Biomedical Applications Based on Marine Collagen Obtained from the Jellyfish Species Rhizostoma Pulmo Extracted from the Black Sea

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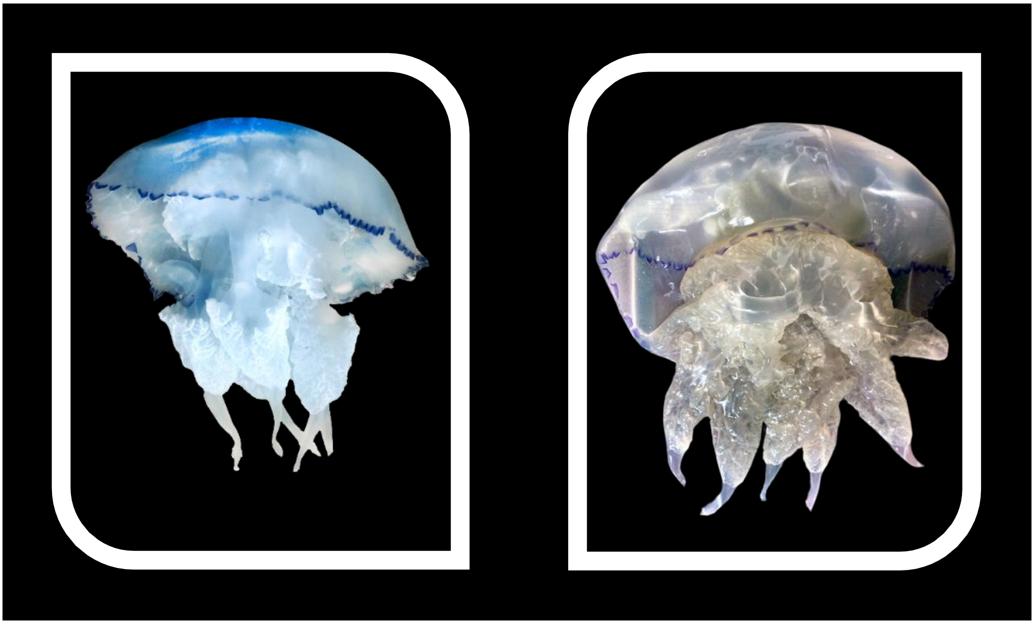
Abstract

Due to its unique properties, collagen is used in various emerging fields such as the pharmaceutical and biomedical device industries, as well as in related fields: nutraceuticals, cosmetics, food, beverages and nutritional supplements. Marine gelatin, one of the biomaterials involved in food and medicinal research, is denatured collagen produced from acid, alkaline, or enzyme hydrolysis. Gelatin is a crucial ingredient for the food, pharmaceutical, medical, biomedical focusing on versatile applications. Jellyfish collagen is a valuable resource for bioplastics and biomaterials used in various health sectors. Recently, marine organisms have been considered viable sources of collagen because they do not harbor transmissible diseases. In particular, fish biomass, as well as the catches of other types of organisms, such as small fish, jellyfish, starfish, sea urchins, sponges, possess a significant content of collagen. The collagen extracted from the species Rhizostoma pulmo from the Black Sea basin is also part of the bioresources that can be used to obtain natural marine collagen from this type of invertebrates. The use of discarded or hither to unused biomass could contribute to the development of a sustainable collagen extraction process with a positive impact on the ecosystem. In the future it is desired to approach a world strategy that minimizes the amount of waste and that supports all three general objectives of sustainability: sustainable economic well-being, social well-being and environmental protection.

Keywords: Rhizostoma pulmo, jellyfish, marine collagen, bioactive compounds, biomaterials, medical apllications

Introduction

Collagen of marine origin can be obtained from various animals, whether they are marine vertebrates - the source of collagen is fish skin, bones, cartilage, including scales, but also from other types of animals such as invertebrates, they are bioavailable compared to collagen bovine or porcine and have a higher absorption capacity (up to 1.5 times more efficient in the body) [1] and faster circulation in the bloodstream due to the low molecular weight and small particle size [2]. Another positive side of collagen of marine origin is the fact that it possesses similarity with conventional bovine and porcine collagen in terms of amino acids in composition and biocompatibility [3].



Rhizostoma pulmo – personal archive photo

Jellyfish collagen can be obtained from different parts of the jellyfish such as the umbrella, oral arms or even from the whole animal. The sources of marine collagen are diverse: fish, jellyfish, sea sponges, sea urchins, starfish, certain species of shells, together forming a biocompatible, natural alternative, without barriers of race, religion, degree of pollution or other inconveniences that standard animal collagen sources can meet them see Fig. 1.

Jellyfish are a rich source of minerals and protein, and collagen is a major protein in these gelatinous sea creatures. Cartilaginous tissue was isolated from jellyfish, later shown to be a type II collagen that can lead to the differentiation of mesenchymal stem cells. From a therapeutic point of view, it is TGF-3 cells in the form of nanoreservoirs that have helped to achieve combined cartilage [4].

Figure 1 Marine collagen sources

The objectives of the study consist in highlighting collagen sources, methods of collagen extraction from these sources and enumerating the most important biomedical applications of collagen from marine resources.

Materials and Methods

Marine collagen extraction methods

In jellyfish, it is common to separate the mouth arms from the umbrella and then divide the umbrella into mesoglea, exumbrella and subumbrella [5].

Reducing the size of these samples is essential to facilitate subsequent chemical (pre)treatment actions, used to remove proteins, pigments or non-collagenous fats. The common method involves the use of basic pretreatment with sodium hydroxide (NaOH), which does not cause structural changes to collagen chains, alcohols (namely butyl alcohol or ethanol) and oxygen peroxide in the process of removing non-collagenous proteins, fats and respectively of pigments [6]. In addition, to remove non-collagenous proteins from cod skin, the use of sodium chloride (NaCl) as an alternative to NaOH has also been proposed [6].

Moreover, to improve the extraction of collagen from bones, cartilage and scales, ethylenediaminetetraacetic acid (EDTA) is recommended for demineralization purposes [8]. For the extraction phase, it is well known that the solubility of collagen in cold water is poor due to the presence of strong crosslinks in its triple helix structure. There are two different conventional methods widely used: acid solubilized collagen extraction and collagen solubilized pepsin extraction see Fig. 2. Using these two methods, the yield, chemical composition and characteristics of the collagen extract differ from each other. The entire extraction phase is carried out at 4 ◦ C. When collagen extraction is performed using only acid, the product is called acid soluble collagen (ASC). For the extraction of collagen from marine animal tissues, acetic acid is the most used, dilute acid is used (generally at the final concentration of 0.5 M), but also citric acid and lactic acid [7].

About 95% of marine invertebrates such as jellyfish are made up of water, which affects collagen soluble in acetic acid. Therefore, homogenization or lyophilization of jellyfish is necessary to improve the solubility of collagen in dilute acids and consequently increase the extraction yield.

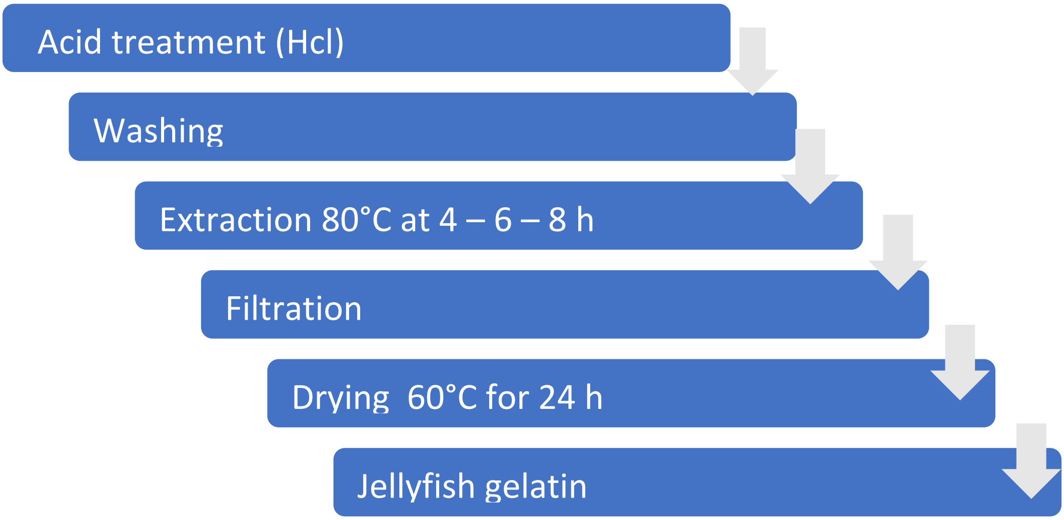


Figure 2 Jellyfish gelatin extraction

Recently, it proposed a new method to extract colagen from aquatic animals, in which acid treatment is combined with a sequence of physical and mechanical treatment, including pH adjustments, homogenization, mixing, and sonication [8]. By increasing physical intervention in jellyfish, the extraction yield increased significantly compared to conventional extraction processes [9].

When the enzyme pepsin is added to the extraction process, the extracted collagen is referred to as pepsin-soluble collagen (PSC). This treatment is very useful because proteases cleave the telopeptide of the cross-linked region without breaking the integrity of the triple helix and thus hydrolyze some non-collagenous proteins and increase the purity of collagen [10]. In most cases, enzymes are used to obtain specific protein products, high yield and reduced waste, as well as a decrease in antigenicity caused by telopeptides [11]. However, when a large amount of pepsin is used for a long time, the yield of PSC may be lower because the collagen is probably cleaved, affecting the integrity of the triple helix. [12].

During the recovery step, collagen is precipitated, generally by adding NaCl to a final concentration of 2.3–2.6 M. The precipitate is collected by centrifugation, dissolved in 0.5 acetic acid, dialyzed, and lyophilized [12].

From jellyfish, collagen is generally extracted by a methodology based on solubilization in 0.5 M acetic acid solution (usually for three days), followed by dialysis against a Na2HPO4 solution. The precipitated collagen is separated by centrifugation, solubilized in acetic acid, and purified by reprecipitation by adding solid NaCl to a concentration of 0.9 M. ASC can also be digested with pepsin to obtain atelo-collagen [12].

Results and Discussions

Pepsin-soluble collagen treated with ultrasound for 15 minutes showed the highest recovery yield (23.8%) as well as the highest amino acid content (18.2%) [13].

Ultrasonic extraction is an efficient and rapid technique to produce collagen from jellyfish in large quantities. Advantages of ultrasound collagen extraction are:

- obtaining food or pharmaceutical products with a high percentage of collagen
- high molecular weight collagen
- good extraction yields
- preserving the composition of amino acids
- fast processing
- easy operation

Ultrasonic extraction can be used in combination with various acid solutions to release acid-soluble collagen (ASC) from jellyfish. Ultrasonic cavitation promotes mass transfer between the jellyfish substrate and the acid solution through cellular structural disruption and acidification of the substrate. Thus, collagen as well as other targeted proteins are transferred into the liquid. In a subsequent step, the remaining jellyfish substrate is treated with enzymes (eg, pepsin) under sonication to isolate pepsin-soluble collagen (PSC) [10].

Sonication is recognized for its ability to increase enzyme activity. This effect is based on ultrasonic dispersion and agglomeration of pepsin aggregates. Homogeneously dispersed enzymes provide an increased surface area for mass transfer, which is correlated with higher enzyme activity. In addition, powerful ultrasonic waves open collagen fibers, so collagen is released see Fig. 3. Research has shown that an ultrasonically assisted enzymatic extraction (with the addition of pepsin) gives higher yields and an extraction process with a shorter execution time [13].

Figure 3 UIP4000hdT (4 kW) ultrasonic extraction system

https://www.hielscher.com/ro/ultrasonic-collagen-extraction-from-jellyfish.htm

In order to use salted jellyfish-derived products as raw material, the presence of 20-25% [13] residual salt from the salting process must first be removed. Traditional washing by desalination with water several times and overnight soaking is required before using it for food [12]. This takes a lot of work and time. Washing machines have been used to reduce the time, but the washing process generates a massive amount of waste water [13]. Research has shown that ultrasound can be used to desalt proteins. The application of ultrasound and microwave pretreatment in the desalination of salted egg white or duck proteins can reduce the salt content from 7.80 to 0.62% [13].

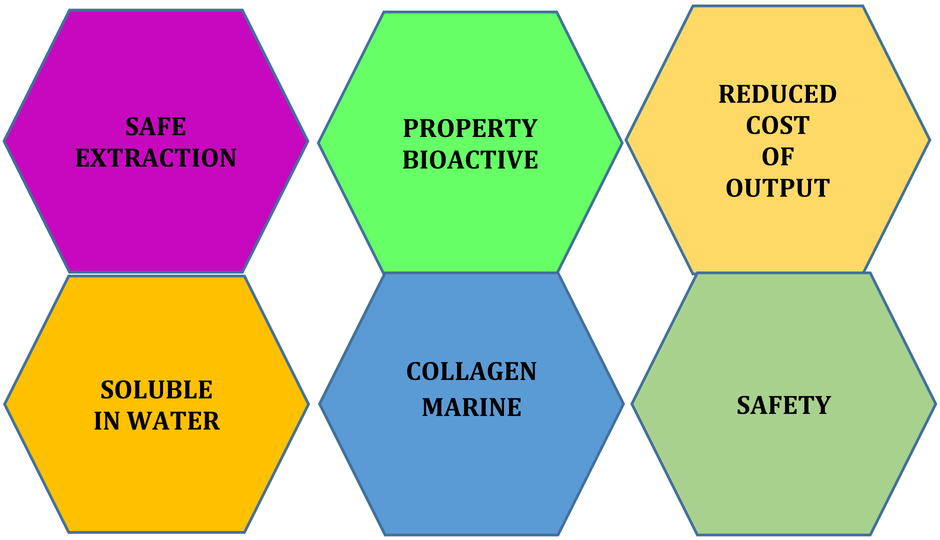
Nowadays, ultrasound, a non-thermal process that generates a sound wave at a frequency of 20-100 kHz, can be applied in food processing, such as in homogenization, emulsification, extraction, crystallization, degassing, marinating or cleaning. Ultrasound has also been investigated for pesticides, mycotoxins, heavy metals and allergen removal [13].

The frequency of ultrasound produces cavitation, crushing, vibration, mixing and heating, thereby reaching the mass transfer to induce the rapid collapse of bubbles and produce shear forces to break covalent bonds in materials [13]. Apply this green technology, ultrasound could be an alternative for removing the remaining salts in salty jellyfish by-products. However, no application of the ultrasonic method for desalination of such a jellyfish by-product sample has been reported.

The structure of type I collagen was observed in the SDS-PAGE pattern of collagens and no structural change occurred during the extraction process. The presence of the helical structure in collagen samples was confirmed by UV and FTIR spectra. The ultrasound cavitation advanced the viscosity of collagens and resulted in excellent solubility in acidic environments and lower salt concentrations.

Ultrasound-treated samples suggested superior water holding capacity along with emulsifying, and foam attributes. The improved gel strength of collagens was parallel with increasing the time of ultrasound up to 15 min. Free radicals scavenging ability and ferric reducing power of collagens were positively stimulated by increasing the time of the ultrasound up to 15 min[13]. Regarding the study it contributes to the development of green technology and the promotion of by-product utilization for collagen recovery as a potential practical protein in biomaterial, wound dressing, drug delivery, food, and cosmetics products[14].

Figure 4 The qualities of marine collagen



Applications of collagen extracted from different species of jellyfish

Jellyfish collagen is an important source of antioxidants. Recently, it was shown that peptide fractions from R. pulmo collagen were able to prevent oxidative stress, collagen peptides exhibit antioxidant and antifatigue activity, they were also identified in R. esculentum [15], as well as collagen hydrolyzate with several activities (including superoxide anion scavenging and melanogenesis inhibition activities) based on the ability of the hydrolyzate to chelate copper by inhibiting intracellular tyrosinase activity [16].

In the case of both jellyfish, it was found that collagen and its hydrolyzate function as protectors against UV radiation, suggesting their possibilities for use in skin care industries [17]. Similarly, collagen peptides from S. meleagris have been shown to be an effective tyrosinase inhibitor by acting on glutathione (GSH) levels [18].

According to recent studies, type II collagen extracted from Rhizostoma pulmo has been used to develop a collagen-based biomaterial. It was implemented using nanoreservoirs containing the growth factor TGF-3 and human stem cells, building a new adaptive device for articular cartilage repair [4].

The immunostimulatory effect of N. nomurai collagen stimulated immunoglobulin and cytokine production, not only in the specific human hybridoma cell line HB4C5, but also in the peripheral blood lymphocyte (PBL) line. In addition to these effects, tumor necrosis factor (TNF) and interferon (IFN) levels were increased in PBL [19].

Thrombosis and hypertension are among the leading causes of cardiovascular disease-related death [20], so research for new treatments remains an active field. In this case, Rhizostoma pulmo collagen was used to fabricate a sensor suitable for the clinical detection of thrombin in blood. Collagen was cross-linked to the designed thrombin amine aptamer using glutaraldehyde.

This hybrid sensor displayed a detection limit of 6.25 nM, largely below the imposed clinical limits, suggesting an interesting future involvement of collagen as a promising candidate for clinical thrombin analysis [20].

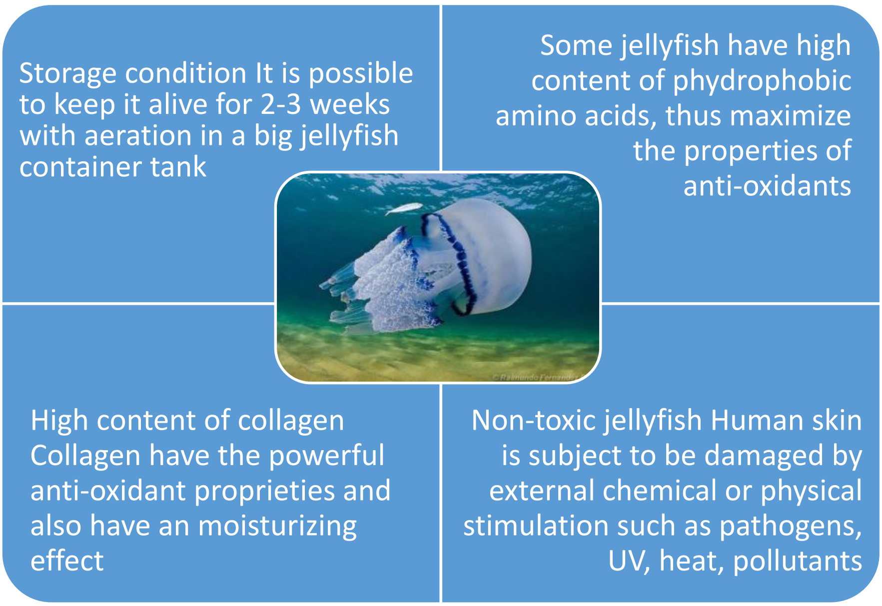


Figure 5 The advantages of collagen extracted from jellyfish

Conclusions

The contemporary world is faced with the urgent need to find alternative, sustainable and ecological resources due to overexploitation of land resources and waste disposal problems [21]. Today, people are living longer than their ancestors, which means they need more support from the medical, pharmaceutical, nutraceutical and biomedical systems to increase people's quality of life and longevity. Collagen from marine sources such as that extracted from jellyfish avoids major problems arising from cultural practices and religious beliefs, which may limit the use of bovine and porcine products by some consumers and in certain parts of the world [22].

Jellyfish collagen is a source of a great number of antioxidants. Recently, it was demonstrated that peptide fractions from *R. pulmo* collagen were able to prevent oxidative stress in HEKa cells treated with H2O2 [23].

Collagen has several applications in different fields, including nutraceuticals, cosmeceuticals, biomedicals, biomaterials and the food industry. Such a large variety of applications means that collagen can be key for the health and well-being of humans. To date, the sources of collagen mainly relied on terrestrial organisms, but they are becoming limited due to the spread of diseases and increasing alternative dietary choices of humans. This review highlights how marine organisms and their wastes can be a sustainable, eco-friendly source of collagen for the applications aforementioned.

Currently, collagen has become a necessary ingredient toward the healthy food development. The production of collagen in the body decreases with age and with an unhealthy diet. As a result, collagen has been added to a variety of foods [24]. Collagens are usually used as food additives to improve the rheological properties and reduce the fat consumption.

Collagens are used also to ensure the presence of adequate amount of animal nutritive fibers [25]. Collagen-based edible films and coatings have already been proposed to protect, maintain and extend the shelf life of different food products. The film or coating acts, in this case, as a barrier layer against the migration of oxygen, moisture and solutes, providing structural integrity and vapor permeability to the food product [26]. Moreover, it prevents fat oxidation, discoloration, microbial growth and preserves the sensory qualities.

In conclusion, research in recent years has focused on marine organisms in order to find new sources, alternatives to those already known and that can be exploited to the maximum with low energy consumption, labor and pollution. Over the past 20 years, more than 28 natural products and 175 chemicals have been found in marine entities, and hundreds of new compounds are still being discovered each year, likely due to advances in collection techniques and molecular biology [26]. To date, there are seven drugs of marine origin approved for clinical use and approximately 26 natural products in phase I to phase III clinical trials [27].

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