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Review

Diving into Fish Valorisation: Review Opportunities and Analyzing Azorean Fish Data

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Abstract: In response to the exponential growth in world population, there has been a striking surge in the volume of discarded fish worldwide. This surge is particularly evident in the fish processing industry, where a substantial amount of waste is generated, posing significant environmental concerns. Consequently, the repurposing and utilisation of these waste materials have emerged as pivotal processes for the preservation of marine resources. By employing innovative strategies, valuable products can be extracted from these fish by-products, offering not only economic advantages but also contributing to mitigating environmental impacts. This comprehensive literature review focuses on exploring diverse avenues for using fish waste and extracting high-value materials such as bioactive peptides, collagen, and enzymes, elucidating their potential applications across various industries. The literature review also demonstrates the possibility of extracting various bio-compounds from highly diverse fish waste. It has been observed that there is a need for optimisation of extraction protocols, as the variation in extraction methods and respective conditions significantly affects the extraction yields of the products. Moreover, considering our specific interest in the fish species endemic to The Azores, a meticulous characterisation will be conducted, as there is limited knowledge about waste utilisation processes specific to this archipelago.

Keywords: fish waste; fish by-product valorisation; sustainable marine sources; the Azores; circular economy; blue economy

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1. Introduction

The global population, surpassing 8 billion, poses a significant challenge for humanity to ensure both sustenance and livelihoods. Simultaneously, concerns about climate change and environmental degradation have heightened as natural resources are being depleted at alarming rates. The Food and Agriculture Organisation (FAO) reports that fish accounts for approximately 17% of animal protein consumption and 6.5% of human protein consumption. Additionally, the FAO states that fisheries and aquaculture products contribute around 20% of animal protein intake [1]. The consumption of fish and fishery products is driven by their high nutritional value, as they are rich sources of essential amino acids and Omega-3 polyunsaturated fatty acids, which have been linked to various health benefits, including cardiovascular protection. Additionally, fish is low in cholesterol and saturated fatty acids, reinforcing the importance of eating this type of protein [2]. According to data provided by FAO, fish production corresponds to around 171 million tons, with aquaculture representing about 47% of the total produced [1]. Aquaculture is one of the sectors of animal production that is exponentially growing. This growth is mainly due to increasing demand for this type of food by consumers. In 1961, t per capita fish products consumption was around 9 kg, while in 2018 this value rose to around 20.5 kg [3]. The increase in fishery production has led to a corresponding rise in waste generation. Based on FAO data from 2018, it is estimated that over 20 million tons of fishery by-products such as red meat, skin, head, bones and fins, offal, shavings, and scales were generated [3]. The management of this waste falls under the purview of the Commission decision 2014/955/EU-European Waste List, specifically, chapter 02, which encompasses wastes from agriculture, horticulture, aquaculture, forestry, hunting, food preparation, and processing [4]. Given the waste management hierarchy and the potential value of these by-products, their valorisation should be prioritised. Consequently, this literature review will primarily focus on exploring the key methods of valorizing fish waste and extracting biomaterials from it. Furthermore, considering our particular interest in the fish species of The Azores, a careful characterisation will be carried out, and since to our knowledge waste valorisation processes of fish waste are very scarce at the Azorean archipelago, we will also analyse the potential for implementation of identified valorisation routes.

1.1. Blue Economy

The way humanity interacts with the oceans and utilises its resources has undergone significant changes over time. The oceans have emerged as an indispensable source of food, energy, and valuable products, including medicines and enzymes. Concurrently, our understanding of total non-marketable marine goods and services has expanded. Society is now more attuned than ever to the ocean's finite nature, realizing that increasing cumulative human impacts hinder regeneration and impede sustainable economic growth. Undoubtedly, the oceans and seas play an undeniable role in sustainable development. They actively contribute to poverty eradication by creating sustainable livelihoods, managing food and mineral resources, generating oxygen, absorbing greenhouse gases, and mitigating the effects of climate change. Additionally, with approximately 80% of global trade volume traversing the seas, they serve as crucial connections in global supply chains, ensuring market access for all nations.

The concept of the Blue Economy encompasses the vast wealth potential retained within the oceans. It revolves around the responsible and efficient use of natural resources, resulting in no harm to ecosystems. This approach integrates present and future utilisation of ocean resources with regional considerations, industry needs, and societal well-being. By aligning interests and reconciling expectations, the Blue Economy contributes to the sustainable development of communities. Nevertheless, the blue economy's potential faces various challenges. Throughout history, aquatic ecosystems have been regarded as boundless resources and dumping grounds for waste. However, it is increasingly evident that these resources are finite, and the world is witnessing the consequences of this unsustainable mindset. Blue Economy emphasises inclusive and environmentally sound economic growth that safeguards natural resources from depletion.

1.2. Fish Industry in Portugal

Portugal is situated in the westernmost region of Europe, along its west coast, boasting a coastline spanning approximately 1214 km. Notably, Portugal ranks among the countries with the highest per capita fish consumption globally, making fishing a significant economic activity within the nation [1]. The Direction of Sea Policy (Direção-Geral da Política do Mar-DGPM) leads the development, assessment, and periodic updating of the National Strategy for the Sea. Furthermore, the organisation assumes responsibility for fostering both national and international cooperation in maritime affairs [2]. As reported in the 2021 Fishery Statistics compendium by Portugal Statistics and the Directorate-General for Natural Resources, along with Safety and Maritime Services (DGRM), overall fish capture in Portugal was 185,417 tons, corresponding to an increment of 13.2% relative to 2020 [3]. The main species caught were sardines (26,697 tons), mackerel (16,634 tons), tuna (11,781 tons), and anchovy (9630 tons) [3]. On the other hand, concerning data from the latest Monthly Bulletin of Agriculture and Fisheries (April 2023) produced by Statistics Portugal, the volume of catches decreased by 13.87% in 2022 compared to 2021 [4].

Regarding the Fish catches in the Azores amounted to 10,201 tonnes in 2022, showing a decrease of 13.93% in overall volume compared to 2021. However, the profitability in 2022 increased by 9.25% compared to the previous year [4]. More than half of the catches correspond to tuna specimens. However, as seen in the rest of the country, tuna fish catches showed a significant nominal decrease (27.76%) regarding 2021 values. Nevertheless, their profitability only decreased by 6.52% which demonstrates the increment in the tuna price [4].

1.3. Environmental Impacts Associated with Fish Industry

Extensive descriptions and reviews have been conducted regarding the environmental effects stemming from fisheries. Fishery activities can have adverse effects on marine populations, diminishing their abundance and spawning potential, thereby inducing changes in population parameters such as growth and maturation [5]. Effective regulation of fisheries is imperative due to the direct ecosystem, social, and economic consequences of overfishing. Species such as tuna and cod, which enjoy high demand for consumption, as well as long-lived and slow-to-mature species such as sharks and deep-sea fish, are particularly susceptible to overfishing, leading to a significant reduction in their numbers within the ocean [6]. Furthermore, fishing operations often unintentionally capture non-target species that hold no economic value, either due to their small size or lack of commercial popularity. This lack of selectivity in fishing practices results in the unnecessary loss of individual animals, exerting negative impacts on the marine food chain. These discards of fish in the ocean can occur even when fishermen adhere to catch licenses for specific species [7]. The use of fishing gear can bring about enduring changes in living and non-living ecosystems. Inadequate gear, such as trawls, can inflict damage on delicate coral reefs [8].

To minimise these practices, the European Parliament and the Council of the European Union (EU) established Regulation nº 1380/2013 of the European Parliament and of the Council of 11 December 2013. In this, the Regulation is settled as a system for the conservation and exploitation of fisheries resources under the Common Fisheries Policy (CFP. The "landing obligation" is a ban on discarding unwanted catches at sea. Nevertheless, this obligation, introduced in 2015, is in force since 2019. This obligation aims the fisher sensibilisation to fish more selectively and to minimise unwanted catches [9].

Additionally, the deployment of toxic substances in fishing, as exemplified in the Philippines by using sodium cyanide to capture tropical fishes for the aquarium trade, leading to the destruction of coral reefs and the decline of the overall fish food chain. Although many of these practices are officially prohibited, they persist due to limited alternative livelihood options for individuals engaged in these activities [6]. In 2009, Europe embraced the "Green Paper" to initiate a reform of the Common Fisheries Policy (CFP) aimed at defining objectives for ecological, economic, and social sustainability. This "Green Paper" seeks to offer short-term guidance while ensuring the long-term viability and environmental sustainability of fisheries. The Common Fisheries Policy (CFP) presents the possibility of curbing overfishing while providing a viable and ecologically sound alternative for the industry [6,10].

2. Gifts from the Sea: Azorean Fish

The Azores archipelago comprises nine volcanic islands and it is located about 1500 km from Portugal's mainland. According to Silva and Pinheiro (2007), these islands are considered an "oceanic seamount ecosystem area" [11,12] Seamounts exhibit significant biodiversity, attracting numerous marine species that gather densely for spawning or feeding purposes. They also serve as crucial sanctuaries for various deep-sea organisms [12]. In the Azores archipelago, fishing stands out as the primary maritime activity, along with agriculture, comprising the most influential economic sectors in the region [13]. Therefore, Table 1 described the main fish captured and associated profits. The data described in Table 1 showed that the main fishes caught in the Azorean Ocean, in the period 2017–2020, are tuna species. However, the fishes caught from the *Pagellus bogaraveo* and the *Beryx decadactylus* species are mainly from the Azores, corresponding to 92% and 89% of the specimens caught at a national level in 2020.

Table 1. Nominal catches by species in the Azores [13–16].

Main Species	2017				2018				2019				2020			
	Portugal		Azores		Portugal		Azores		Portugal		Azores		Portugal		Azores	
	t	1000€	t	1000€	t	1000€	t	1000€	t	1000€	t	1000€	t	1000€	t	1000€
Marine Fish	99,834	191,800	6048	26,572	107,996	191,107	11,204	32,280	119,534	201,760	6960	23,788	92,606	179,629	6890	24,266
Fork-beard fish (*Phycis phycis*)	309	1374	103	576	282	1298	97	579	289	1294	98	580	325	1373	129	671
Megrim (*Lepidorhombus whiffiagonis*)	160	451	ə	ə	138	387	ə	2	124	369	ə	1	151	411	ə	2
Bigeye tuna (*Thunnus obesus*), blue fin tuna (*Thunnus thynnus thynnus*), bullet tuna (*Auxis rochei rochei*), skipjack tuna (*Katsuwonus pelamis*), yellowfin tuna (*Thunnus albacares*)	8236	21,845	2052	4299	13,229	27,481	7335	12,309	9966	25,491	3390	5440	6822	19,231	3507	6942
Badejo (*Mycteroperca fusca*)	19	108	2	14	16	91	2	9	18	72	1	8	21	84	1	6
Axillary sea-bream (*Pagellus acarne*)	596	2774	37	167	672	2956	36	108	533	2838	14	63	486	2308	22	92
Bogue (*Boops boops*)	605	171	64	45	604	144	81	37	368	92	15	8	341	80	13	8
Tope (*Galeorhinus galeus*)	131	416	75	174	101	321	41	81	88	292	27	50	93	310	21	42
Offshore rockfish (*Pontinus kuhlii*)	600	3149	374	2207	502	2826	312	1962	374	2327	214	1588	339	1934	169	1191
Blue jack mackerel (*Trachurus picturatus*)	4573	3128	602	1318	3738	2920	848	1478	3635	2677	1040	1336	3472	2853	854	1352
Chub mackerel (*Scomber japonicus*)	19,482	8282	197	305	33,564	10,401	202	267	46,314	17,878	227	304	23,666	9348	299	394
Wreck-fish (*Polyprion americanus*)	215	3674	128	1975	174	3223	89	1477	157	3134	80	1423	172	2927	81	1201
Conger eel (*Conger conger*)	1302	3422	318	748	1012	2930	211	566	975	2854	173	517	1001	2666	163	440
Dory (*Zeus faber*)	352	4132	19	232	328	4052	10	138	384	4469	4	46	359	4087	4	53
Blacktail comber (*Serranus atricauda*)	80	435	76	408	69	367	62	324	38	253	31	209	29	200	25	176
Red sea-bream (*Pagellus bogaraveo*)	568	8006	499	7030	504	7458	446	6449	510	7197	473	6550	534	7054	491	6328
Red bream (*Beryx decadactylus*)	169	1420	149	1152	179	2003	157	1655	148	1785	138	1598	156	2220	139	1966
Red porgy (*Pagrus pagrus*)	277	3764	131	1510	239	3012	83	919	179	2355	39	478	152	1979	41	467
Scabbardfish (*Lepidopus caudatus*)	152	796	100	367	98	517	73	283	104	497	65	170	171	812	88	181
Black scabbard fish (*Aphanopus carbo*)	4342	14,053	63	205	3940	13,972	14	47	4565	15,450	17	51	4505	14,740	ə	ə
Blue ling (*Molva macrophthalma*)	1494	4895	10	44	1499	4267	11	47	1917	4906	10	51	1902	5627	9	33

Table 1. *Cont.*

Main Species	2017				2018				2019				2020			
	Portugal		Azores		Portugal		Azores		Portugal		Azores		Portugal		Azores	
	t	1000€	t	1000€	t	1000€	t	1000€	t	1000€	t	1000€	t	1000€	t	1000€
Skate (*Raja (Dipturus) batis*), painted ray (*Raja (Raja) microocellata*), long-nosed skate (*Raja (Dipturus) oxyrinchus*)	1213	3019	69	110	1167	3139	60	95	1175	3144	41	74	1289	3198	60	85
Red gurnard (*Aspitrigla cuculus*)	346	651	1	1	322	604	1	1	334	609	ə	ə	341	594	ə	ə
Salema (*Sarpa salpa*)	263	155	6	5	252	136	6	4	171	96	2	2	297	123	1	1
Red stripped mullet (*Mullus surmuletus*)	171	2371	10	117	180	2604	14	151	175	2759	8	103	152	2371	6	58
Sardine (*Sardina pilchardus*)	14,557	23,868	32	73	9694	21,873	25	39	9700	19,039	22	37	14,526	22,087	22	39
White sea bream (*Diplodus sargus cadenati*)	921	4120	70	245	809	3411	79	238	767	3446	49	149	684	3027	43	127
Thick-lipped grey mullet (*Chelon labrosus*)	280	386	27	68	530	491	29	63	342	469	16	50	289	433	13	38
Monkfish (*Lophius piscatorius*)	544	3196	6	11	341	2265	4	12	308	2071	3	9	604	3671	2	5
Atlantic pomfret (*Brama brama*)	2	6	ə	1	2	5	ə	1	6	18	ə	1	1	4	ə	1

t (ton); 1000€(Economical profitability generated in thousands of euros with the fish tons captured).

Furthermore, although the species of Blue jack mackerel (*Trachurus picturatus*) and Chub mackerel (*Scomber japonicus*) correspond to the second and fourth highest fish catches at a national level, they are not the most relevant in terms of the Azores, corresponding in the Azores to 25.6% and 1.26% regarding the total national catch of these species.

Moreover, it is possible to conclude that the most economically profitable fish in Moreover, it is possible to conclude that the most economically profitable fishes in the Azores were the tuna species the Red sea bream (*Pagellus bogaraveo*), and Red bream (*Beryx decadactylus*). Furthermore, although few specimens were caught, Wreck-fish *(Polyprion americanus)* was the third most economically profitable fish species.

3. Fish Waste Valorisation

The issue of waste and its management holds significant social implications that affect society. Therefore, there is a pressing need for improved fish waste management to address environmental concerns and harness the full potential of fish by-products, which hold considerable commercial value. In last years, an increasing interest in exploring alternative uses for fish by-products raised, contributing to economic growth and sustainable development. The Council of the European Communities defined waste in 1975 as "any substance or object that the holder disposes of or is required to dispose of according to national law" [14]. This directive recognised the importance of adopting techniques for waste recovery, reuse, and recycling. The process of fish processing involves several steps, such as grading, removing slime, stunning and de-heading, washing, scaling, fin cutting, gutting, filleting, and separating meat from bones. The quantity of waste produced varies based on the level of processing and the type of fish, ranging from 20% to 80% of the overall volume [15]. It is crucial to recognise that the residual biomass should not be considered waste, but rather raw material or valuable by-products. Embracing sustainability in its entirety is of paramount importance, as it carries moral and economic implications.

3.1. Fishery Environmental Impact

Marine-derived by-products contain valuable components such as proteins, lipids, enzymes, pigments, minerals, vitamins, and more. The proportion of these by-products varies among different fish species due to variations in processing yields. For instance, canned and loin products, which primarily utilise light muscle, result in approximately 60–70% of the generated by-products [2]. When it comes to protein-rich by-products, they encompass various parts such as cut-offs, heads and backbones, skin, stomachs, viscera, roe, and blood. The quantities, chemical composition, and properties of the protein and lipid fractions in these by-products depend on factors such as species, season, and fishing location (see Figure 1). This information is crucial for the industry to effectively utilise these by-products. Fish roe, for example, is a protein-rich component of the by-products, constituting approximately 16–30% of their composition. In contrast, the backbone, which accounts for about one-third of the dry weight, is primarily composed of minerals (60–70%) and proteins (30%).

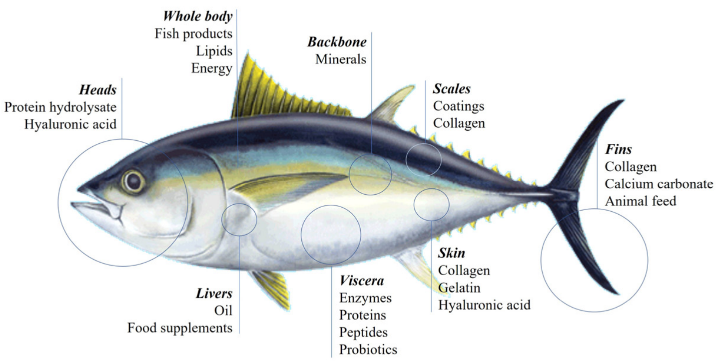

Figure 1. Fish by-products.

3.2. Biomass Valorisation

Fish waste undergoes various utilisation methods, including its conversion into fishmeal or fertilisers, as well as its direct incorporation as raw material for aquaculture feed [16,17] Regarding human food applications, fish discards can also be valorised through surimi production [18]. For instance, surimi refers to deboned, minced, and washed fish meat, serving as a range of seafood's key ingredients [19]. It is utilised to create imitation seafood items such as crabsticks, chunks, and flakes. Furthermore, surimi has shown the potential in maximising the utilisation of less popular and underutilised fish species for human consumption improvement [20].

Biomass-fish valorisation also includes the production of fish meals and silage, which have emerged as cost-effective and valuable options. Fish silage involves the conversion of fish by-catch and processing by-products into a liquid mixture of nutrients composed of lipids, hydrolyzed proteins, and minerals. It possesses excellent digestibility and absorption properties for both terrestrial and aquatic animals. Notably, fish silage enables the recovery of fish biomass, resulting in a low-cost, highly nutritious product with long-term storage capabilities. Additionally, fish silage can serve as a natural fertiliser for crop cultivation [21–23]. Some authors, such as Kuley (2020), have studied the employment of specific bacteria strains to silage to improve organic acids in fish-based silage [24].

3.3. High-Added Value Biomaterials

Fish processing involves various operations, resulting in the generation of by-products including offal, heads, roe, and shells. These surplus materials, whether edible or non-edible, are considered by-products [23,25]. In recent years, numerous studies have focused on utilizing compounds derived from fishery industry by-products. These biomaterials have found applications in diverse sectors such as functional foods, pharmaceuticals, nutraceuticals, biomedicals, livestock, aquaculture feed, agriculture, biodiesel, and other chemicals [23]. The literature emphasises that significant investments have been made in producing items for human consumption, particularly in the extraction and purification of bioactive peptides, enzymes, and biopolymers for biotechnological or pharmaceutical purposes, as they offer high profitability [26]. This approach aligns with the principles of the circular economy by not only providing marketable products but also promoting sustainability within the aquaculture and fishing industries. By reducing the impact of human exploitation on marine resources and preserving coastal environments where these activities are concentrated, this approach contributes to a more sustainable future [27]. Consequently, it is crucial for the fishing and food processing industry to establish comprehensive strategies for utilizing captured and processed waste to develop new products [23]. Recent research has focused on exploring various biomaterials from seafood by-products, including proteins, lipids, chitin/chitosan, derivatives, minerals, enzymes, pigments, and aromatic compounds, with the aim of harnessing their potential for producing valuable products [23].

3.3.1. Hydroxyapatite

Hydroxyapatite, having the chemical formula $Ca_{10}(PO_4)6(OH)_2$, is highly regarded as a material for biomedical implants used in bone filling. This is not only due to its chemical similarity to bone tissue but also because of its bioactivity, biocompatibility, high osteoconductivity, and non-toxic osteoactivity [27].

Synthetic hydroxyapatite, with its chemical formula and properties resembling the main inorganic component of bones and teeth, has found wide application as a biomaterial in orthopedic and dental fields. It serves to repair or replace hard tissues and deliver drugs. Hydroxyapatite-based biomaterials have been extensively studied for creating artificial bone grafts, either solely composed of hydroxyapatite or as surface coatings [28]. Fish bones, containing approximately 70% inorganic matter consisting mostly of minerals and hydroxyapatite, are a significant source of calcium. The extraction of hydroxyapatite from fish bone waste typically involves thermal extraction, which includes removing residual proteins, drying, and high-temperature calcination. The proportions of hydroxyapatite and b-tricalcium phosphate (b-TCP) produced depend on the specific fish bone characteristics and process temperature [29].

3.3.2. Fish Protein Hydrolysate

In recent years, there has been significant research on improving the production of fish protein hydrolysates from fish industry wastes. This involves converting fish waste into peptides containing 2 to 20 amino acids. These studies have highlighted the relevance of these hydrolysates as functional ingredients in dietary supplements due to their various biological activities, such as antihypertensive, antioxidant, antimicrobial, immunomodulatory, and anticancer effects. Commercial nutraceuticals based on fish hydrolysate are available, aiming to support muscle and vascular functions, reduce blood pressure, and manage weight disorders [30]. Large-scale production of fish hydrolysate involves chemical and biological processes. While chemical methods are cost-effective and easy to implement, they often result in peptide mixtures with reduced nutritional quality. Enzymatic hydrolysis is considered the most viable strategy for obtaining food-grade protein hydrolysates with bioactive properties. During the enzymatic hydrolysis process, the raw material is heated to inactivate endogenous enzymes. Exogenous enzymes are carefully selected and added to optimise the cleavage of proteins into peptides. After reaching the desired hydrolysis level, the enzymatic reaction is halted by heating or acidification. The resulting mixture is then separated into different fractions through centrifugation. Purification techniques such as ultrafiltration, gel filtration, ion exchange chromatography, and HPLC are crucial for improving the quality and biological activity of the peptides for commercial use. These steps ensure the removal of impurities and enhance the overall efficacy of the fish hydrolysate as a source of bioactive compounds [31,32].

3.3.3. Collagen and Gelatin

Collagen, which constitutes a significant proportion of the body's dry weight, is the primary protein present in the extracellular matrix of tissues. Tropocollagen serves as the fundamental unit of collagen and possesses a helical structure. It consists of three α chains that repeat a characteristic chain motif (Gly-X-Y)n, where X and Y are commonly occupied by proline and hydroxyproline, respectively. The self-assembly of these triple helices leads to the formation of collagen fibrils [23,27].

Marine organisms offer a secure and convenient source of high-quality collagen, especially when compared to collagen derived from land animals. Various marine species such as crustaceans, mollusks, annelids, and different parts of fish such as their skin, scales, bones, and fins have been extensively studied as alternative collagen sources [23,33]. Consequently, researchers have explored the utilisation of bio-waste, particularly from the organic fraction of fish waste, to discover additional raw materials for collagen production [27]. Gelatin, which is derived from collagen, a fibrous protein, is a heterogeneous mixture of water-soluble proteins with a high molecular weight. The global demand for gelatin has been on the rise in the past decade. Similar to collagen, gelatin sourced from marine animals is considered a viable substitute for mammalian-derived gelatin due to its ability to address concerns related to religious perspectives, safety, and stability of use [34].

Extraction Process

Different techniques can be employed for collagen extraction from marine resources. However, the general procedure for isolating collagen involves three main steps: preparation, extraction, and recovery [35]. During the first phase, the initial by-product is cleaned, and size reduction techniques such as cutting or chopping are applied to facilitate subsequent pre-treatment. Chemical pre-treatment is then carried out to enhance extraction efficiency and remove other substances. Depending on the raw material and extraction method, various pre-treatments can be employed, including acid and/or alkaline treatments, which involve partial hydrolysis while preserving the integrity of collagen chains [36]. In the acid pre-treatment, the solution penetrated the collagen structure allowing the expansion from two to three times its initial volume, leading to cleavage of non-covalent intra and intermolecular bonds [35]. Alkaline pre-treatment, typically utilizing sodium hydroxide and calcium hydroxide, is preferred due to its greater swelling capacity and improved extraction efficiency [37]. Demineralisation of raw materials using EDTA or hydrochloric acid is necessary before the extraction phase, particularly for collagen extraction from mineral-rich fractions such as bone, cartilage, and scales [38]. To solubilise collagen proteins and isolate them, specific techniques are required as collagen fibers exist in a triple helix structure with stable hydrogen bonds, making them insoluble in water [39,40]. Common methods for collagen extraction from fish by-products include acid solubilisation, pepsin solubilisation, deep eutectic solvents, and supercritical fluid extraction [39,40]. Parameters including time, temperature, and solvent concentration greatly influence the extraction yield and need to be carefully optimised [35]. Gelatin, a soluble form of collagen, can be obtained by heating collagen in an acidic or alkaline solution or through enzymatic hydrolysis. Enzymatic hydrolysis enhances the degradation of gelatin into smaller peptides, while heat treatment disrupts the collagen's triple helix configuration, converting it into a coiled conformation and facilitating solubility [34]. Thus, hot water is the general procedure used to solubilise collagen and extract gelatin [23].

Applications

The use of collagen/gelatin is increasing in various fields, such as pharmaceutical, biomedical, cosmetic, and food [23,41]. As mentioned earlier, recent studies have indicated marine organisms as the most beneficial and safest source for obtaining high-quality collagen. Additionally, it has great potential for applications in biomaterials, due to its low risk with regard to biological toxins, and a good absorption capacity [23,42]. Thus, there is no known risk of disease transmission, and there is also a risk of minimal inflammatory responses. In addition, there is no religious or ethical restriction on its potential application in any area. Marine collagen can be used in several applications compared to other sources of collagen. Regarding the biomedical area, specifically tissue engineering, collagen of marine origin already exceeds collagen of origin in mammalian animals [23,43]. Collagen has been extensively used for cosmetic formulations, skin repair, and regeneration [27]. Regarding gelatin, it can be used as a food emulsifier, edible film, thickener, stabiliser, and foaming agent, in the preparation of medical and pharmaceutical products, since its low gelation temperatures offer new areas of potential applications. One of the main applications of fish gelatin is in the microencapsulation of pharmaceutical additives, namely vitamins. The use of fish gelatin soft capsules is quite common in nutritional supplements [23].

3.3.4. Fish Oil

Fish oils have gained significant popularity in the field of nutrition due to their abundant levels of long-chain polyunsaturated omega-3 fatty acids, which have been recognised for their positive impact on human health for over four decades. As a result, they continue to attract commercial interest and are the subject of numerous studies. The primary omega-3 fatty acids of utmost importance are docosahexaenoic acid and eicosapentaenoic acid, which are initially synthesised by microalgae and subsequently accumulated in phytoplankton, forming part of the fish's diet [27]. The diagram below illustrates the structure of these key omega-3 fatty acids. Furthermore, seafood, in addition to fish oil, possesses a favourable lipid composition that varies based on the specific dietary intake of phytoplankton or zooplankton [44]. Marine fish oil derived from by-products demonstrates significant market potential, especially when prepared using molecular distillation, enzymatic processes, or other specialised methods developed for the purification of end products [45].

Extraction Process

The process of extracting and purifying fish oil involves several steps. Initially, the oil is separated from a protein-rich solid waste through cooking and pressing. Cooking breaks down the fat cells and facilitates the release of oil, typically carried out at temperatures between 95–100 °C for a duration of 15–30 min. The resulting suspension is then pressed to extract the liquid from the sludge. The recovered water/oil emulsion is further separated through centrifugation to obtain the oil while removing the water. The subsequent stages of refinement include degumming, neutralisation, bleaching, and deodorisation. Crude fish oil contains gums primarily composed of phospholipids that act as emulsifiers and increase viscosity. Degumming is performed by mixing the fish oil with acids such as phosphoric, acetic, citric, or oxalic acid at temperatures around 60–70 °C. This step protects the gums and helps remove bivalent metals by generating water-soluble species that can be washed away [46]. Neutralisation follows degumming and aims to remove acidity and free fatty acids present in crude fish oil. It involves mixing the oil with alkaline solutions, such as approximately 10% aqueous sodium hydroxide, at temperatures of 40–60 °C [47]. The next stage, bleaching, aims to produce a light-coloured oil by eliminating pigments, traces of metals, and other contaminants through adsorption [46]. Fuller's earth, activated carbon, and synthetic silica are commonly used for this purpose. During the refinement process, there is a risk of undesirable changes in flavor quality due to the presence of secondary lipid oxidation products [48]. Deodorisation, a crucial step, involves vacuum distillation of the oil at 5–50 mmHg and carefully controlling parameters such as temperature and time (<200 °C, 1 h) to prevent degradation of polyunsaturated fatty acids [49]. To produce docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) on an industrial scale with concentrations exceeding 95%, the omega-3 fish oil industry utilises extraction technologies such as molecular distillation, supercritical fluids, and supercritical fluid chromatography. Other ways to recover fish oil can be obtained from industrial by-products using various techniques including chemical, enzymatic, and supercritical extractions. Chemical methods involve solvent-based protocols such as the Soxhlet method, the Folch method, the Bligh & Dryer method, and acid digestion. Nonpolar solvents such as petroleum ether, hexane, chloroform, or mixtures of polar and nonpolar compounds are used. Enzymatic procedures utilise enzymes such as Alcalase, Neutrase, Ultra Lecitase, Protex, and Protamex to break down the protein portion of the residue, enabling oil recovery through centrifugation [27].

Applications

Marine oils have diverse applications encompassing food, feed, aquaculture, and nutraceuticals [50]. Fish oil, commonly employed in the food industry, finds its use in edible products such as bakery items. Additionally, marine oils are utilised in the preparation of various food products such as bread, baby food, maternal drinks, margarine, and salad sauces [51]. Notably, fish oils have gained immense popularity in the realm of nutrition, owing to their numerous health benefits. They have garnered commercial interest and have been extensively studied for their efficacy in combating various diseases [50]. The presence of long-chain polyunsaturated fatty acids, particularly omega-3 fatty acids, in fish oils confers valuable disease-fighting properties, such as blood pressure reducer, preventing coronary heart disease, and improving the well-being of cancer patients and individuals with autoimmune conditions [23,52]. In the realm of aquaculture, the incorporation of fish oil into the marine food chain offers a valuable means of increasing the omega-3 levels in the diet of fish species, which is significant [23]. Furthermore, the production of biodiesel represents an excellent application for fish oil. Recent research has explored the feasibility and performance of biodiesel derived from fish oil, particularly from fish waste sources [53]. Notably, successful experiments involving salmon oil have been conducted by the Marine Institute of Memorial University of Newfoundland, Canada [54]. This conversion of fish oil from discarded fish into an affordable and environmentally friendly fuel source has the potential to enhance air quality and reduce dependency on imported fuels [53]. Fish oil, enriched with polyunsaturated fatty acids (PUFAs), exhibits improved fluidity and flow characteristics, particularly at lower operating temperatures [53]. Therefore, utilizing fish processing by-products for bioenergy production presents an effective solution to address environmental concerns associated with fish waste, while simultaneously contributing to pollution reduction and energy sustainability [53].

3.3.5. Chitin and Chitosan

Chitosan is a hydrophilic biopolymer produced from the alkaline hydrolysis of chitin. Chitin is a polymer made up of β-(1,4) molecules coupled to d-glucosamine (deacetylated form), randomly arranged between N-acetyl-d-glucosamine molecules (acetylated form) [55]. This biopolymer is a structurally important part of the cell wall of some fungi, the exoskeleton of arthropods and insects, in addition to being part of the constitution of several marine beings, such as crustacean shells, cephalopod molluscs and some fish [56]. Chitin is widely studied due to the versatility of its properties, and having applications in areas such as medicine, pharmacy, agriculture, and environment [55]. Taking as an example, the biocompatibility and antimicrobial and antioxidant properties associated with the biopolymer, allows its use in medicine. The study by Mami et al. (2020) allowed the association of the use of oral therapy with chitin to treat symptoms associated with multiple sclerosis [57]. Chitosan is formed by removing the acetyl groups from chitin, which results in a biopolymer soluble in most acids. However, during the deacetylation process, the release of acetyl groups (-$COCH_3$) occurs, giving chitosan cationic properties [56]. Currently, the largest source of chitin production comes from residues from the fishing industry, namely from the handling of crustacean shell waste, such as shrimp and crab shells [58]. Seashells are made up mostly of chitin, minerals, and proteins. While chitin serves as a skeleton for organisms, minerals make the shells robust. In turn, the protein part of the shells results from a complex with chitin [59]. Regarding the extraction of chitin from residues from the fishing industry, it is first necessary to remove the mineral and protein fraction from the organisms. Then, chemical or biological methodologies can be adapted to extract chitin and process it in order to obtain chitosan [60].

Chitin Extraction and Purification

The chitin extraction and purification processes are fundamental for the enhancement of this polysaccharide, in addition to determining the efficiency of chitin extraction that can be extracted from each source. Thus, chitin extraction can be carried out according to two different approaches, chemical and biological. The chemical process of chitin extraction comprises two initial stages, namely the treatment of the material with acid to proceed to demineralisation and the treatment based on deproteinisation [61]. Subsequently, a bleaching and deacetylation step of the material must be added to obtain chitosan [27]. As previously mentioned, the traditional chemical extraction process begins with the treatment of the residue with hydrochloric acid (HCl) followed by sodium hydroxide (NaOH) treatment. The entire process must be conducted at a controlled temperature, 60–90 °C, to avoid the degradation of chitin [61]. As observed in the case studies described in the following table, it is important to notice that each treatment protocol (concentration, time, and temperature) is adapted to each residue. Consequently, for residues with a high protein and mineral content, such as seafood, the acidic and basic treatment must be adjusted, in order to eliminate this source of contamination, to obtain a final product, chitosan, crystalline [60]. However, despite being the conventional extractive process, it generates the production of chemically concentrated effluents, which require neutralisation and detoxification treatment [62]. In addition, the use of strong chemical agents makes the process more expensive and can contaminate the final product, reducing its purity [63]. In contrast, the biological process of chitin extraction is an environmentally more sustainable and economical method that uses specific microorganisms to produce enzymes and organic acids capable of mimicking the chemical process of deproteinisation and demineralisation [61,63]. This type of methodology, in addition to generating high-quality products, also has the advantage of using low-cost production materials. Additionally, they are more environmentally sustainable extraction methods, since they do not form such toxic residues, compared to the chemical process [61,63]. The main biological methods of chitin extraction include enzymatic deproteinisation and fermentation. Enzymatic deproteinisation is a “green” method that uses proteolytic enzymes, such as proteases to promote the deproteinisation of waste from the fishing industry during the chitin extraction process. There are several sources of production of these proteases, such as plants, animals, and some microorganisms. However, some of these proteases need to be purified before the deproteinisation step, which makes the process a little more expensive. However, this method remains energetically more advantageous than the chemical process [60]. Although the use of proteolytic enzymes makes the process more environmentally sustainable, it has a lower efficiency rate than the chemical method, so it may require an additional treatment step based on eliminating the traces of proteins that may be bound to chitin [63].

In the process of fermentation, specific microorganisms are utilised during the deproteinisation phase to reduce costs associated with purified proteolytic enzymes. Fermentation can be classified into two primary categories: acid-lactic fermentation and non-lactic acid fermentation [63]. Acid-lactic fermentation of crustacean shells involves the use of the strain *Lactobacillus* sp., which produces proteases and lactic acid. Lactic acid is obtained by converting glucose, leading to a decrease in pH and inhibiting the growth of harmful microorganisms. The efficiency of fermentation relies on various factors, including glucose concentration in the medium, the quantity and composition of the microbial inoculum, initial pH, carbon source and concentration during fermentation, and the duration of fermentation time [60]. The significant advantage of this process is the recovery of valuable by-products such as proteins, enzymes, and pigments that can find applications in the food industry. This microbial extraction approach is gaining prominence in biotechnology and bioremediation research [59,62]. On the other hand, non-lactic acid fermentation of crustacean shells can be achieved using fungi and bacteria such as *Bacillus* sp., *Pseudomonas* sp., and *Aspergillus* sp. [63]. Several studies have highlighted the potential of proteolytic enzymes for deproteinizing shellfish residues. Recent research has focused on isolating and identifying various protease-producing bacteria, characterised as metalloenzymes with diverse properties such as solvent, surfactant, and bleach stability. These proteases also demonstrate thermal stability and excellent compatibility with certain commercial liquid detergents. Additionally, fermentation has been shown to be a promising method for chitin production, yielding chitin with superior physicochemical characteristics compared to chemical methods. The recovery of secondary products such as proteins, pigments, and minerals further enhances the economic benefits [59].

Chitin/Chitosan Applications

Several studies analyzed the applications of chitin/chitosan in biomedical, cosmetic, and agriculture, as well as water treatment and food packaging. The following figure (Figure 2) demonstrates how to obtain chitosan and its main areas of application.

Regarding agriculture, chitin can be used as a feed additive, demonstrating a positive effect on the growth and carcass characteristics of chickens [64]. In the cosmetics area, these polymers are frequently used in creams, makeup, lotions, hair products (shampoo, spray, colouring), and toothpaste, among others [63]. In biomedicine, this biopolymer has numerous applications, associated with its antioxidant, antimicrobial, and biocompatibility properties. As an example, the use of chitin was tested in the production of a structural biomaterial capable of helping wound healing, demonstrating its usefulness in the engineering of tissues [65]. Another study allowed associating the use of oral therapy with chitin to treat symptoms associated with multiple sclerosis [57]. In addition to biomedicine, composites based on chitin and chitosan have also been explored in other areas. A mixture of lignin and chitosan was used to promote the removal of the methylene blue dye in water treatment [66]. In turn, other studies tested with success a carboxymethyl chitosan/polyvinyl alcohol crosslinked network to be applied in food packaging films. In this study, researchers found that adding the mixture to the packaging induced an improvement in the mechanical and antimicrobial properties of the product [67].

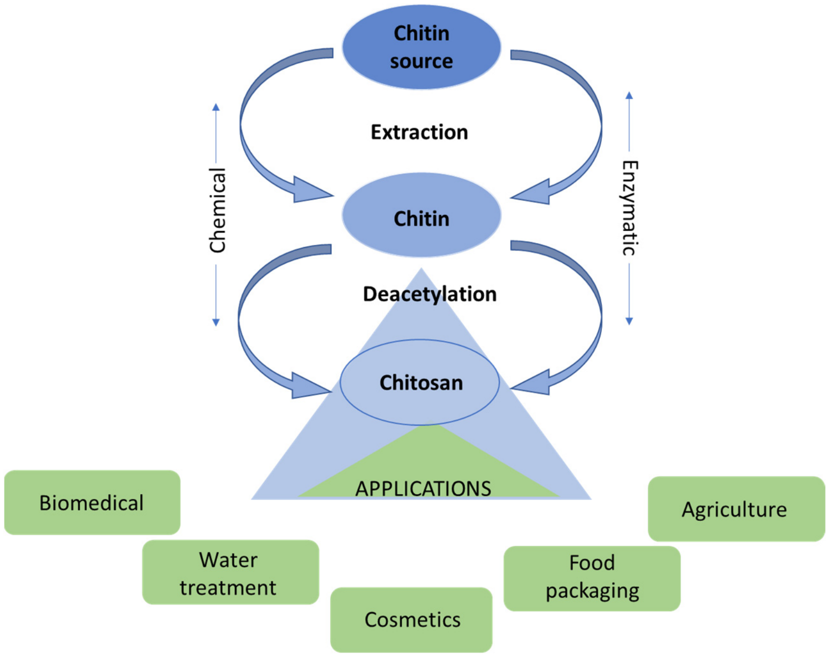

Figure 2. Chitosan production and main applications.

Table 2 presents a summary of the main articles analysed, showing the fish waste used, extraction protocol, and extracted materials.

Table 2. Resume of studies performed on marine biomaterials extraction.

Waste	Biomaterial	Protocol (Briefing)	Reference
Mussel Perna viridis; Tropical oyster Crassostrea iredalei;	Chitin	Chemical • Demineralisation with 1 M HCl for 2 h at 75 °C; • Deproteinisation with 1 M NaOH for 2 h at 80 °C; • Chitin deacetylation (chitosan production) with 50% NaOH solution for 2 h.	[68]
Shrimp, crab, and squid shells	Chitin	Biological • Brevibacillus parabrevis TKU046 bacteria isolation; • Liquid fermentation (bacterial inoculum with 3% marine residue, 0.05% magnesium sulfate heptahydrate, and 0.1% dipotassium phosphate) for 5 days at 37 °C, at 150 rpm;	[55]
Pinna bicolor pen shells	Chitin	Chemical • Pre-treatment with 3% sodium hypochlorite (NaOCl) at 100 °C for 10 min; • Demineralisation with 1 M HCl, at 75 °C, for 15 min; • Deproteinisation with 1 M NaOH, 100 °C, for 20 min; • Storage of the extract for 5 days at 60 °C.	[56]
Cephalopod *Uroteuthis duvauceli*	Chitin	Chemical • Sun bleaching and addition of 1 M NaOH (18 h); • Demineralisation with 1 M HCl, 50 °C, 15 min; • Deproteinisation with 1 M NaOH, at 100 °C, for 20 min.	[69]

Table 2. *Cont.*

Waste	Biomaterial	Protocol (Briefing)	Reference
Shrimp shells	Chitin	Biological • Demineralisation and deproteinisation with fermentation process (Production of lactic acid by the strain *Lactobacillus plantarum subsp. plantarum* and proteolytic enzymes by *Bacillus subtilis subsp. subtilis*); • Fermentation (bacterial inoculum with residue from autoclaved shrimp shells and sterile glucose solution) for 5 days at 30 °C.	[70]
Shrimp shells	Chitin	Chemical • Pre-treatment with boiling and washing the material; • Demineralisation with 3 M HCl and microwave radiation; • Deproteinisation with 5% NaOH, (during time and variable radiation); • Deacetylation of Chitin with NaOH (20–50%) and microwave radiation (90–650 W)	[71]
Turbot fish By-products	Fish hydrolyzate	Alcalase hydrolysis • Hydrolysis of turbot waste in a controlled pH system (NaOH 5 M). • Mix with distilled water and Alcalase (0.2%/*w*/*v*) • Hydrolysis at 60 °C for 3 h and at pH 8.5 • Filtration (100 µm); • Centrifugation (15,000× *g*/20 min) • Protease deactivation was achieved by heating (90 °C/15 min) • Freeze drying	[72]
Tuna By-Products	Fish oil	Enzymatic Extraction • Wet pressing (T = 95 °C, 15 min) and centrifugation. • Enzymatic extraction with alcalase (T = 56 °C, 120 min); • Centrifugation (20 °C, 10 min).	[73]
Salmon By-products	Fish oil	Enzymatic Extraction • Sample preparation; • Mix the sample with the enzyme and distilled water, keeping it at 30 °C; • Incubator digestion (100 rpm 2 h); • Heat treatment to stop digestion (70 °C 10 min); • Centrifugation (3000 rom 30 min); • Oil decantation and storage at −80 °C.	[74]
Tuna By-Products	Fish oil	Supercritical carbon dioxide extraction • A high-pressure pump is used to pump CO_2 into the extraction vessel at the desired pressure; • Conditions: Pressure 25 MPa, Temperature: 40 °C, flow: 10 kg CO_2/h; • Separation and storage at −20 °C, in a dark container with nitrogen.	[75]
Tilapia scales	Hydroxyapatite	• Washing and hydrolysis with 1% NaOH, 2 h; • Washing and sterilisation (121 °C 15 min) • Drying (110 °C, 5 h), calcination (1000 °C, 5 h) and milling;	[76]
Fish scales	Hydroxyapatite	Eutectic solvents • Preparation of the eutectic solvent; • Addition of the sample to the solvent (solid:liquid 1:15 (g/g)); • Reaction: 65 °C for 2 h; • Centrifugation; • Hydroxyapatite remains insoluble and precipitates; • Addition of silver nitrate to obtain the crude extract; • Dissolution with 5% sodium hydroxide 1:5 at 70 °C, 5 h; • Washing and drying.	[77]

Table 2. *Cont.*

Waste	Biomaterial	Protocol (Briefing)	Reference
Flounder fish skin	Collagen	• Pre-treatment with 0.3 M NaOH 1:10 (p/v), 4 h (change solution every 60 min); • Wash with distilled water to neutral pH; • Remove the fat for 30 h, keeping it in 20% butanol at the rate of 1 g in 10 mL (change solution every 10 h) • Washing; • Addition of acetic acid; • Filtration; • Addition of 0.05 M Tris-HCl and NaCl; • Adjust to slightly basic pH with 5 M NaOH; • Centrifugation 130× *g*, 40 min; • Precipitate is dissolved in 5 mL of acetic acid and dialyzed against 1 L of 0.1 M acetic acid for 24 h (10°C); • Subsequently dialyzed in distilled water for another 24 h (10 °C).	[78]
Nile tilapia skin	Collagen	• Stirring for 48 h at 4 °C in an aqueous solution of 0.1 mol/L NaOH, 1:20 (skin: solution); • Washing with distilled water up to pH 7; • Deproteinisation (20% ethanol in water at 1:20 (skin:solution)) and stirring for 24 h at 4 °C; • Extraction: acetic acid at 0.35 mol/L for 65 h at 20 °C; • Purification (addition of NaCl to filtered Collagen, precipitation, and centrifugation): • Resolubilisation in acetic acid 1:5 (skin:solution) and again precipitation and centrifugation; • Dialysis; • Freeze drying.	[79]
Medusa skin	Collagen	• Pre-treatment: washing with demineralised water and cutting into small parts; • Extraction at 4 °C; • Removal of non-collagenous proteins, soaking the sample (10 g) in 0.1 M NaOH (1:30 *w*/*v*) for 36 h; • Washing to remove the alkaline solution; • Deproteinisation suspended in 10% butyl alcohol (1:30 *w*/*v*) for 48 h; • The pretreated skin is suspended in 0.5 M mm of acetic, citric, hydrochloride, lactic, tartaric, formic, and sulfuric acid (1:25 p/v) at 4 °C, for 24, 48, and 72 h; • Centrifugation (10,000× *g*, 4 °C); • Precipitation with 2.0 M NaCl; • Centrifugation (10,000× *g*, 4 °C); • Dissolution in 0.5 M of the acid (1:5 *w*/*v*); • The solution was dialyzed against cold demineralised water for 48 h.	[80]
Atlantic cod skin	Collagen	Supercritical Fluids • Washing the skin with distilled water; • The skin is placed in a high-pressure container (30 cm^3) with distilled water (1 g/20 mL); • Heating to 37 °C, pressurizing with CO^2 up to 50 bar; • Extraction takes place for 3 h; • Fast depressuriastion; • Filtration (2 times).	[81]

3.4. Azorean Fish Waste Potential Valorisation

As mentioned before, the most economically profitable fishes in the Azores in the last four years (2017–2020) belong to the tuna species and the Red sea-bream (*Pagellus bogaraveo*) and Red bream (*Beryx decadactylus*) species. These organisms generate more than 50% of waste, being composed essentially of skin and bones and head and viscera [15]. Considering the main fish waste produced in the Azores and the potential added-value biomaterials mentioned in the previous sub-chapters, Figure 3 presents a summary of the main extraction methods and technologies that can be used in The Azores fish species to obtain high-commercial value compounds from fish by-products.

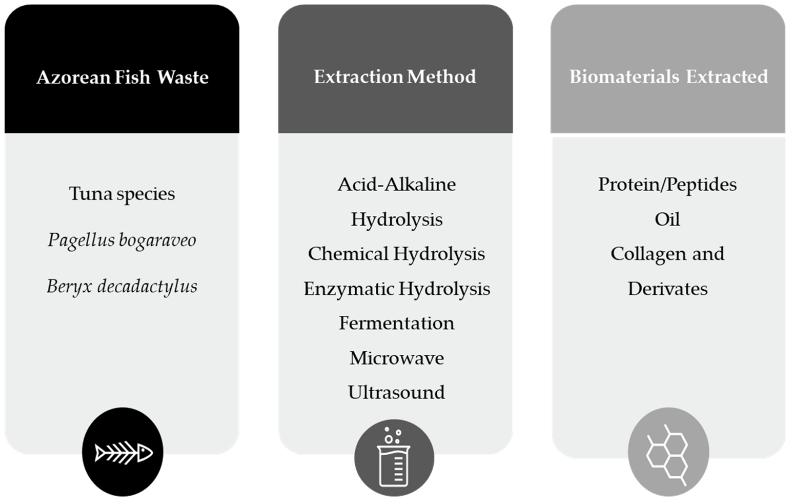

Figure 3. Summary of the main extraction methods and technologies that can be used in The Azores fish species to obtain high-commercial value compounds from fish by-products.

4. Final Remarks

The sustainable management of marine resources is crucial due to their inherent limitations. Recognizing these limitations, coupled with the growing concern over environmental pollution, underscores the urgent need to optimise the utilisation of these resources. Fish waste represents a significant environmental challenge and a substantial economic loss, necessitating improved waste management practices to address these critical issues. This aligns with the objectives outlined in the Waste Framework Directive, which aims to minimise waste generation and promote its reuse, recycling, and recovery as valuable resources. By harnessing fish by-products, it is possible to develop high-value products, thereby contributing to economic growth.

This review highlights the wide range of compounds that can be derived from fish discards and by-products, showcasing the tremendous potential of this waste as a valuable resource for various applications. Analysis of extraction methods reveals that chemical-based processes still dominate the traditional approach to extracting biomaterials, despite the advantages offered by biological methods in terms of reducing toxic waste. However, the lower popularity of biological processes is primarily due to their lower extraction yields. Additionally, this review characterises the economically viable species found in the Azorean Ocean and explores processing technologies that can be employed to obtain high-value compounds from fish by-products in the region. It is worth noting that the literature lacks comprehensive information on the valorisation of fish waste in the Azores, necessitating urgent evaluation of more sustainable processes to achieve more efficient utilisation of local marine resources. Finally, the adoption of circular economy models, particularly within the framework of the blue economy concept, has become a pivotal component of EU directives. This review focuses on the application of these concepts and economic models in the context of marine resources, with specific emphasis on fish waste. By embracing these principles, it is possible to establish a more sustainable and efficient utilisation of marine resources, further contributing to the overall well-being of our ecosystems and economies.

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