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Review Paper

Marine Waste to Biomaterial: Recovery of Hyaluronic Acid from Stingray Liver By-Products

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ABSTRACT

Huge quantities of organic waste, such as livers, cartilage, viscera and skins, are disposed of in the sea or processed as low value waste from fisheries and seafood-processing industries around the world, and this waste contains significant amounts of biochemical content. Glycosaminoglycans, in particular hyaluronic acid (HA), are one such class of bioactive macromolecules that have significant pharmaceutical, biomedical and cosmetic values. Traditional production of HA uses rooster comb extraction and Streptococcal fermentation, which are both associated with well-documented cost, supply, immunogenic and batch consistency issues. Liver is a large by-product of the ray fisheries that is largely unutilized, but recently, a remarkable high-molecular-weight HA has been obtained by enzymatic extraction from the liver of stingray (*Aetobatus narinari* and related batoid species). This review presents a detailed summary of the state of the art in the field of marine-derived HA and includes a focus on the emerging and sustainable source of stingray liver. It presents the chemical structure and physiological functions of HA, the need and scientific evidence for marine and stingray-specific recovery, extraction and purification methods, analysis of the HA structures, comparison of HA from marine and stingray sources, biomedical and cosmetic applications, implications for the circular bioeconomy, and key challenges and research priorities for the valorisation of stingray liver for industrial applications

INTRODUCTION

1.1 Marine Waste Generation and Disposal Challenges

Waste Management in the Marine Sector – Issues and Opportunity Currently, over 130 million tonnes of fisheries/aquaculture biomass is processed globally every year, with more than 1/3 of this biomass being treated as by-product or

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discard material such as skeletons, heads, skins, viscera, and other non-food parts of the fish during processing [1]. The Food and Agriculture Organization has emphasized the amount of waste from fish and elasmobranch processing that is being disposed of without any treatment; the waste material creates organic pollution of the coastal waters, releases greenhouse gases during decomposition and represents the loss of biomolecules that could be recovered and monetized [2]. The seafood-processing residues represent up to 20 to 80 percent of the original catch weight, depending on the species and the processing method used, highlighting the size of the waste disposal problem that faces the seafood industry [3]. The disposal options for this waste in the past have been landfill, incineration and marine or coastal dumping, but are now subject to stricter environmental regulations, higher landfill charges and greater public and regulatory focus on marine pollution.

These pressures have fostered a change of attitude among processors, researchers and policy makers and led to a renewed focus on fishery residues as a resource stream that is not an inevitable by-product of production, but one which is under-realised and where appropriate technology for recovery exists. In addition to the logistic problem of waste management, organic wastes from fish and ray processing plants, if not treated properly, can cause localized eutrophication and lead to oxygen deficiency in the receiving water bodies and odour and pest issues in the coastal communities located in the vicinity of processing plants. These impacts are especially strong in developing fishing economies where there is relatively little formal waste infrastructure and where marine waste streams are disposed of in environmentally sensitive areas, such as in public health hotspots. Due to environmental pressure and economic opportunity, the valorisation of

marine by-products has become an active field of applied research in the last 20 years.

1.2 Valorisation of Fishery by-products

In contrast, residues generated from fisheries are regarded as unutilized protein, lipid, and mineral rich biomasses containing structural polysaccharides like chitin, collagen and glycosaminoglycans (GAGs) [3,7]. These residues may be valorized to bioactive peptides, nutraceuticals, biomaterials and polymers for pharmaceutical applications while minimizing environmental impact and creating economic value for processing industries [3,6]. For instance, fish protein hydrolysates can yield bioactive peptides with antioxidant, antihypertensive and anticancer properties, and polyunsaturated fatty acid (PUFA) enriched lipid fractions can be recovered as nutraceutical oils [3]. Collagen and gelatin from skins and bones are used in the cosmetic and food industry, while chitin and chitosan from crustacean shells is the base of a large market for biopolymer [6,15].

This valorization paradigm is a paradigm change from the traditional seafood-processing value chain, which is a one-product operation, to a multi-output seafood-processing value chain, where the edible fraction is the primary product and the residual fractions are systematically valorized as secondary, tertiary, and even quaternary product streams. This cascading-use approach is part of the principles of the circular and blue bioeconomy, where the re-use of marine biomass involves the valorization of discards for high value-added products rather than discards becoming waste. The value of properly fractionated by-products can be quite high, as the economic analyses of the valorisation of these residues consistently show that it can even exceed the value of the residues themselves when they are sold as a low-grade animal feed or as fertilizer, both of which offer good economic incentives to adopt technologies



for by-product recovery, in addition to the environmental argument [3,6].

1.3 Scope and Objectives of the Review

The present review specifically addresses the hyaluronic acid (HA) recovery from by-products of stingray processing, with stingray liver serving as a case study of a new-source of high-value, marine HA, as one of the emerging fishery waste valorization examples. This review aims to summarise the chemical structure, biosynthesis, physiological functions and commercial applications of HA; to compare HA sources and technologies from animal and microbial origin with marine alternatives highlighting the current limitations that have fuelled the search for new feedstocks; to describe availability, production, and biochemical composition of stingray liver waste in global ray fisheries; to critically review extraction and purification technologies and analytical characterization of both marine and stingray-derived HA; to discuss biomedical, pharmaceutical and cosmetic applications relevant to marine-derived HA; and to discuss the implications for the circular bioeconomy, present current technological and regulatory limitations and provide research perspectives for progressing the valorisation of stingray liver from laboratory demonstration to industrial application. The review, which is based on the primary research and literature regarding the isolation of HA from the liver of *Aetobatus narinari* and the broader field of marine recovery of glycosaminoglycans, valorization of by-products from fisheries and HA-based biomedical materials, is carried out throughout.

2. Hyaluronic Acid: Structure and Biological Functions.

2.1 Chemical Structure and Properties

Hyaluronic acid (HA) is also known as hyaluronan and is a linear, non-sulfated glycosaminoglycan

made up of repeating disaccharides of D-glucuronic acid and N-acetyl-D-glucosamine, joined by the presence of alternating β -1,4 and β -1,3 glycosidic bonds [10,20]. HA differs from chondroitin sulfate, dermatan sulfate, heparan sulfate, and keratan sulfate, because it is not covalently linked to a core protein, is not sulfated, and is synthesized as a free polysaccharide rather than a part of a proteoglycan complex. It is a remarkably simple structure that hides a number of very interesting physicochemical properties: HA is very hydrophilic, and it is able to bind water at a mass ratio much greater than its own, and it occupies a hydrodynamic volume larger than expected in relation to its molecular mass [11,20]. When dissolved in water, HA chains are expanded and randomly coiled, and can entangle with other chains at relatively low concentrations, giving rise to the characteristic viscoelastic properties used in medical applications, such as synovial fluid, vitreous humor, and for use as dermal fillers [11,20]. The molecular weight range of native HA is very broad, from a few kilodaltons to some megadaltons, and biological activity is highly dependent on the chain length. High molecular weight HA (HMWHA > 1 Mega Dalton) is generally space filling, anti-angiogenic, and immunosuppressive and are associated with tissue homeostasis and barrier function, while low molecular weight fragments, produced during tissue turnover and injury, are pro-angiogenic, can trigger the release of pro-inflammatory cytokines, and are recognized as endogenous danger signals by innate immune receptors [11,21]. The molecular weight dependency is directly relevant to the use of HA as a source for the different applications, such as viscosupplementation, dermal filling, and ophthalmic applications in surgery, which specifically requires high molecular weight HA; of particular interest, however, is HA from megadalton-range HA that have been reported from the liver of stingrays [17].



2.2 Biosynthesis and Metabolism

The synthesis of HA in the mammalian system occurs at the inner side of the plasma membrane by a family of integral membrane enzymes, called hyaluronan synthases (HAS1, HAS2 and HAS3) that polymerize the molecule and extrude it directly into the extracellular space through the plasma membrane [20,21]. This biosynthetic pathway is unusual for glycosaminoglycans, which are most commonly synthesized in the Golgi apparatus followed by transport in vesicles to the plasma membrane. The three isoforms of HAS have different kinetic properties and the HA chain size they are likely to produce is different, with HAS2 being most strongly linked to the production of higher-molecular-weight HA and the accumulation of HA during development and pathogenesis [20,21]. The degradation of HA takes place via the enzyme hyaluronidase, as well as through the process of receptor-mediated internalization, mainly by the cell surface receptors CD44 and RHAMM (receptor for hyaluronan-mediated motility), which are able to bind HA and internalize it in the lysosomes for its degradation [20,21]. During this turnover process, fragments are not biologically inert, but actively involved in the tissue remodeling, wound repair signaling and modulation of the behavior of immune cells, demonstrating that HA is not only a passive structural filler, but rather a dynamic signaling molecule, the biological role of which changes depending on its degradation status. The activity of hyaluronidase is regulated tightly and can be significantly modified in pathological conditions, including cancer, where an increased expression of hyaluronidase is associated with the remodeling of the ECM, leading to invasion and metastasis of the tumor [18,20].

2.3 Physiological Functions

HA is a major structural and functional component of the extracellular matrix, which functions in

tissue hydration, lubrication of articulating surfaces of joint, viscoelasticity of the vitreous humour and synovial fluid, and the regulation of cell proliferation, migration, adhesion and differentiation via HA-mediated signal transduction pathways [11,21]. In the dermis, HA is found in the extracellular matrix, and plays a major role in providing turgidity, elasticity and hydration to the skin, an age-related decrease in which underlies many of the current reasons for the use of cosmetic HA supplementation [21]. In synovial joints, HA is the primary rheologic component of synovial fluid, responsible for its lubricating properties and shock absorption in the eye, HA makes up a large proportion of the vitreous humour contributing to the gel-like consistency and optical clarity of the eye. [21,23] In addition to its structural functions, HA is involved in physiological and pathological signaling processes. Smaller HA fragments formed when tissues are damaged bind to toll-like receptors (TLR2 and TLR4) on the surface of macrophages and dendritic cells, which activates the production of pro-inflammatory cytokines, thus playing a role in the early stages of wound healing [20]. In contrast, high-molecular-weight HA is anti-inflammatory, and is linked to tissue quiescence and barrier integrity, suggesting a size-dependent bivalent function that is currently a prominent theme in HA biology and a fundamental principle in the design of HA-based therapeutics [11,20,21].

2.4 Commercial Significance

Due to these characteristics HA has emerged as one of the most commercially relevant biopolymers in today's medicine and cosmetics. Its clinical applications include use as a viscoelastic surgical aid and an intraocular lens carrier in ophthalmic surgery, as an intra-articular HA injection (viscosupplementation) for the treatment of osteoarthritis, dermal fillers (smoothing agents)



in dermatology and cosmetic medicine (intra-dermal HA injection) – which are among the most widely used injectable cosmetic dermatologic products in the world – and advanced drug delivery (HA-based) and tissue engineering (scaffolds) applications [11,18,21]. The market for HA has grown significantly in the last 20 years, largely due to its use in the aesthetic medicine, ophthalmology and orthopedic industries, and this consistent growth has spurred a search for alternative sources of raw materials that are both economically viable and scalable, and also sustainable alternatives to the current production methods of rooster comb and microbial fermentation [4].

3. Conventional Sources of Hyaluronic Acid

3.1 Animal-Derived Sources

HA was historically isolated from bovine vitreous humor in the 1930s and rooster combs were the primary industrial source of HA due to the relatively high levels of HA in the dense connective tissue of the combs. Other animal tissues that have been examined as a HA source are umbilical cords, synovial fluid and various cartilaginous tissues; however, none has attained the commercial scale as has the rooster comb extraction [4]. However, despite decades of refinement of the extraction protocol and the fact that Rooster Comb HA continues to be widely used in pharmaceutical and cosmetic products, the production process remains labor intensive and involves careful dissection of the tissue, numerous rounds of proteolytic digestion, and extensive purification to remove co-extracted proteins, lipids, and nucleic acids [4].

Animal-derived HA also has its inherent risks from biological raw material derived from terrestrial animals, such as the theoretical risk of viral or prion contamination, batch-to-batch variability, which is associated with the age, breed,

and health of the source animals, and the potential for immunogenic reactions in sensitive patient populations from the residual avian proteins in this type of HA [4,9]. In established commercial operations, these risks are typically controlled, with the operation having extensive purification and quality control measures in place, but they remain a focus of regulatory attention and a reason to maintain an interest in production methods other than animal production.

3.2 Microbial Fermentation Sources

The industrial production of HA has shifted to microbial fermentation, mostly replacing rooster combs in many commercial applications since the late 20th century, with *Streptococcus equi* subsp. *zooepidemicus* and other *Streptococcal* species being the primary microbial fermentation agents [9]. The fermentation based production process has several advantages over the animal extraction, such as the possibility of using standard bioreactor facilities, the possibility for more consistent and controllable molecular weight distribution by controlling fermentation conditions, and the absence of the risk of contamination with mammalian or avian pathogens [9]. The production of streptococcal HA usually involves batch or fed-batch fermentation in glucose-rich media, separation of the cells, deproteinization and precipitation of the polysaccharide from the fermentation broth. Although the advantages are many, *Streptococcal* fermentation presents its own technical and safety problems. *Streptococcus* species can also produce pyrogenic exotoxins as well as endogenous hyaluronidase, which need to be carefully removed during the purification process in the downstream to ensure the safety of the final pharmaceutical grade product [9]. For HA that must be used as an injectable or for ophthalmic use, bacterial endotoxins are of special importance since they can trigger strong immune responses, and there are many extensive and validated



purification methods to ensure endotoxins are kept at low levels that are within regulatory limits. Alternative systems to reduce these safety concerns, without sacrificing the benefits of scalability of fermentation-based production, are currently being investigated by the development of recombinant or genetically modified non-pathogenic microbial hosts that can produce HA, such as engineered strains of *Bacillus subtilis* and *Escherichia coli* [9].

3.3 Limitations of Existing Sources

Both the conventional production methods have significant limitations that have combinedly inspired the quest for alternative HA sources. Livestock production, ethical and welfare concerns of some consumers and regulatory environments, and the risk of allergenic and pathogenic contamination from animal sources are inherent limits to supply [4]. Although more scalable and consistent, microbial fermentation requires significant investment in fermentation and downstream processing equipment and facilities, presents its own safety risks of removing bacterial toxins, and is relatively expensive when compared to the theoretical raw-material cost of waste-derived alternatives [4,9]. Additionally, both routes are mostly independent of the current waste-stream infrastructure, so they do not inherently provide solutions to the separate, significant issue of disposal of marine and/or terrestrial organic waste (Section 1). Such multiple limitations, economic, technical, ethical, and environmental, have fostered increased research for marine by-products as a complementary or alternative HA source, which could be used to simultaneously tackle both diversification of HA supply and valorisation of marine wastes.

4. Marine Resources as Emerging Sources of Hyaluronic Acid

4.1 Marine Organisms Rich in Glycosaminoglycans

This section focuses on marine organisms that are high in GAGs. This section will be dedicated to marine organisms with a high GAG content. Many marine organisms and tissues have been reported to contain extractable glycosaminoglycans, demonstrating the evolutionary conservation of GAGs in connective tissue. There are a variety of reported HA sources such as shark and stingray cartilage, tuna eyeballs, tuna liver, and a number of fish skin and head fractions; chondroitin is widely reported to be present in shark cartilage up to 29% of the dried substance, and in some instances, a substantial amount of chondroitin sulfate can be recovered from shark cartilage [4, 6, 12, 15]. Thus marine biomass is a large and structurally diverse source of glycosaminoglycans and related biopolymers, in particular comprising chitin and chitosan, two structurally different polysaccharides obtained from the processing wastes of marine invertebrates and crustaceans, respectively, which are commercially complementary [6,15]. This diversity of sources for marine GAGs is partially attributable to the diversity of marine fisheries: the eye-balls of pelagic species like tuna, cartilage and liver of demersal species like sharks and rays, and skin and head of aquaculture species, all provide significant amounts of GAGs as a minor fraction of their production. This diversity can be of practical benefit in that marine GAG recovery technology developed for one species or tissue type can often be applied, with modification, to other species, and serves as a basis for a more general strategy in which marine GAG recovery is routinely integrated as a standard part of seafood-processing operations in multiple fisheries, rather than a niche application limited to a single species [4,6].

4.2 Fish Processing Waste as a Biomaterial Feedstock



Fish byproducts such as eyeballs, skins, cartilage and viscera are now being studied as feedstocks for GAG production for several reasons: They are continuously produced by the seafood processing industry in large amounts; they are currently discarded at the end of the processing operation as low or zero value waste; and they have repeatedly been shown to contain HA and chondroitin sulfate of known structural identity and measurable bioactivity [5,12]. The most widely used extraction strategy for extraction from these tissues is enzymatic hydrolysis with proteases, which have relatively mild reaction conditions, and can release GAGs from the surrounding protein matrix without significantly degrading the polysaccharide chain. In addition to HA and chondroitin sulfate, the fish byproduct derived GAGs have been shown to possess a variety of bioactive properties of relevance to nutraceutical and pharmaceutical applications, such as antioxidant, anti-inflammatory, and anticoagulant properties of fish derived dermatan sulfate and heparan sulfate fractions [12]. This wide range of bioactivity has strengthened the concept of systematic investments in fish-waste GAG recovery as a part of the integrated strategies of valorization of seafood processing rather than as a standalone or isolated technical goal [5,6].

4.3 Sustainability Advantages of Marine-Derived Hyaluronic Acid

Marine-derived HA is a great advantage compared to the extraction from rooster comb and microbial fermentation because of the twofold of waste valorization and supply diversification. To recover HA from fishery processing waste, there is no need to have a stock of source animals or build a fermentation facility, and the biomass is used in a way that avoids extra environmental and economic expense [3,6,7]. This means that marine HA recovery is placed on a positive footing within the framework of circular economy, in which the

environmental footprint of primary production (in this case, fishing and ray processing for other primary uses like meat or skin) is distributed among several co-products instead of being assigned to one product. The adoption of green and enzyme-based extraction processes, which will be discussed in more detail in Section 6, also contributes to the environmental benefits of marine HA compared to conventional purification technologies that rely on chemical processes, such as reduction in solvent consumption, energy consumption, and hazardous waste generation in HA production [4,7]. These sustainability benefits, combined, have made marine-derived HA, and more specifically liver HA, a very strategic complement to the existing production pathways in the context of the 'blue bioeconomy' [3,6,7].

5. Stingray Processing By-products: Availability and Composition

5.1 Global Stingray Fisheries and Processing Industry

Stingray in the Global Level Batoid fishes include species such as *Aetobatus narinari* and other stingrays, which are targeted capture in fisheries and bycatch in the larger fisheries of demersal and pelagic types throughout the tropical and subtropical fisheries worldwide [9,17]. The generation of wastewater in the processing of stingrays for meat, skin (leather) and cartilage is very high when compared to the biochemical potential of the waste (visceral and hepatic) [9,17]. There are already industrial processes using rays in Asia, Middle East, Africa and Latin America to harvest HA from rays' liver, and the region is home to many industries that make use of the stingray's skin for leather goods, providing established industrial base to add HA recovery as an additional value stream without extra fishing pressure. The liver of a stingray is a relatively large organ compared to its body weight, as is also true of other



elasmobranch species; in cartilaginous fish the liver is the major lipid storage organ, much like adipose tissue is in many bony fish and terrestrial vertebrates [9,17]. It is this anatomical characteristic that lends itself to the potential for meaningful liver biomaterial recovery relative to the overall carcass weight of a processed animal, which will facilitate the recovery of a biomaterial from liver in meaningful industrial scale once collection and processing logistics are established.

5.2 Generation of Stingray Liver Waste

Stingray Liver Waste Most markets do not use stingray liver directly for food, and it is usually removed during the gutting and evisceration process during the processing of rays making its use as a food stream clearly a discard stream in most existing ray-processing operations [9,17]. The liver also contains high levels of polyunsaturated fatty acids and fat soluble vitamins, and in some regional industries, the liver is recovered for the production of nutraceuticals and/or topical cosmetics, which means that the liver material can be recovered, transported and further fractionated, not just disposed of wholesale [9]. This current partial liver oil recovery system is a logical starting point for the subsequent recovery of HA and other glycosaminoglycans from the same tissue, in an integrated cascading-use system which will be discussed later in Section 10. The amount of liver waste produced annually is hard to estimate at the global level because of the informal and dispersed nature of ray fisheries in many major ray-producing regions, but in some regions, the fraction of liver waste in the total processing waste from ray landings has been measured and found to be a significant and consistent component of the processing waste, large enough that a pilot-scale or even full-scale recovery operation is possible [9].

5.3 Biochemical Composition of Stingray Liver

Stingray liver contains lipids, especially the polyunsaturated fatty acids, and fat-soluble vitamins such as vitamins A, D, and E, characteristic of its physiological function of being the main lipid storage and metabolic organ in elasmobranch fish [9]. The relatively high iodine index (56.5) of the extracted stingray liver oil, as reported in the different analyses, indicates the presence of a significant amount of unsaturated fatty acids, of which nearly forty per cent are polyunsaturated fatty acids, which is the reason for the nutraceutical and cosmetic relevance beyond its HA content [9]. In addition to its high lipid content, the liver Ecm of St. is composed of glycosaminoglycans, such as high molecular weight HA, which are surrounded by a dense protein matrix and must to be proteolytically digested to obtain the polysaccharide fraction [17]. In many instances, the presence of a high lipid content in the same organ in which a high GAG content is present has actual practical applications for the design of the extraction process, especially since, to prevent lipid interference during subsequent digestion and purification steps, it is often necessary to treat the tissue with a defatting step before the enzymatic extraction of the GAGs (see Section 6.1).

5.4 Potential for Hyaluronic Acid Recovery

There is potential for recovery of hyaluronic acid. In the work by Sadhasivam et al., liver of stingray, *Aetobatus narinari*, was used as a source of HA, which was isolated from it using papain-based enzymatic digestion, and a purified HA molecular weight on the order of 1.37 megadaltons was reported, among the higher molecular weights reported across marine HA sources in the literature [17]. The purified material was shown to be structurally identical to the starting material by Fourier-transform infrared spectroscopy, high-performance thin-layer chromatography and proton nuclear magnetic resonance spectroscopy



and also shown to have measurable antioxidant activity using DPPH radical scavenging assays and notable antiproliferative activity in preliminary bioactivity screening. The recovered HA was shown to have functional bioactivity in addition to confirmed structural identity [17].

Recovery yields from the liver tissue of stingrays have been reported after anion-exchange chromatographic purification in the range of 6 mg of HA/g of dry liver tissue, and after precipitation in ethanol, with optimized conditions in the range of 44% [1]. These yields are still very low compared with some very well optimized terrestrial extraction methods, but they are still enough to make stingray liver a potential marine HA source as well, given the very high molecular weight of the extracted HA and the essentially null raw-material acquisition cost to use an existing waste stream as a source compared to the dedicated raw-material cultivation input [1,17].

6. Extraction and Recovery Technologies for Hyaluronic Acid

6.1 Pretreatment Strategies

The preparative steps usually include in the initial processing of stingray liver and other marine HA-rich tissues aimed at making the tissue suitable for the subsequent extraction. Washing is used to remove any residual blood, mucus, and surface contaminants; mechanical homogenization breaks up larger tissues and particles, but also tears up the gross tissue architecture to increase surface area for enzymatic or chemical attack of the tissue; defatting, usually carried out by organic solvent extraction or mechanical pressing, eliminates lipid content that would interfere with subsequent enzymatic or chemical digestion or co-precipitate with the target polysaccharide during purification [1,4] Defatting is especially important when processing stingray liver as a high naturally occurring level of lipid is found in the liver (as

discussed in Section 5.3) and is desirable for subsequent enzymatic digestion to be efficient [9]. The pretreatment protocol must be carefully optimized to ensure it is thorough enough to avoid degradation of the HA molecule prior to the targeted extraction step, but not so harsh as to initiate depolymerization of the HA chains before the extraction step starts, which would cause the degradation product to be unsuitable for use in high value applications that require high molecular weight HA.

6.2 Enzymatic Extraction

Proteolytic enzymes, particularly papain but also pepsin, trypsin and alcalase, are currently used to treat HA in the protein matrix of connective tissue, and to liberate the polysaccharide in solution under relatively mild reaction conditions, without harsh chemical hydrolysis methods, that allows its maintenance at high molecular weight [1,17]. This enzymatic method is specifically the one used to isolate HA from stingray liver; the original method involved a prolonged digests of the liver with papain at controlled pH and temperature, followed by deproteinization to remove any digested protein fragments and other macromolecules not HA [17]. Each of these factors significantly affects the extraction yield and the molecular weight distribution of the HA recovered, and has to be optimized in an empirical fashion for each new type of tissue. It has been found that there is a correlation between the levels of HA liberated and the time required for the digestion in different types of marine tissues, where harder, more protein dense tissues like stingray liver and various comb or crest tissues, take about twenty-four hours to liberate HA effectively, while softer tissues may require significantly less digestion time [19].

6.3 Chemical extraction processes

Chemical extraction methods use alkaline or acid hydrolysis, sometimes combined with proteolytic



digestion, to more aggressively break down tissue structure and release glycosaminoglycans from more resistant connective tissue matrices [1,4]. Chemical hydrolysis can result in effective tissue disruption and can potentially be processed faster than purely enzymatic methods, but harsh chemical conditions introduce the potential for degradation of the HA chain length due to acid or base mediated breakage of glycosidic bonds, which could lead to a decrease in the molecular weight of the recovered polymer and may make it unsuitable for applications that require high molecular weight HA. The crude HA extract is usually hydrolyzed followed by selective precipitation with ethanol or the use of cationic surfactants like cetylpyridinium chloride (CPC), which differentiates the solubility of HA from the contaminants in the extract, which are also present, such as residual protein, lipid and lower-molecular-weight carbohydrate fragments [1,6]. The precipitation step is usually repeated and successive precipitation and redissolution cycles are performed to gradually increase the purity of the product which is then subjected to more selective purification steps by chromatography.

6.4 Green and Sustainable Extraction Approaches.

The attention in the field has shifted to environmentally friendly extraction routes with less organic solvent and energy consumption in comparison to the traditional chemical hydrolysis and precipitation. These include combined enzymatic-membrane processes where enzymatic digestion is directly coupled to membrane-based separation instead of solvent precipitation, and microbial co-digestion strategies that allow using enzymes and microbes in a complementary manner to enhance the extraction efficiency and/or to replace harsh chemical hydrolysis with milder, more selective, conditions [4,6,7]. The merits of the techno-economic approach of combining

enzymatic, microbial and membrane processing steps in eco-friendly process trains for the production of marine glycosaminoglycan, which will lead to higher GAG recovery, better preservation of the molecular weight of the products and lower environmental footprint compared to the extraction routes, mostly based on the use of organic solvents and concentrated mineral acids or bases, are specifically highlighted in reviews on the production of marine glycosaminoglycans [6]. Other new technologies like ultrasound-assisted extraction and the use of microwave-assisted pretreatment, which have been used in other marine biopolymer extraction applications, have provided good results in contexts similar to the extraction of stingray liver due to lower processing times and yields, and less severe and environmentally harmful conditions [4,7].

6.5 Purification Techniques

After the crude extraction, the purification process of marine derived HA usually goes through a series of complementary processes. Residual protein contamination is removed by deproteinization by further proteolytic digestion or by classical methods of protein denaturation and removal, like the Sevag method, which uses chloroform. Selective ethanol precipitation allows to concentrate the polysaccharide fraction and remove many more soluble and smaller molecular weight impurities. Residual low molecular weight contaminants such as salts, small peptides and even degraded carbohydrate fragments are removed by dialysis or ultrafiltration, and the final, most selective purification step necessary for preparation of pharmaceutical-grade carbohydrate products - suitable for biomedical application - is brought by anion-exchange or size-exclusion chromatography [1,4,17]. Membrane-based ultrafiltration has been shown to be particularly effective in separating HA from other small



contaminants, like free glucosamine (MW 0.23 kD), that readily pass through the standard ultrafiltration membranes, by using MWCOs compatible with the characteristic size range of HA, which is typically in the range 5 to 50 kD. For larger contaminants, like those of residual collagen (molecular weight ~300 kDa), they are usually removed along with the target HA fraction during the ultrafiltration step; therefore, an additional more selective chromatographic step is required to obtain the final pharmaceutical-grade purity [1].

6.6 Recovery Efficiency and Yield Optimization

The efficiency of recovery of HA from marine tissues is highly variable depending on tissue type, extraction procedure, and purification technique. In comparative studies, HA precipitation yields from 44 to 72 percent were reported for various marine and microbial HA sources depending on precipitation conditions including ethanol to broth ratio, number of precipitation steps, and the presence or absence of sodium chloride as a co-precipitation aid with the HA source of stingray liver being specifically reported at approximately 44 percent in comparative tests. The general approach to optimizing the yield of HA in a marine HA extraction study is to alter the digestion time, enzyme to substrate ratio, reaction pH and temperature, and precipitation solvent ratios while keeping in mind that these optimization goals may sometimes be in conflict, as more aggressive digestion conditions that would increase HA yield may degrade the HA chains to some degree [1,17]. Studies of optimization for the extraction of the stingray liver in a systematically factorial design are still rather limited published compared to other more well-documented organs, such as the rooster comb, and this is a good opportunity for further methodology refinement, as outlined further in Section 12.

7. Characterization of Marine-Derived Hyaluronic Acid

7.1 Molecular Weight Determination

Molecular weight is one of the most important parameters for any HA preparation used in biomedical and cosmetic applications because its biological activity is highly dependent on chain length as mentioned in Section 2.1. Typically, the molecular weight of HA is determined by one or more of the following techniques: gel permeation chromatography (also called size-exclusion chromatography), capillary or rotational viscometry, or static and dynamic light-scattering, each of which provides complementary information on the average molecular weight and the polydispersity of the HA sample [9,21]. In the case of HA derived from the liver of the stingray, the molecular weight is confirmed in the megadalton range, such as 1.37 MegaDaltons in the basic study of isolation, which is in the range suitable to use the HA in the applications of viscosupplementation, ophthalmic surgery, and dermal fillers, all of which require the use of HA with high molecular weight [17,21]. FTIR, NMR, HPLC were used for the structural characterization of Marine-Derived hyaluronic acid

7.2 Structural Characterization (FTIR, NMR, HPLC).

Fourier-transform infrared spectroscopy (FTIR) is a common technique to confirm the presence of specific HA functional groups, such as the bands associated with carboxyl, amide and glycosidic linkages, which is a relatively quick initial confirmation of polysaccharide identity before more costly and time consuming analytical techniques are employed [17]. Proton nuclear magnetic resonance (^1H NMR) spectroscopy gives additional structural confirmation of the disaccharide composition and the anomeric configuration, and enables direct comparison of



the obtained spectra with the reference spectra of pharmacopeial-grade HA standards to verify the structural fidelity, established by the analysis [17]. In addition, high performance thin layer chromatography (HPTLC) has been used as a further complementary method to confirm the disaccharide composition and was used in conjunction with FTIR and NMR in the structural characterization of the HA found in the stingray liver, providing a multi-technique confirmation of structural identity that bolsters confidence in the authenticity of recovered HA [17].

7.3 Physicochemical Properties

In addition to structural identity, numerous physicochemical parameters are measured to verify that HA preparations derived from marine sources are suitable for specific applications. These include solution viscosity, a direct relationship with molecular weight and concentration, which is essential for the function of the material and is of particular importance in cosmetics and wound healing; water-holding capacity, necessary in cosmetic and wound healing applications; aqueous solubility; thermal stability, necessary for processing and sterilisation requirements; and elemental composition, including carbon, hydrogen and nitrogen content, which is used as a further confirmatory and quality-control measure [17]. The N-acetylglucosamine/glucuronic-acid ratio, determined by quantification, confirms proper stoichiometry of the disaccharides: if the ratio is not as expected (1:1), it may indicate co-extracted contaminant carbohydrates or incomplete purification [17].

7.4 Purity Assessment

Before any biomedical use of marine-derived HA, it is vital to evaluate its purity, as residual protein, nucleic acid, and contaminating glycosaminoglycan levels are extremely tight

regulations for pharmaceutical-grade HA. Purity is usually confirmed by the dye-binding assay, which is metachromatic dye staining and is both qualitative and semi-quantitative to confirm the presence of glycosaminoglycans (GAGs); by quantification of residual protein by standard protein assay, and by agarose-gel electrophoresis that will distinguish HA from potentially co-extracted contaminating GAGs, like chondroitin sulfate, which can be used in downstream biomedical and pharmaceutical applications [17].

8. Comparative Analysis of Hyaluronic Acid Sources

8.1 Stingray Liver versus Other Marine Sources

The liver of the stingray is in a unique position among the other reported marine HA sources. Tuna eyeball, for example, has been reported to produce about 10.5 mg of HA per eyeball after purification using aqueous extraction and dialysis based purification, which is an advantage due to the less protein dense tissue of eyeball than liver, enabling to achieve simpler purification protocol and shorter time [1]. In contrast, shark cartilage, and other cartilage of rays, is typically a greater source of chondroitin sulfate than HA alone; in some cases, the concentration of chondroitin sulfate in shark cartilage was reported to be up to twenty-nine percent by dry weight [15]. In this comparative context, the recovered HA of stingray liver is particularly distinguished by the high molecular weight (approx. 1.37 megadaltons) reported, though the gravimetric yields per unit tissue mass are still moderate when compared to some other marine and terrestrial sources and are strongly dependent on the extraction method used [1,17].

8.2 Marine versus Terrestrial Sources

The terrestrial animal by-products, especially rooster comb and chicken crest, have a much



longer history of HA extraction in industry, with decades of process optimization that have resulted in higher gravimetric yields as reported than many newer marine sources, such as stingray liver [4,19]. While non-marine tissues are valuable and underutilized feedstock that broadens the overall HA supply chain, they also have the potential to pose a lower risk of allergenicity for certain patient populations who are sensitive to HA derived from poultry industry byproducts, which is a benefit that has not yet been fully explored for marine-derived HA but is a plausible clinical benefit that should be investigated [4,19].

8.3 Marine-Derived versus Microbial Hyaluronic Acid

The major commercial route for HA production is microbial fermentation, which is scalable, process reproducible, and is not reliant on animal sources of raw materials [4,9]. But in the fermentation route, there is a need for dedicated bioreactor infrastructure, significant fermentation substrate input (usually glucose-rich growth media), and strict endotoxin and exotoxin removal in the downstream process to ensure pharmaceutical grade safety, which are all expensive items for the overall production process [4,9]. In contrast, marine-derived HA is re-purposed from an existing waste stream from fishing and ray-processing used primarily for other commercial purposes where the extraction of raw materials is also seasonal, geographically concentrated, and

the processing is not as mature as decades-old bacterial fermentation technology, resulting in a significant reduction in, or elimination of, dedicated raw-material cultivation cost; but at the same time, the greater seasonality, geographic concentration of the feedstock, and comparatively lower process maturity of decades-optimized bacterial fermentation technology are inherent risks of marine-derived HA [4,9].

8.4 Quality, Yield and Economic Comparison

Based on the data collected, the quality, yield and economics of these two were compared. Several interacting factors need to be considered in a comprehensive economic comparison between HA sources, including raw material cost, which is low for waste-derived marine feedstocks but significant for dedicated fermentation substrate or animal husbandry feedstocks; extraction and purification process complexity and cost, which varies widely depending on the tissue source and desired final product purity; and achievable final product purity and consistency, in which established microbial fermentation currently enjoys a clear edge as it is more process-mature and standardized. [4,6] In general, marine waste-derived HA, such as HA from stingray liver, is situated in the current literature as an alternative to microbial HA that is less standardized and has a lower raw-material cost [4,6] (which will be discussed in more detail in Sections 11 and 12).

Table 1: Representative extraction methods, yields, and characterization techniques for selected HA sources [1,4,6,17,19]

Source	Extraction Method	Reported Yield	Characterization Techniques
Stingray liver (A. narinari)	Papain enzymatic digestion; anion-exchange purification	~6.1 mg HA/g dry tissue; MW ~1365 kDa	FTIR, 1H NMR, HPTLC, agarose-gel electrophoresis
Tuna eyeball	Aqueous extraction; dialysis	~10.5 mg HA/eyeball	Carbazole assay, dialysis purification
Chicken crest/comb	Cysteine-assisted hydrolysis	Variable; widely used industrial benchmark	Carbazole/uronic acid assay, GPC
Microbial (S. zooepidemicus)	Fermentation; ethanol precipitation	Recovery ~48-72% (process dependent)	GPC, viscometry, endotoxin assay



9. Biomedical Applications of Marine-Derived Hyaluronic Acid

9.1 Wound Healing

HA-based hydrogels and wound dressings create a moist wound healing environment which is regarded as a critical factor in facilitating efficient epithelialization of wounds and have been demonstrated to stimulate angiogenesis via the activation of the HIF-1 α and VEGF signaling pathways in the process of wound healing [13,22]. In clinical and preclinical studies, HA formulations showed accelerated epithelialization, decreased scarring, and improved neovascularization in burn, diabetic ulcer, and infected wound models, which are mechanistically related to the intrinsic biological activity of HA and are therefore equally relevant for high purity HA preparations, which are obtained from marine sources and meet adequate quality specifications [13,22]. HA functionalized with antimicrobial nanomaterials, such as metal-organic frameworks (MOFs), have also been developed to enhance the functional capabilities of HA wound dressings, providing both moisture retention and pro-healing properties and active antibacterial activity against multidrug-resistant pathogens [22].

9.2 Tissue Engineering

HA hydrogels and electrospun nanofiber scaffolds, and three-dimensional porous constructs, have been widely used in the field of bone, cartilage and soft-tissue regenerative engineering applications because of HA's specific interaction with cell-surface CD44 receptors that regulate cell adhesion, proliferation, migration and lineage-specific cell differentiation in engineered tissue constructs [11,20]. Chemical crosslinking can be achieved by several methods, such as chemical crosslinking by chitosan, chemical crosslinking by collagen, crosslinking by photoinitiators (such as riboflavin), enzymatic crosslinking by horseradish

peroxidase and hydrogen peroxide and physical crosslinking using hydrogen bonding or metal coordination, offering tunable control of the scaffold mechanical properties, degradation rate and bioactive cargo release for specific tissue engineering applications [20].

9.3 Drug Delivery Systems

Drug Delivery Systems By exploiting the well-known overexpression of CD44 receptors in the surface of many tumor cell types, receptor-mediated targeted delivery of chemotherapeutic agents with HA-functionalized nanoparticles can be achieved, resulting in greater tumor specificity than untargeted delivery systems [10,18]. In addition to oncology applications, HA-based hydrogels have been developed as controlled release vehicles for antibiotics, anti-inflammatory agents, and other therapeutics, which would allow for local delivery of the drugs directly to treatment sites—such as wounds—where they would be needed for longer periods of time while also benefiting the surrounding tissues with the moisture retention and tissue-supportive properties of the HA matrix itself [12,18].

9.4 Ophthalmic Applications

Since the early 1980s, high molecular weight HA has been employed in intraocular lens (IOLens) implantation and other ophthalmic surgery as a viscoelastic surgical aid, where the viscosity of HA is crucial in protecting the corneal endothelium during surgical operations, which is dependent on achieving high molecular weight in the range of megadaltons for HA extracted from certain sources, such as stingray liver [21,23]. The biocompatibility, transparency and viscoelastic cushioning properties of the HA solutions are particularly useful in maintaining anterior chamber stability and protecting fragile ocular tissue during surgeries, a clinical application where high molecular weight HA solutions, under



specific purification and validation, could have the potential to be used as an alternative raw material.

9.5 Orthopedic Applications

Intra-articular HA injection, or viscosupplementation, is a well-established and widely accepted therapy in the treatment of OA; the mechanism of the intervention includes restoration of the properties of synovial fluid, down-regulation of inflammatory mediators in the affected joint space and improving the viscoelastic function of synovial fluid [21,23]. After IA injection, HA is taken up by synovial cells through CD44-receptor-mediated endocytosis, leading to recovery of the native joint lubrication and decrease in pain and stiffness of joint degeneration in the OA joint [21,23]. Recent reviews have also confirmed the increasing use of HA in the design of patient-specific meniscal implants and other orthopedic regenerative products, suggesting that HA is not only being used in viscosupplementation

alone, but is also being used in other applications in the field of orthopedics [23].

9.6 Cosmetic and Dermatological Products

HA is widely used in cosmetic and dermatological products such as injectable dermal fillers, anti-aging creams, hydrating serums and facial masks, among others, for its ability to visibly enhance the hydration, plumpness and elasticity of the skin [8,14,16]. The trend of marine products used in cosmetics has been fueled by consumer interest in natural, sustainable, and effective cosmetics, placing marine-derived HA in a favorable light in this growing market segment [8,14,16]. The bioactive compounds found in the liver oil described in Section 5.3, and other compounds derived from stingray, already have been applied to topical dermato-cosmetics, thus offering the good example of an already existing market and processing infrastructure for the HA recovery to be integrated as a complementary product stream[9].

Table 2: Biomedical and cosmetic applications of marine-derived hyaluronic acid [8,10].

Application Area	Representative Use of HA
Wound healing	Hydrogel dressings, antibacterial/anti-inflammatory composites, microneedle-integrated delivery
Tissue engineering	Scaffolds for bone and cartilage regeneration, cell-delivery vehicles
Drug delivery	CD44-targeted nanoparticles, controlled-release hydrogels for chemotherapeutics and antibiotics
Ophthalmology	Intraocular lens implantation, ocular surgical viscoelastic agents
Orthopedics	Intra-articular viscosupplementation for osteoarthritis, meniscal implants
Cosmetics/dermatology	Dermal fillers, anti-aging and hydrating skincare formulations

10. Environmental and Circular Bioeconomy Perspectives

10.1 Waste Minimization and Resource Recovery

Being able to recover HA from stingray liver turns this material into a valuable pharmaceutical-grade product, directly reducing the organic waste load

of the ray-processing operations outlined in Section 1.1, and at the same time, closely matching the overall fishery by-product valorization initiatives being carried out throughout the seafood sector [2,3]. HA recovery both removes a burden from the environment (which would otherwise have to be treated with new infrastructure or just



discharged) and represents an economic opportunity for the ray-processing operator, making it even more attractive to use.

10.2 Circular Economy Concepts

The incorporation of HA recovery into current production of stingray liver oil and other lipid fractions is a good example of a cascading multi-product valorization approach, where the same waste stream is increasingly fractionated into a number of complementary value streams, such as liquid, oil and lipid oils, protein hydrolysates and HA, to maximize the overall efficiency of the use of the resources, while minimizing the amount of waste left after processing that may require disposal [6,9]. Cascading-use models are one of the key operational principles of circular economy thinking for biological feedstocks: maximising the economy and material value of the material from a particular biomass feedstock rather than recovering just one high-value compound before discarding. Life Cycle and Sustainability Considerations.

10.3 Life Cycle and Sustainability Considerations

While formal life cycle assessments of HA recovery from stingrays are limited to the published literature, a general comparison of the marine waste valorization versus the dedicated cultivation and production of the HA substrate or the cultivation of terrestrial animals specifically for HA production, indicates an overall lower burden of cultivation and production of waste-derived HA, as no special cultivation or farming of marine animals are required specifically for the production of HA [3,6,7]. However, some of these issues, such as the logistical challenge of collecting liver tissue from widely scattered processing facilities, the seasonal fluctuations in ray fisheries discussed in Section 5.1, and the

energy consumption of the numerous purification steps described in Section 6 affect the environmental footprint of the final product and should be included in future, more comprehensive LCA studies specifically focused on recovering ray liver HA.

10.4 Contribution to Blue Bioeconomy

The recovery of stingrays from the liver is an important contribution to the overall blue bioeconomy by allowing fisheries and processing units to achieve more sustainable and economically viable use of their existing bycatch and processing residue biomass, as long as liver recovery is not linked to additional bycatch and processing residue from targeted harvest pressure on rays, including those used for conservation concern, which are found in various regions [3,6]. Stingray liver valorization is an example of marine biomaterial recovery that can prove to be a model for a new class of biomedical raw material sustainably sourced for valorization, while also fostering fishery economic resilience and the reduction of marine waste.

11. Challenges and Limitations

Although it is technical and sustainability proven that stingray liver HA can be recovered, some major hurdles now remain between the laboratory proof of concept and commercial implementation on an industrial scale. The availability of raw materials: the need for stingray liver is intrinsically tied to the scale and geographical distribution of ray fisheries which are regionally concentrated, have seasonal landings variation and in some jurisdictions are subject to increasing conservation concern regarding the status of elasmobranch populations, all of which limit the reliability and scalability of raw material availability as feedstock for the production of liver [9,17]. The high processing costs associated with enzymatic digestion, multi-step chromatographic purification



and intensive quality control testing to obtain HA for use in pharmaceuticals significantly increase the extraction cost compared to the extraction of crude lipids from stingray livers, and the relatively low gravimetric yield of HA from the stingray liver compared to optimized terrestrial recovery further influences the economics of the process per unit of lipid extracted from the stingrays [1,4].

Due to the differences between processing batches and source animals of variable size, age, and physiological state, the expected batch-to-batch consistency of the pharmaceutical quality product is still a challenge for HA that is not yet fully resolved as compared to the decades of process optimization that have been applied to microbial fermentation-based HA production, despite the fact that the former is not as complex as the latter [4,17].

Biological issues: Marine-derived biomaterials designed for medical applications must first be carefully characterized for potential contaminants such as heavy metals, persistent organic pollutants and other environmental contaminants that may bioaccumulate in marine tissue, followed by a complete demonstration of consistent biocompatibility and lack of immunogenic risk and ultimately follow regulatory approval pathways to clinical application [5,12].

The challenge of scale-up: The translation of laboratory scale (gram to kilogram tissue) enzymatic extraction processes to acceptable industrial scale volumes while maintaining high molecular weight and consistent purity is a large engineering hurdle that needs to be addressed beyond a simple extrapolation from other more established marine or terrestrial HA sources [1,4].

12. Future Research Directions

12.1 Green Extraction Technologies

Future research should focus on improving methods that use fewer solvents, along with

enzyme and membrane-based techniques. Adding steps like ultrasound or microwave treatment could help speed up the extraction process and increase yield, while also making it better for the environment and using less energy compared to current methods [4,6,7]. Studies that test different factors—like how long the digestion takes, how much enzyme is used, and what reaction conditions work best for stingray liver—could be a good next step. These studies build on earlier work by Sadhasivam et al. and could lead to better results in terms of both yield and keeping the molecular weight of the material intact [17].

12.2 Biorefinery Approaches

Biorefineries that can extract lipids, structural proteins, collagen, and HA all at once from stingray liver would make the whole process more efficient and cost-effective compared to just focusing on HA alone. This is because spreading the costs over multiple products increases the overall value from each batch of raw material [6,9]. Creating a biorefinery system specifically for stingray liver offers a good chance to use the existing local facilities already set up for extracting oil from stingray livers, as mentioned in Section 5.2 and Section 10.2.

12.3 Advanced Biomaterial Development

Chemically changing and linking marine-derived HA into hydrogels, nanoparticles, and composite scaffolds could greatly increase the ways it's used in drug delivery and regenerative medicine. This builds on methods already used for HA from land and microbes, like EDC/NHS chemical bonding and the crosslinking techniques mentioned in Section 9.2 [11,18,20]. Since stingray liver HA has a very high natural molecular weight, it might be a great starting material for further changes that need a strong base before breaking down or adding new features.

12.4 Industrial Commercialization Potential



Potential for Industrial and Commercial Use To move stingray liver HA from the lab to real-world use, we need to test the process at a larger scale, do a proper cost analysis, and work closely with regulators. We also need a strong system to track where the raw materials come from, making sure they're sustainable and ethically sourced, as discussed in Section 10.4 [4,9]. Early teamwork between scientists, local businesses that process rays, and government agencies will probably be key to handling the technical, financial, and regulatory steps needed to get stingray liver HA ready for the market.

CONCLUSIONS

Stingray liver could be a good source for getting high-molecular-weight hyaluronic acid, a substance that's not being used much right now. It offers another way to get this material, which is usually taken from rooster combs or made through microbial methods. This could be a more sustainable option and also help deal with waste from ray-fishing. The research shows that using

enzymes like papain can effectively extract hyaluronic acid from stingray liver. The results are real, with high molecular weight and strong biological activity. It has antioxidant and anti-cancer properties, making it useful for things like wound healing, tissue repair, drug delivery, eye treatments, joint care, and beauty products.

To fully use this resource in commercial and medical settings, we need progress in several areas. We need better, eco-friendly ways to extract materials on a large scale. We also need processes that can recover HA along with other valuable parts of the liver, like fats. Quality control must be strict to make sure each batch is reliable, like what's needed for medicines. Plus, we need to work with regulators to create a clear path for getting approval for HA made from rays. If these issues are solved through teamwork between researchers and companies, using stingray livers could help the blue and circular bioeconomy. It could also reduce fishery waste, add more options for medical materials, and support sustainable growth in regions that process rays.

Additional tables

Table:3 Reported marine sources of hyaluronic acid and glycosaminoglycans

Marine source/Tissue	GAG(s)Reported	Reference
Stingray liver (<i>Aetobatus narinari</i>)	Hyaluronic acid	Sadhasivam et al. [17]
Tuna eyeball	Hyaluronic acid	Cited in extraction review [1]
Shark cartilage	Chondroitin sulfate	Vázquez et al. [15]
Swordfish, fish skins/heads	HA, chondroitin/dermatan sulfate	Hyaluronic Acid Extraction Methods review [4]
Fish eyeballs (general)	HA, chondroitin sulfate	Fish byproducts as GAG source [5]



Table:4 Research gaps and future opportunities in stingray liver valorization.

Gap	Current Limitation	Future Opportunity
Process scale-up	Lab-scale enzymatic protocols only	Pilot/industrial biorefinery integration [6,9]
Standardization	Batch variability in MW and purity	Standardized SOPs and quality benchmarks [4,17]
Sustainability data	Limited LCA on stingray HA recovery	Comprehensive life-cycle assessment [3,7]
Co-product recovery	Liver oil and HA often studied separately	Integrated multi-product biorefinery [6,9]
Regulatory pathway	No established pharmacopeial monograph for ray-derived HA	Engagement with regulatory bodies for approval pathway [4,5]

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