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Nano-crystalline hydroxyapatite from scales of the reef fish *Epinephelus malabaricus* (galkossa)

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Fish scales comprise of collagen and hydroxyapatite (HAp). The latter of which holds potential for biomedical applications. In Sri Lanka these scales are discarded into the environment as waste. The objective of this research was to develop a method to prepare nanocrystalline HAp from scales of the reef fish *Epinephelus malabaricus* (Gal Kossa). Scales were collected from Ja-Ela, Sri Lanka, washed, dried and subjected to acid deproteinization with hydrochloric acid (HCl) under varying conditions (concentrations: 0.1 M, 0.2 M; temperatures: room temperature, 60 °C; treatment periods:1 h, 2 h). The product was isolated, washed and dried. The best conditions were treatment with 0.2 M HCl at 60 °C for 2 h. The product was subjected to alkaline deproteinization under different conditions (NaOH gave better results than KOH; NaOH concentrations: 2%, 3%, 4%, 5% w/v; temperature: 60 °C, 70 °C; treatment periods: 1, 2 and 3h.). The product was isolated, washed and dried. The best condition was treatment with 4% NaOH at 70 °C for 3 h. The product was subjected to alkaline heat treatment using NaOH under different conditions. (concentration: 20%, 25%, 30% w/v; temperature: 60 °C, 80 °C; treatment period: 1h, 2 h, 3 h). The product was isolated, washed and dried. The best condition was 25% NaOH at 80 °C for 2 h. Under the best conditions for acid deproteinization, alkaline deproteinization and alkaline heat treatment, the yield was 43%. Characterization using FT-IR and XRD analysis of the product confirmed the product as hydroxyapatite. According to EDX, Ca/P ratio was 1.82. SEM showed agglomerated particles within 100 nm range. The FT-IR suggested it to be a B-type carbonated hydroxyapatite (CO_3^{2-} groups at 1462, 1417 and 873 cm^{-1}) where CO_3^{2-} ions substituted in place of certain PO_4^{3-} ions. TGA confirmed the carbonated form. Therefore, this method can be used as a cost-effective method to produce pure carbonated hydroxyapatite. It also helps to reduce the environmental pollution due to fish waste.

Keywords:

Fish scales; nano-crystalline; hydroxyapatite; deproteinization; biocompatibility