Shark cartilage (*Prionace glauca*) by-products as collagen source for biotechnological application

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Blue shark (*Prionace glauca*) is one of the most widespread species in the epipelagic zone. Its geographical distribution is within the fishing areas of tuna and swordfish, so it has always been an incidental catch of those fisheries. The total catch reported for this species by ICCAT for the Spanish fleet in 2017 was 38.843 tons. Its meat and fins are used in gastronomy, skin for leather, liver for oil and the by-products generated in its processing, normally used in the fish meal industry (FAO).

Cartilages from trunks result from the processing operations made by the industry, the main valorisation for this by-product is cartilage powder or chondroitin sulphate. However, cartilage is also a source of collagen and this could be also another potential valorisation alternative.

Collagen is the fibrous protein of the connective tissue that gives unique physiological functions of tissues in the skin, tendons, bones, cartilage and is associated with muscle toughness. Nowadays, one of the main problems associated with the human aging population is related with osteoarthritis pathologies. Some research efforts have been devoted to find strategies which can be used to improve the conditions of these patients, and among others, collagen oral supplementation or implants to substitute damaged cartilage, are currently investigated. Collagen type II is usually found in cartilages and scaffolds made from collagen has been shown to be useful to promote cartilage regeneration.

Although many studies and industrial production is focused on Collagen type I, which is usually obtained from skins and bones of mammals, especially pork and cow, these sources of collagen has been regarded as risky by the potential transmission of bovine spongiform encephalopathy (BSE), transmissible spongiform encephalopathy (TSE) and foot-and-mouth disease (FMD). In addition, pig skin and bone collagen cannot be used due to religious restrictions. For these reasons, attention has been paid to alternative sources of collagen, such as skins and fish bones, by-products of the fishing industry. Furthermore, some studies have shown that marine origin collagen showed lower immunogenicity than the mammalian ones.

The aim of this study is the isolation of collagen from the cartilage of blue shark and characterization for its potential in different applications as alternative to collagen of mammals. The trunk cartilage of the blue shark was provided by Protea, a fish processing plant in Marín, Spain and was used as the raw material for the isolation of collagen. The cartilage was washed with distilled water and was subdivided in two parts: proper cartilage and vertebral part or centrum. Both parts were homogenized by a mixer and stored at -4ºC until used. The cartilage was pre-treated with 0,1 M NaOH with a solid/alkali solution ratio of 1:10 (w/v) by continuous stirring for 24 h, the pre-treated cartilage was washed until neutral pH. Then a demineralization step was carried out with 0.5 M ethylendiaminetetraacetic (EDTA, pH 7.4) with a solid/solution ratio of 1:10(w/v) for 48h with continuous stirring and water changes every 8h; the demineralized cartilage was washed with 20 volumes of water during 10 minutes

for 3 times. Acid soluble collagen (ASC) and pepsin soluble collagen PSC) were extracted by the method of Kittiphattanabawon et al (2010) with slight modifications. For the isolation of ASC the pre-treated cartilage was soaked in 0.5M of acetic acid (1:15, w/v) for 72 h with continuous stirring. The extracts were precipitated with NaCl in the presence of Tris (pH7,5) and then were centrifuged at 17000 g for 60 minutes. The pellet was dissolved in 0.5M acetic acid, dialyzed against 25 volumes of distilled water and finally was freeze-dried. For the extraction of PSC, the residue obtained after acid extraction was soaked in 0.5 M acetic acid (1:15, w/v) with pepsin (40 unit/g of residue) during 48 hours, and then the previous procedure was followed.

Cartilage and vertebral parts were characterized by proximate analysis and the collagens obtained were characterized by amino acid contents, SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and Fourier transform infrared (FTIR) spectroscopy.

The moisture content was the main component (80,41-69,76 g/100g), followed by nitrogen content (18,84-18,51 g/100g) and ash (2,05-12,92 g/100g). ASC and PSC extracted from cartilage and centrum had similar amino acid profiles, all of them had Glycine as the main amino acid (32,78-31,09% for ASC and PSC from cartilage, and 31,73-30,50% for ASC and PSC from centrum) and were rich in alanine (around 11% for collagen of cartilage and 10% for centrum collagen), proline and hydroxyproline (around 10% and 7,5% respectively in all collagens obtained).

SDS-PAGE patterns of ASC and PSC of cartilage presented β- and α- chains as major components with traces of ϒ-chain. ASC presented α1 and α2 chains and in the PSC patterns the α2 was very weak. The results revealed that these fractions could contain two types of collagen, including type I and II, as indicated in previous works.

FTIR spectra showed the major absorption bands in the amide band region, including the peak of amide I, II and II and amide A and B, as a typical pattern of collagens from cartilages.

ASC and PSC can be isolated from the cartilage of blue shark, both from the proper cartilage and the centrum. Higher yield was obtained for PSC from cartilage in comparison with PSC for centrum and ASC. Both ASC and ASC showed similar amino acid composition, similar secondary structures and could contain type I and type II collagens. Due to these characteristics, both collagens could be used as alternative to mammalian collagen in different applications. The extraction process must be improved in order to increase the yield of collagen in the future.

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