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Chondroitin sulfate and hydroxyapatite from *Prionace glauca* shark jaw: Physicochemical and structural characterization

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ABSTRACT

In the present work, the potential of the *Prionace glauca* jaw as a source of both chondroitin sulfate and bioapatite is explored. The sandwich-type structure in cross section of the jaw based on alternate layers with prevalence in organic tissue or mineralized is shown and these bands respectively confirmed as CS or hydroxyapatite -enriched zones. As result of this, an optimized process in sequential steps for the recovery of both biomaterials and their purification process is proposed, by combining enzymatic proteolysis, chemical precipitation and separation using ultrafiltration membrane for CS production together with controlled thermal treatment for hydroxyapatite obtaining. The purified CS was characterized by Gel Permeation Chromatography, Nuclear Magnetic Resonance and Strong Anion Exchange Chromatography, revealing a polymeric material with a molecular weight of 67 kDa, and prevalent 6S-GalNAc sulfation (68%), followed by 4S-GalNAc (13%), a significant proportion of disulfated disaccharides (12%) and only 7% of non-sulfated units. In the case of the bioapatite a purified biphasic 60:40 porous calcium phosphate of hydroxyapatite: whitlockite/β-TCP was confirmed. Hydroxyapatite as major component (85%) was also obtained for jaws directly subjected to the thermal treatment. This proved the influence of the enzymatic hydrolysis and centrifugation on the composition of the mineral fraction.

1. Introduction

The marine environment represents a rich source of biomaterials for biomedical, pharmacological and nutraceutical applications [1–3]. Some of these compounds may occur in rare and unexploited organisms, but many marine biomaterials originate from commonly fished species. This is the case of blue shark (*Prionace glauca*), a wide-ranging oceanic chondricthyan dispersed across temperate and tropical waters. This species represents a relatively abundant source of fish by-products, mostly heads and skeletons, amenable to be processed to obtain valuable biomaterials [4].

Apatites and, in particular, hydroxyapatite (HA) is a bioactive bioceramic which belongs to the family of calcium phosphates and fits the ideal formula $Ca_{10}(PO_4)_6(OH)_2$. This basic composition enriched by groups and elements in trace concentrations, as CO_3^{2-}, HPO_4^{2-}, Na^+, Mg^{2+}, Sr^{2+}, K^+, Cl^- and F^-, constitute the mineral fraction of bone tissue. The rest of the tissue is formed by collagen with other proteins and water. This composition gives the bone tissue a great consistency, hardness, elasticity and resistance to compression [5–7]. Moreover, as a bioactive material, HA has the ability to induce its biological integration in living tissues, so that it interacts chemically with the organism, giving rise to a layer rich in phosphorus and calcium on its surface in the form of bioapatite [6,8]. It is, therefore, a property of great interest to promote the activity of bone tissue forming cells, since the presence of hydroxyapatite and the bioactivity generated activate the adhesion, proliferation and osteoblast activity of osteoblasts.

In recent years HA-based materials of biological origin as bone substitutes have attracted greater interest than those of synthetic origin, given that their behaviour is biologically more active compared with synthetic equivalents [9–13]. Moreover, in contrast to commercial synthetic HA the biological version (mainly bovine, porcine origin) is enriched, as in human bones, with essential elements (such as Mg^{2+}, K^+, Na^+, etc.) that have specific roles of relevance in bone defect healing, and it has lower crystallinity with disordered nanostructures providing unique chemical and physical properties [10,11,14]. New biological sources are being investigated seeking to guarantee sustainability. One such approach is exploring the potential of the marine environment, especially discards from the fishing industry.

Chondroitin sulfate (CS) is a sulfated polysaccharide of the glycosaminoglycan family formed by alternating β1–3 and β1–4 glycosidic bonds between glucuronic acid (GlcA) and *N*-acetyl galactosamine (GalNAc). The basic building block of the polymer is the disaccharide thus formed, which can be sulfated at different positions on both rings. In animals, CS participates as regulator of functional proteins, either at the cell surface or in the extracellular matrix [15], being

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