically useful amino acids and peptides can all be found in fish waste. In general, the fish scale is thought to be useless, impractical, and a waste. However, fish scale, on the other hand, is known to include a variety of important organic and inorganic components, primarily collagen and hydroxyapatite, which have commercial worth for application in functional meals, cosmetics, and biomedical products.

HYDROXYAPATITE:

Hydroxyapatite (HAp) is a calcium-phosphate compound that has found widespread use in a variety of sectors, including medicine, pharmaceuticals, and dentistry. The chemical formula of hydroxyapatite is (Ca_{10} $(PO_4)_6$ OH_2). Hydroxyapatite, commonly known as hydroxylapatite (HAp), is a calcium apatite mineral that occurs naturally. It is the hydroxylated form of phosphate minerals known as apatite. It has a hexagonal structure which is the same as bone apatite. The properties include bio-compatibility, biodegradability, osteogenic capacity, osteoconductive, inert, non-toxic, and non-inflammatory. The hydroxyapatite produced by the synthetic method has poor crystallinity and inhomogeneous composition. As a result, the synthesis of HA from natural sources remains the most efficient and cost-effective option. HAp manufacturing from bio-waste is both ecologically and economically sound.

HYDROXYAPATITE FROM FISH SCALE:

Natural materials such as coral and fish bone, as well as fish scales, are used to make Hap. The cheapest source to produce biological hydroxyapatite is fish scales and bones. Fish scales are the most common by-product of the seafood processing sector, accounting for 30-40% of total volume, and handling these by-products is generating issues for businesses. By weight, waste accounts for nearly half of the total waste generated by the fish processing industry, with fish scale accounting for around 4% of the waste. In general, the fish scale is thought to be useless, impractical, and a waste. Collagen, connective tissue proteins, water, and the remaining 41% to 84% of other proteins make up most of the fish scales. Scale creation follows the same method as the formation of teeth and bone throughout evolution. Due to their strong biological reaction in the physiological environment, various kinds of calcium phosphate salts are found in fish scales. To obtain hydroxylapatite, fish species such as salmon, carp, Japanese anchovy, sardine, tilefish, and tuna have been utilized. When compared to commercially available mammalian-derived hydroxyapatite, fish-derived is zoonosis-free and has no religious or ethical implications. As a result, fish scales may be used as a low-cost source of collagen and HA in biological applications.

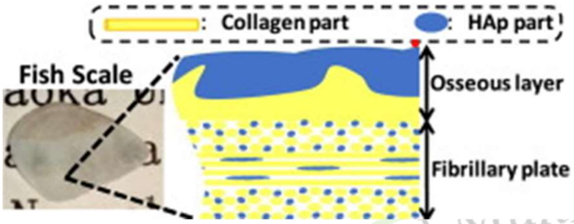

Fig 2: Hydroxyapatite in fish scale

EXTRACTION OF HYDROXYAPATITE FROM FISH SCALES:

Alkali heat treatment: After washing, fish scales are put in an oven for 60 minutes to eliminate water and moisture content. External washing with 0.1 M HCl and numerous washes with distilled water deproteinized the scale. To remove all protein attached to the scale, the remaining protein was treated with 5% (weight/volume) NaOH and heated at 80 °C for 5 hours with careful stirring. The fine white precipitates were washed with distilled water and dried for 48 hours at 60˚C.Sodium hydroxide (50 percent w/v) was added to the treated scale, which was heated to 300 °C and stirred for 1 hour. The HAp powder was carefully rinsed with deionized water until the pH of the rinsed solution was neutral. Prior to usage in the oven, the HAp was dried for 24 hours at 60˚C.

Microwave irradiation method: After washing, fish scales were boiled in water for 30 minutes and then baked for 60 minutes to eliminate the water and moisture content. The dried fish scale is first heated for 1 hour at 300°C at a rate of 3°C min^{-1}. The temperature was raised at a rate of 10°C/min and held at 900°C for 3 hours before being sintered. Then the furnace was cooled to room temperature after the black powder had changed into white powder (calcium oxide). After that, 0.1M EDTA was added to form a CaEDTA complex. The liquid mixture was then agitated for 15 minutes after 0.06 M Na2HPO4 (50 mL) was added dropwise to the Ca-EDTA complex. The liquid mixture's pH was maintained at 13. Microwave heating (2.45 GHz, 1100 W) at 50% power capacity was applied to the combinations until they dried. The precipitate was washed several times with deionized water to remove Na and EDTA residues before being dried in a vacuum oven (80 °C for 6 hours) to create HAp powder.

Thermal treatment: The cleaned scales were deproteinized by soaking them in a 1 N HCl solution (2:1 volume/weight, water HCl) for 24 hours at room temperature (30.2) °C. After that, the deproteinized fish scales were carefully rinsed with distilled water multiple times. Fish scale proteins were treated with a 1 N NaOH solution to remove any remaining protein. The filtered fish scales were carefully cleaned with distilled water and dried for many hours at 60 °C in a hot air oven. For hydroxyapatite production, treated fish scales were calcined at 1000 °C.

Ionic liquid pre-treatment: To remove the HAp from biological sources, all of the above technique employs acids, alkalis, and heat. Furthermore, all of these techniques result in the loss of other important components, such as collagen, which has been recognized for a variety of uses. The fish scales were washed to remove debris and then dried and grinded. In a 30 mL reagent bottle, 10.0 g of 1-butyl-3-methylimidazolium acetate was added, followed by 0.5 g of crushed fish scales and heated for 12 h at 100˚C in an oil bath. After the dissolving process, an equivalent amount of water was added to the reaction mixture, followed by 0.5 M NaOH solution to bring the pH to 9. Then they are centrifuged at 11,000 rpm for 30min. NaCl 2M and Hcl 0.5M are added to the supernatant. The resulting acidic supernatant was neutralized with a 0.5 M NaOH solution before being evaporated using a rotary evaporator. Acetone and dichloromethane were used to extract the ionic liquid, which was then filtered and evaporated.

Calcination method: Fish scales were removed from the skin with the use of a fish scale remover. The scales were then cooked for 1 hour in an aqueous solution at 100°C. The scales were repeatedly rinsed with tap water to remove any adherent fish flesh, and the samples were then dried in a 60 °C oven for 3 hours to eliminate any moisture. Finally, the samples were calcined by heating them for 2, 5, and 7 hours at 900 °C and allowing them to cool naturally in the furnace.

APPLICATIONS OF HYDROXYAPATITE FROM FISH SCALES:

BONE TISSUE ENGINEERING:

Hydroxyapatite (HAp) is utilized as a filler, augmentation, artificial bone graft, and scaffold material in bone tissue engineering. Aside from collagen, fish scales include a lot of HAp, which is a bioactive ceramic that looks like a bone mineral. In most cases, a very high heat treatment (1250°C) is utilized to isolate HAp from the fish scale. This temperature provides the HAp structure more strength, resulting in a good biocompatible inorganic material. When compared to synthetically produced hydroxyapatite, hydroxyapatite derived from fish scales are in the form of flat-plate nanocrystals with a porous shape, greater surface area, and higher surface roughness, resulting in increased cell adhesion, and proliferation and has higher mechanical characteristics. In combination with a polymeric substance like collagen, hydroxyapatite serves as the inorganic basis for bone substitutes. Because hydroxyapatite derived from fish contains salt and magnesium, it is particularly helpful for stimulating bone repair. They also have a higher capacity to promote apatite production. The adherence to osteoblastic cells is also higher in fish-derived hydroxyapatite. Thus, the hydroxyapatite derived from fish scales has a greater chance of being used in bone tissue engineering.

WASTEWATER TREATMENT:

The challenge of removing harmful heavy metal ions from wastewater is crucial. Hydroxyapatite (HAp) is a high-quality sorbent material with an inorganic ion lattice, it can be employed in wastewater treatment and valuable element recovery. Because of its excellent sorption capacity for heavy metal ions, low water solubility, availability, low cost, and good stability, hydroxyapatite is regarded as a potential material for the adsorbent. It is viewed that the mechanical strength, biocompatibility and biodegradability of hydroxyapatite are improved in combination with other organic materials such as rice husk. Modification of the absorbent by the inclusion of amino, silica, and phosphate groups also gives unique binding characteristics for potentially dangerous contaminants such as ammonia. At 210 minutes of contact time, hydroxyapatite recovered by thermal breakdown showed the highest effectiveness, removing 79 percent of ammonia. Within 10 minutes, fish scale powder had removed more than 90% of the lead ion, and after 20 minutes, it had nearly completely removed it.

ANTIMICROBIAL ACTIVITY:

Hydroxyapatite (HAp) has been considered a valuable, innovative, eco-friendly medicinal application that is largely targeted for its antibacterial characteristics. Resistance to antibacterial agents develops as a result of biofilm development, resulting in failures in the elimination of biomaterial-associated infection. The use of metal composites such as zinc to impart antibacterial properties to HAp-based materials gives an ideal answer to bacteriabased diseases. The antibacterial action of hydroxyapatite is due to the generation of reactive oxygen species (OH, H_2O_2, and O_2) on the surface of the hydroxyapatite nanoparticles, which is associated with bacteria death.

OTHER APPLICATIONS:

A popular field of research is targeted drug delivery systems with the ability to control the rate and duration of drug release. Because of the large quantity of hydroxyl on the surface and the polar charge, hydroxyapatite has the characteristics to bind and retain DNA and peptides, making it an excellent drug carrier. Hydroxyapatite is a calcium phosphate that has also long been used to separate proteins and DNA in chromatographic separations.

ADVANTAGES OF HYDROXYAPATITE:

- Hydroxyapatite is biocompatible because it is similar to those found in human bone.
- They are biodegradable and cost-effective.
- Simplicity in modification and surface functionalization.
- Hydroxyapatite has a well-adherent capacity to the polymer.
- High level of chemical uniformity.

DISADVANTAGES OF HYDROXYAPATITE:

- The flexibility of the compound is low.
- They are very fragile.
- Sensitive to high pH.

CONCLUSION:

Fish scales which are a common by-product of seafood processing could be viewed as a cost-effective and easily available raw material for producing high-quality HAp. Because the chemicals required in the synthetic HA process are costly, alternate natural sources for HA synthesis, such as fish scales, have been used. As fish scales are the major byproduct of the fish processing industry, extraction of hydroxyapatite from scale waste is a better choice for producing high-quality hydroxyapatite and a good approach to execute the waste into wealth. HAp particles have several benefits, making them a potential agent for biological applications. On the other hand, there are drawbacks such as limited mechanical strength and a delayed medication release.

REFERENCES:

Amin Shavandi, Alaa El-Din A. Bekhit, Azam Ali, Zhifa Sun,2014, Synthesis of nano-hydroxyapatite (nHA) from waste mussel shells using a rapid microwave method, Materials Chemistry and Physics (2014) 1-10

Behrad Ghiasi, Yahya Sefidbakht and Maryam Rezaei, 2019, Hydroxyapatite for Biomedicine and Drug Delivery

D.Ayala-Barajas, V. González-Vélez, M. Vélez-Tirado, J. Aguilar-Pliego, 2020, Hydroxyapatite extraction from fish scales of Tilapia.

Gao, L. X., Li, Z., Wang, M., Huang, L., Pi, K. W., & Wei, X,(2012), The Research on Treating Waste Water Containing Copper by HAP, Advanced Materials Research, 550-553, 2146–2150.

Liu, W.-K., Liaw, B.-S., Chang, H.-K., Wang, Y.-F., & Chen, P.-Y, (2017), From Waste to Health: Synthesis of Hydroxyapatite Scaffolds From Fish Scales for Lead Ion Removal. JOM, 69(4), 713–718.

Mondal, B., Mondal, S., Mondal, A., & Mandal, N,(2016). Fish scale derived hydroxyapatite scaffold for bone tissue engineering. Materials Characterization, 121, 112–124.

Niladri Nath Panda, Krishna Pramanik ,Lala Behari Sukla,2013, Extraction and characterization of biocompatible hydroxyapatite from fresh water fish scales for tissue engineering scaffold. Bioprocess Biosyst Eng ,DOI 10.1007/s00449-013-1009-0