Chapter

The Hydrolysates from Fish By-Product, An Opportunity Increasing

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Abstract

The fishery industries have continuously increased over the last decade. This growth comes accompanied by a high volume of by-products released to environment, because these industries discard between 60 and 70% of their production as waste. This waste includes fish whole or part from these such as fillet remains (15–20%), skin and fins (1–3%), bones (9–15%), heads (9–12%), viscera (12–18%) and scales (5%). This by-products are rich in proteins and lipids which of several nature, which can be recovered to obtain compounds of high added value. In this chapter, some methods to recover compounds from fish by-products will be discussed. Among others, will be discussed topics about postharvest of fish, by-product releasing, enzymatic hydrolysis of by-product and bioactive peptide obtaining from fish waste.

Keywords: bioactives peptides, enzymatic hydrolysis, protein revaluation

1. Introduction

Production levels of fishery and aquaculture have been increasing for the last 30 years, as fish is an important protein source for human consumption and it is expected to reach a production of 196 mt by 2025 [1]. As a result, more and more people depend on fish or other fisheries production, capture, processing and marketing. By 2018, aquaculture production in the world was estimated to reach over 178 million tons [2], whereas marine capture fisheries have been around half the global production [3].

A huge waste volume has been produced along with that production increase, too. Around 70% of fish is processed before final sale, producing between 20 and 80% of fish waste, depending on the fish type and its transformation technology [4]. Furthermore, important amounts of water are required for those processes [5]. That situation represents a challenge from an environmental perspective because around 50% of that fish waste is discarded without being used [6]. Most of it is buried or deposited in water sources, either in the ocean, rivers, or streams. In the case of landfills, it can lead to saturations that cause odor and leachate problems. As for dumping in water sources, aerobic bacteria use organic matter by the action of oxygen, releasing large amounts of phosphorus, nitrogen and ammonium, affecting pH, causing algae growth, and

Process	Organic byproduct	%	Goal
Stunning	N/A		Decrease agony time to reduce undesirable compound production
Classification	Whole fish		Separate by size or species
Slime removal	Aqueous fluid		Reduce microbial contamination surface
Scaling	Scales	5	Reduce bacterial contamination
Washing	Washing water	100	Remove micro-organisms and contaminants
Head removal	Heads	9–32	Remove non-edible or low-value parts
Evisceration	Viscera	12–18	Remove internal organs to reduce microbial contamination
Fin Cutting	Fins	1–2	Remove non-edible parts
Skinning	Skin	3	Remove non-edible parts
Filleting	Fillet remains	15–20	Separation of dorsal and abdominal meat from fish
Bone/meat separation	Bones and skeletons	9–15	Separate meat from ribs and bones

Table 1.
Processes used for fish preparation after capture.

turbidity. The absence of oxygen in water results in the release of hydrogen sulfide, carbon dioxide, organic acids, methane, and ammonium [7].

These wastes contain important nutrient levels [8] and their composition depends on species, source organs or obtaining processes, as seen in **Table 1**. On the other hand, some of those nutrients represent an opportunity from an economic perspective, as in the case of the protein, which can be recovered to obtain high added-value compounds.

Among the methods used to add value to fish residues, there are protein hydrolysis, silage, and collagen recovery [9]. In the first hydrolysis tests evaluated, chemical processes and extraction with organic solvents were used, showing that they affected the nutritional quality of proteins and amino acids. For this reason, commercial enzymes have been increasingly applied to intend to obtain hydrolyzed protein of this substrate type [10]. These latter processes have moderate operating conditions, show greater reproducibility, and are more controllable and selective than chemical processes. Besides, they deliver products with techno-functional properties, excellent digestibility, rapid absorption, and amino acid balance, in addition to high levels of bioactive peptides [11].

This chapter will address the issue of protein residues used in fish processing aiming to obtain bioactive peptides through enzymatic hydrolysis using commercial enzymes. The basic concepts of fish processing, the characteristics of the waste generated, their use by enzymatic hydrolysis, and bioactive and functional peptide production will be addressed.

2. Fish post-harvest

Once the fish is harvested, it undergoes different processes intending to improve conservation conditions, separate the non-edible or low commercial value parts, and

leave the product ready to deliver to the consumer. **Table 1** lists, in general terms, the stages of fish processing, many of which release some type of organic by-product [3, 6].

3. Bromatological characteristics of the Main fish-farmed by-products

Fish by-products are made up of different compound types with food importance [12]. The major components are moisture, fat, and protein. However, the bromatological composition varies depending on the species, age, and gender of the fish, in addition to the part of the fish from which the by-product comes, or the processes to which it has been subjected [13]. Thus, **Table 2** presents the bromatological composition of different fish by-products, for different species, fish parts, and processes.

As **Table 2** shows, these residues contain mainly proteins, fats and water, but they may also contain high added-value compounds such as collagen and gelatin, polyunsaturated fatty acids (EPA and DHA), monounsaturated such as palmitic and oleic, in addition to minerals and enzymes such as pepsin, trypsin, chymotrypsin and collagenase [3]. Because of their nutrient richness, inappropriate dumping of these residues affects not only the area where they are directly discharged, but it can also alter natural ecosystems in a wider area. In this sense, phosphorus and dissolved nitrogen release can be favored and thus increase biochemical demand for oxygen (BDO), because at least 80% of the nutrients in fish residues are potentially eutrophic substances. This leads to the higher growth of macroalgae in aquifers [31].

In some regions of the world, alternatives to use by-products have been sought. That is how the demand for complete fish heads and skeletons as food for humans has increased in Asia and Africa [32]. Bones, which contain the highest protein levels among the residues (41–84%), are a good source of collagen and gelatin. Besides, their mineral content has been used in the manufacture of food products for schoolchildren (85 mg/kg zinc, 350 mg/kg iron, and 84 g/kg calcium) [32]. Whereas skeletons contain significant amounts of meat remaining after filleting, whose protein is highly digestible and can be extracted for different purposes since it has better nutritional properties than plant proteins, and better essential amino acids balance than other animal protein sources [33] but are more sensitive to heat [34]. On the other hand, fish skin, provides gelatin [32], such as, Nile Tilapia skin has been used to produce collagen [35], which can be used for tissue regeneration [36].

A fish by-product that has gained the most attention in recent years is the viscera (12–20% of the fish), which comprise all organs of the main body cavity, including gills, heart, liver, spleen, swim bladder, stomach, gonads, intestines and their contents [6]. This residue has an average composition of 8–21% proteins, 2–12% lipids, 60–81% humidity and 1–5% ash [6]. The high protein content, in addition to being an excellent enzyme source, makes them a potential source of added-value products with exceptional properties for different industrial applications [37].

Between 70 and 80% of fish muscle is a structural protein, between 20 and 30% sarcoplasma proteins, and the remaining 2–3% of proteins are insoluble connective tissue. The main food protein is myofibrillar, with 66–77% of the total in fish meat. This protein comprises between 50 and 60% myosin and 15–30% actin [38]. Myosin fibers are connected by actin molecules and can be cut at one end by trypsin and chymotrypsin, while at the other end by papain, to form they divide into two forms of meromyosin, heavy and light, with different functional properties [39].

Type of waste	Protein	Fat	Moisture	Ash	Reference
Freeze-dried Viscera of Yamú (Brycon siebenthalae)	19.19	79.49	0.48	—	[14]
Argentine hake (Merluccius hubbsi) gonad	21.95	10.92	68.72	11.61	[15]
Raw Viscera Tilapia of (Oreochromis spp.)	4.03	32.93	61.36	0.67	[16]
Tilapia (Oreochromis nilotica) skeleton (D.B)	50.6	30.6	65.3	15.3	[17]
Raw Viscera of Trucha (Oncorhynchus mykiss)	9.14	31.15	56.93	1.51	[10]
Argentine hake (M. hubbsi) liver	16.38	29.71	55.79	1.61	[15]
Viscera of Catla Catla	8.52	12.46	76.25	2.50	[18]
Cape hake (Merluccius capensis) by products	18.0	1.1	78.5	1.9	[19]
Tilapia (Oreochromis spp.) scales (D.B)	67.96	—	15.18	32.08	[20]
Greenland halibut (Reinhardtius hippoglossoides) skin	15.95	10.62	55.44	17.63	[21]
Tilapia (Oreochromis spp.) viscera	7.87	26.08	62	1.19	[22]
Blue shark (Prionace glauca) skin	22.79	0.24	76.03	4.24	[21]
Rainbow trout (O. mykiss) viscera	15	13	71.7	2.7	[23]
Atlantic salmon (Salmo salar) viscera	8	44	60	1	[24]
Tilapia (Oreochromis spp.) defatted viscera	10.04	1.88	83.21	1.71	[22]
Yellowfin tuna (Thunnus albacares) skin	32.38	3.22	0.67	62.57	[21]
Red tilapia (Oreochromis niloticus) head, skeleton, and tail	14.6	5.5	66.6	8.9	[25]
Tilapia del Nilo (O. niloticus) skin	29.68	13.89	54.91	1.61	[26]
Black Sea anchovy (Engraulis encrasicholus) head	13.39	10.02	70.94	5.00	[27]
Atlantic salmon (S. salar) head (D.B.)	13	22	39	44	[24]
Tilapia (Oreochromis spp.) spines (D.B.)	55.54	—	53.46	22.91	[20]
Tilapia (Oreochromis nilótica) skeleton	50.6	30.6	65.3	15.3	[17]
Black Sea anchovy (Engraulis encrasicholus) frame	16.47	15.50	59.72	7.60	[27]
Tilapia (Oreochromis spp.) viscera	4.574	33.602	62.693	0.732	[28]
Atlantic salmon (S. salar) skeleton	15	27	42	4	[24]
Tilapia (Oreochromis spp.) defatted viscera	12.644	2.525	82.607	1.462	[28]
Black Sea anchovy (Engraulis encrasicholus) viscera	12.05	23.90	61.50	2.09	[27]
Cuttlefish (Sepia officinalis) viscera	17.45	4.78	74.99	1.95	[29]
Salmon (S. salar) head	13	22	39	4	[24]
Tilapia (Oreochromis spp.) scales	83.9	0.9	—	15.1	[30]
Salmon (S. salar) skeleton	15	27	42	4	[24]

Table 2.
Bromatological composition of fish by-products D.B.: Dry base.

Fish proteins contain between 16 and 18 amino acids, which have an excellent balance, usually 8 essential and 8 non-essential. This makes this type of protein very widely used for animal feed, although they are also used for fertilizer production, silage and in recent decades, bioactive peptide production [30, 40]. **Table 3** shows the aminograms of different residues of several fish species, some raw and others that

Amino Acid	Red tilapia		Mackerel fish			Yamú viscera		
	RTVH	FRTVH	WT	WM	DM	PI	DH9	DH28
Histidine	4.06	1.99	4.5	3.8	5.2	6.629	5.069	5.222
Isoleucine	2.53	2.44	5.5	6	5.6	4.919	4.073	5.221
Leucine	7.99	8.14	9.4	10	8.8	5.445	5.254	5.267
Lysine	7.68	9.91	7.9	8	7.6	3.54	3.33	2.437
Methionine	1.32	0.14	2.7	4.6	2.8	0.944	1.018	0.656
Arginine	3.97	4.44	7.6	5.9	7.1	—	—	—
Valine	5.27	4.43	7.8	6.8	8.5	1.108	1.901	0.874
Phenylalanine	0.91	1.17	4.2	4	3.1	2.407	1.898	2.406
Threonine	8.04	6.06	5.7	5.5	5.5	1.228	1.927	1.898
Tryptophan	—	—	—	—	—	—	—	—
Ac Aspartic	2.31	4.39	11.8	12.2	11.4	1.837	1.799	2.135
Ac Glutamic	6.6	5.84	15.8	18	15.6	4.329	4.797	5.045
Asparagine	2.31	4.39	—	—	—	1.054	0.984	0.627
Serine	4.26	3.94	4.5	5.2	4.1	3.436	3.72	3.398
Glycine	21.38	17.62	6	6.2	4.6	1.516	1.882	1.508
Alanine	3.8	3.82	7.7	7.3	7.3	2.498	3.18	2.89
Tyrosine	3.11	4.23	3.5	3.9	3.4	17.54	17.66	13.824
Cystine	—	—	—	—	—	3.237	4.302	3.706
Hydroxyproline	—	—	—	—	—	—	—	—
Proline	—	—	1.5	4.6	1.5	—	—	—
Glutamine	6.6	5.84	—	—	—	—	—	—
Total	92.14	88.79	106.1	112	102.1	61.66	62.79	57.114
Reference	[11]		[41]			[14]		

RTVH: Red Tilapia Viscera Hydrolysates.
FRTVH: Fraction <3 kDa of Red Tilapia Viscera Hydrolysates.
PI: Yamú Protein Viscera Isolate.
DH9: Yamú Protein Viscera Hydrolysates with 9% of Degree of hydrolysis.
DH28: Yamú Protein Viscera Hydrolysates with 28% of Degree of hydrolysis.

Table 3.
Amino acids content of fish y-products.

have undergone hydrolysis processes [14], atomization drying [40] and membrane fractionation [11].

4. Enzymatic hydrolysis of fish by-product proteins

4.1 Protein hydrolysis

Protein hydrolysis occurs when a peptide bond is broken by water action, in the presence of a catalyst that may be an enzyme or a chemical agent [42]. Low-cost

chemical processes can be by acid or alkaline hydrolysis, but they are non-specific, not reproducible and lead to amino acid denaturation. On the other hand, enzymatic hydrolysis is more expensive but does not deteriorate amino acids [43].

Once the native protein is broken, fragments of the native protein (oligomers) form, which can be a substrate for the subsequent hydrolysis process, so it is a multi-substrate reaction [44], especially in mediums where no pure protein is available, but mixtures of innumerable proteins, such as in fish residues and in general in other agro-industrial waste. Due to the hydrolysis process, the molecular characteristics of the proteins change, because the average molecular weight of the protein fragments present decreases, this increases the surface load, causes the release of hydrophobic groups, and changes functional properties, among other effects [45].

4.2 Enzymatic hydrolysis of protein

This process consists of decomposing proteins into smaller fragments, whose catalysts are enzymes called proteases [11]. This is a set of simultaneous link break reactions, consisting of serial stages, with different species loaded in equilibrium, giving fragments of decreasing size as follows [46]:

$$proteous \rightarrow proteins \rightarrow peptones \rightarrow peptides \rightarrow amino\ acid$$

The catalytic process that occurs is divided into three steps. First, the enzyme (E) should approach the substrate (S) and bind to form the enzyme-substrate complex (ES). Second, the rupture of the peptide bond results in the release of a peptide. Third, the remaining peptide is separated from the enzyme after a nucleophilic attack from a water molecule [11]. Each of these reactions has its speed as described in Eq. (1) [47]. This process can be repeated on any of the peptides formed [46].

$$E + S \underset{K_1}{\Leftrightarrow} ES \underset{K_2}{\rightarrow} EP + H - P' + H_2O \underset{K_3}{\rightarrow} E + P - OH + H - P' \qquad (1)$$

E: Enzyme, S: Substrate, P and P′: Resulting peptides, kx: Constant reaction rate.

This procedure has advantages over chemical hydrolysis as they have high selectivity and low contamination. It is a specific process that is carried out under moderate pH and temperature conditions, which makes it easy to control [30]. The product obtained is called protein hydrolyzate and it consists of peptides generally between 2 and 20 amino acids [48]. However, there are also disadvantages such as the high enzyme costs and long processing times [49].

Critical operating conditions in protein enzymatic hydrolysis include temperature, pH, enzyme type and concentration, substrate and concentration, cofactors, coenzymes, hydrolysis time [50], agitation speed [51], and presence of inhibitors, like fat in fish by-products [11].

On the other hand, variations that enzyme activity may suffer during the reaction should be controlled, such as denaturation, aggregation, or enzyme inactivation, which can be produced by temperature effects, pH shear stress or other substances that interfere with catalysis [12].

4.2.1 Enzymatic hydrolysis kinetics

During the reaction, the enzyme attacks the peptide bond as follows [52, 53]:

Opening of the peptide bond

$$-CHR' - CO - NH - CHR'' - + H_2O \overrightarrow{enzyme} - CHR' - COOH + NH_2 - CHR'' \quad (2)$$

Proton exchange

$$-CHR' - COOH - NH2 - CHR' \rightarrow ' - CHR' - COO^- + N^+H_3 - CHR'' \quad (3)$$

Tritation of amino group

$$N^+H_3 - CHR'' + OH^- \rightleftharpoons NH_2 - CHR'' + H_2O \quad (4)$$

Under neutral or alkaline conditions, the dissociation of the amino group becomes significant, so a decrease in pH may occur due to the accumulation of the protons released, which makes it necessary to add a base to keep pH constant and prevent the enzyme from being affected in its activity [30]. The analysis of the equations above concludes that the amount of hydrolyzed protein is proportional to the amount of base required to neutralize the pH of the reaction medium [30].

4.2.2 Follow-up of hydrolysis reaction

The hydrolysis reaction progress is established by the Hydrolysis Degree (HD), expressed as a fraction or percentage of the number of broken peptide bonds at any given time (h) for the total peptide bonds in the intact protein (htot) (Eq. 5) [54]. Both can be expressed as protein meq/g or as protein mmol/g [30].

$$GH(\%) = \frac{h}{h_{tot}}.100 \quad (5)$$

Methods used to determine Hydrolysis Degree (HD) include the pH-stat method [52], O-phthaldialdehyde (OPA) [54], Trinitrobenesulfonic acid (TNBS) [55], formalin titration, and soluble nitrogen in trichloroacetic acid (TCA) [56]. The fundamental difference between these methods is in the principle that each one is based to measure the number of broken bonds (h) at any given time of the reaction, because htot is usually determined from the analysis of the total amino acid content in the intact protein [57].

4.2.2.1 pH-stat method

This method is based on the fact that in peptide bond hydrolysis, a carboxyl group and an amino group are released. In an aqueous solution, these groups will be more or less ionized depending on pH [55]. At neutral or alkaline pH, carboxyl groups are fully ionized and proton exchange occurs between the carboxyl group and the amino group. At alkaline pH, amino groups will also be partially or fully ionized depending on the pH and amino acid in question, since the pK of the free amino acids N-terminal amino group ranges from 9 to 10.8. The following equations show, in general, the chemical species involved in protein enzymatic hydrolysis [58].

$$P_1 - CO - NH - P_2 + H_2O \xrightarrow{protease} P_1 - COOH + NH_2 - P_2 \quad (6)$$

$$P_1 - COOH \longrightarrow P_1 - COO^- + \boldsymbol{H}^+ \quad (7)$$

$$NH_2 - P_2 \rightleftarrows NH_3^+ - P_2 \quad (8)$$

The resulting free protons cause a pH decrease of the reaction mixture, and a base addition is required to keep pH constant. The amount of base required is directly related to the amount of hydrolyzed peptide bonds, and it can be used to estimate HD. Unfortunately, the relationship between HD and base consumption is not simple and depends on several variables, including pK of the α-amino group released, the temperature of the reaction mixture, and length of the peptide chain [52]. The relationship between the spent base volume and HD has been described by Adler-Nissen, 1986 [55] in Eq. (9).

$$GH(\%) = \frac{BN_B}{M_p \alpha h_{Tot}}.100 \quad (9)$$

where B is the base volume consumed in L to keep pH constant, MP is the protein mass in kg, NB is the base concentration, and α is the dissociation degree of the amino groups released in the reaction. α and pK are calculated with Eqs. (10) and (11), respectively, where T is the temperature (K) [59].

$$\alpha = \frac{10^{pH-pK}}{\left(1 + 10^{pH-pK}\right)} \quad (10)$$

$$pK = 7.8 + \frac{298 - T}{298 * T} * 2400 \quad (11)$$

4.2.2.2 O-phthaldialdehyde method (OPA) and Trinitrobencenesulfonic acid method (TNBS)

Both methods are spectrophotometric, based on the determination of the α-amino groups released, by derivatization with trinitro-bencenesulfonic acid or ortho-phthaldialdehyde, respectively [56]. They are detected in the ultraviolet–visible range for the TNBS method, or fluorescent for the OPA. The absorbance value obtained is then converted into quantitative values using a standard curve prepared with a free amino acid, usually leucine, calculating HD as the percentage proportion of the amino acid released in the hydrolyzed regarding the amino acid amount of the whole protein [54, 55]. In **Figures 1** and **2**, reactions of an amino group with TNBS and OPA, respectively, take place [56].

However, in these methods, derivatization reagents exhibit different reactivity to some amino acids, affecting measurement accuracy. For example, in the case of the OPA method, it will not be accurate when applied on proline- and cysteine-rich hydrolyzates [57].

4.2.3 Proteases most important characteristics

Proteases are the enzymes responsible for catalyzing the hydrolysis reaction of protein-peptide bonds, also known as peptidases [62]. Although, they can be obtained from plants, animals or microorganisms, most commercially viable proteases are obtained from this latter [63], especially Bacillus species, such as Bacillus licheniformis, Bacillus subtilis, and Aspergillus fungal species such as Aspergillus

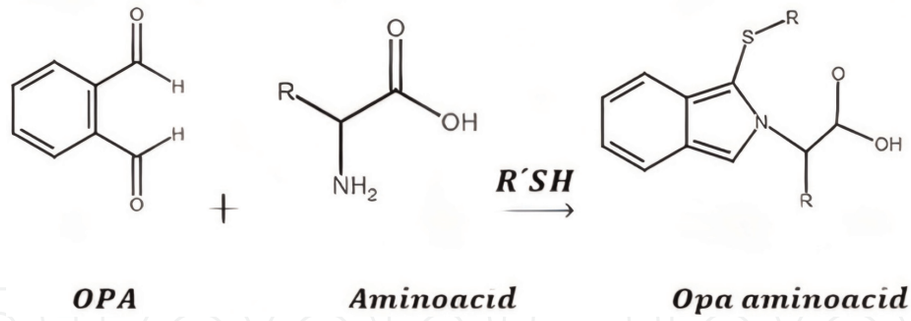

Figure 1.
Reaction of OPA with amino acids. Source [60].

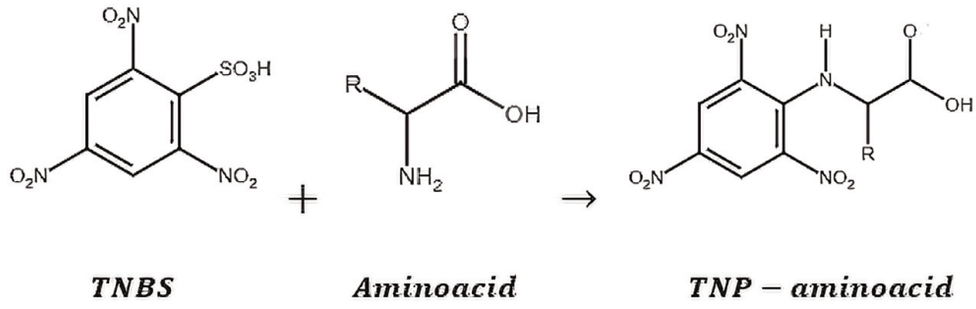

Figure 2.
Reaction of TNBS with amino acids. Source [61].

niger, A. flavus, Ammophilus fumigatus, and A. oryzae [64]. Some of the commercial proteases that have been used to obtain hydrolyzates from fish residues include trypsin, chymotrypsin, pepsin, Alcalase® 2.4 L, Flavourzyme® 500 L, E Properase, pronase, collagenases, bromelain and papain [50].

Proteases belong to the hydrolases group, they constitute a large and complex group of enzymes that differ from each other in their specificity due to substrate, their selectivity, the nature of their active sites, their catalytic mechanism, their stability profile, their pH, and optimum temperature. For these reasons, proteases cannot be classified under the general enzyme nomenclature system, but are classified according to their catalytic action, the nature of their active site, and their optimal pH value [63]. From the point of view of functional groups that have their active site, proteases can be classified into four main groups as follows [62]: Serine Proteases, Aspartic Proteases, Cysteine Proteases, Metalloproteases. On the other hand, when considering its catalytic mode of action, i.e., the excision site of the polypeptide chain, proteases are classified into exopeptidases and endopeptidases [65]. While, based on their optimal pH range, proteases can be classified into alkaline, neutral and acidic.

5. Production of bioactive and techno-functional peptides of fishery by-products

According to the HD achieved, the hydrolyzate obtained will potentially have biological activities or techno-functional properties. HD less than 10% result in improved techno-functional properties, such as emulsification, foaming capacity and greater solubility, whereas a higher HD tend to deliver hydrolyzates with greater potential as bioactive peptide sources [66].

5.1 Bioactive peptides

A bioactive peptide is a sequence of amino acids that is encrypted in the intact protein, in which it remains inactive, but once released, it can interact with certain receptors and regulate the physiological functions of the organism [67]. This may express some kind of effect on metabolic behavior, either human or animal [65]. These peptides can be released from the protein by gastrointestinal digestion, enzyme hydrolysis, or fermentation [68].

Among the most widely studied biological activities, are antihypertensive [69] Antioxidant [11] Antimicrobial [70], antithrombotic [71], anticancer [11] metal chelating agent, anticoagulants, among others [72].

One of the methods currently applied for obtaining bioactive peptides is enzymatic hydrolysis using commercial enzymes, which represents a reproducible, scalable, and industrial-application-capable method [73]. In this technology, biological activities of the peptides obtained may be affected by the operating conditions applied to isolate proteins, hydrolysis degree, protease type, peptide structure, the amino acids sequence, concentration, and the molecular weight of the peptides obtained [74].

The relationship between the peptide's biological activity and their molecular weight has been widely documented [73], so the search for conditions that maximize HD has been one of the priorities in many studies [75] Peptide fractions with molecular weights between 1 and 4 kDa are of the greatest interest for nutritional and/or pharmaceutical uses in particular [75].

5.1.1 Antioxidant peptides

Free radicals and reactive oxygen species ROS [76], can cause DNA, protein, or lipid damage, resulting in human body damage from neurodegenerative, inflammatory, cardiovascular, diabetes, and cancer diseases [76]. This type of effect can be counteracted by substances with antioxidant capacity, which have different mechanisms of action depending on the free radical reduction form, among which are SET (single electron transfer), and HAT (hydrogen atom transfer) [77]. Based on these mechanisms, some methods to evaluate the antioxidant capacity of different substances have been designed. SET-based methods detect the antioxidant's ability to transfer a chemical species such as metals, carbonyls and electrons, the most commonly used methods of this type are ABTS and FRAP. In the case of HAT methods, the antioxidant ability to inactivate a free radical is measured through the donation of a hydrogen atom, in which one of the most commonly used methods is ORAC [77].

On the other hand, some metals such as iron and copper, which are of importance at the physiological level, may also participate in the formation of reactive oxygen species [78], as in the case of hydroxyl radicals (OH), that are formed by the Fenton reaction and can cause damage to different types of tissues (Canabady-Rochelle et al., 2018). In this sense, metal chelation can counteract the formation of metal-catalyzed radicals in some way, which has somewhat been considered as a form of antioxidant activity [79].

Thus, peptide antioxidant activity is related to metal chelating activity and electron donation activity, which facilitates interaction with free radicals and cuts the reaction chain in which they participate [80]. In addition, the presence of hydrophobic sequences in peptides can interact with lipid molecules, eliminating the donation of protons to result in lipid radicals [81]. Thus, the imidazole group in histidine residues

participates in hydrogen atom transfer, electron transfer, active oxygen extinction and capture of hydroxyl radicals [82].

The antioxidant capacity in these hydrolyzates has been attributed to the presence at the N-terminal end of peptide sequences of non-polar hydrophobic amino acids, such as phenylalanine, alanine and proline, and hydrophilic amino acids such as tyrosine, histidine and valine [6]. Thus, capturing the activity of hydrogen peroxide, the chelating activity of Fe2+, and reducing the power of Abalone (Haliotis discus hannai) hydrolyzates was related to hydrophobic amino acids in their peptides [83]. The capturing capacity of radicals has also been attributed to the presence of aromatic residues [84]. While tryptophan and tyrosine have been attributed antioxidant activity mediated by their phenolic and indolic groups, capable of donating hydrogen atoms [85]. The **Table 4** lists several sequences of antioxidant peptides, from different kinds of fish by-products.

5.1.2 Antihypertensive peptides

Hypertension is one of the most important cardiovascular risk factors worldwide, since high blood pressure currently affects about 20% of adults around the world [97]. In these blood pressure-increasing processes, the angiotensin I converter enzyme (ACE) plays a crucial role. This enzyme, a dipeptidyl carboxypeptidase (EC. 3.4.15.1), promotes the conversion of angiotensin I to a powerful angiotensin II vasoconstrictor, and inactivates the bradequinine vasodilator, which is a depressant of the renin-angiotensin system action [97]. Angiotensin II is also involved in the release of the steroid Na-retaining, which also tends to increase blood pressure [97]. For these reasons, a first step in the search for potentially useful substances to control high blood pressure is the ability test to inhibit ACE. In this sense, the search for peptides that can reach therapeutic tests as drugs for blood pressure control should initially be evaluated as ACE inhibitors [97]. The **Table 5** lists several sequences of antihypertensive peptides, from different kind of fish by-products.

Source	Sequence	Reference
Amur sturgeon (Acipenser schrenckii) skin	PAGT	[86]
COD (Gadus macrocephalus) gelatin	TCSP, TGGGNV	[87]
Hoki (Johnius belengerii) Skin	HGPLGPL	[88]
Tilapia (Oreochromis niloticus) skin	EGL, TGDET	[89]
Mackerel (Magalaspis cordvla) viscera	ACFL	[90]
Biuefin leatherjacket (Navodon septentrionalis) Head	WEGPLK, GPP, GVPLT	[91]
Salmon Pectoral fin	FLNEFLHV	[92]
Black Pomfret (Parastromateus niger) Viscera	AMTGLEA	[93]
Skate (Raja porosa) Cartilage	F1MGPY, GPACDY, 1VAGPQ	[94]
Grass carp (Ctenopharyngodon Idella) skin	PYSFK, GFGPQLVGGRP	[95]
Squid (Ommastrephes bartrami) Viscera	WVAPLK	[96]
Salmon (Salmo sp.) Fin	FLNEFLHV	[92]

Table 4.
Amino acid sequence of antioxidant peptides from fish by-products.

5.1.3 Anti-carcinogenic peptides

Cancer (malignant tumor), one of the most common diseases in the world [106], consists of abnormal and uncontrolled growth of cells, with proliferation and spread in surrounding tissues [11]. Thus, inhibition of deregulated cell proliferation is one of the strategies for treating this type of disease [107]. Among the broad list of substances that have been evaluated for this purpose are luteinizing hormone-releasing hormone and Atrial natriuretic peptide, for the treatment of prostate and colorectal cancer, respectively [106].

Various fish-derived proteins have been reported as sources of anticancer peptides [11, 108], as in the case with the antiproliferative activity of protein hydrolyzates of 18 fish species against breast cancer cell lines [109]. In **Table 6**, different fishery sources that have been active against some types of cancer are shown.

There are three ways in which antiproliferative peptides act on cancer cells, apoptosis, necrosis, and cell cycle disturbances [11]. These mechanisms of action change according to structural characteristics such as molecular weight and amino acid composition. Thus, smaller peptides have greater molecular mobility and diffusivity, so they can interact better with the components of cancer cells. This activity has been attributed to amino acid sequences between 3 and 25 residues, with the predominance of hydrophobic amino acids, and one or more residues of Lys, Pro, Arg, Ser, Glu, THR Leu, Gly, Ala and Tyr. Because hydrophobic amino acids improve interactions

Source	Sequence	Reference
COD (Gadus macrocephalus) gelatin	TCSP, TGGGNV	[87]
Pollack (Theragra chalcogramma) Skin	GPL, GPM	[98]
Salmon (Oncorhynchus keta) Skin	GLPLNLP	[99]
Sea Bream Scale	GY, VY, GF, VIY	[100]
Sardinella (Sardinella aurita) Head/viscera	FRGLMHY	[101]
Snakehead fish (Channidae sp) Muscle	LYPPP, YSMYPP	[102]
Small-spotted catshark (Scyliorhinus canicula) Muscle, viscera, skin, and frame	VAMPF	[103]
Lizard fish (Saurida elongate) Muscle	RVCLP	[104]
Lizardfish (Synodus macrops) Scale Gelatin	AGPPGSDGQPGAK	[105]

Table 5.
Amino acid sequence of ACE inhibitor peptides from fish by-products.

Source	Type of cancer	Reference
Ruditapes philippinarum hydrolysates	prostate, breast, and lung cancer	[110]
Squid gelatin hydrolysates	CMF-7, U87	[111]
Oyster protein and anchovy hydrolysates	Colon and prostate cancer	[112]
Blood clam muscle	Prostate cancer	[91]
Krab subproducts	Prostate cancer	[113]

Table 6.
Use of peptides from fish by-products in cancer treatment.

between peptides and the outer surface of the bilayer of the tumor cell membrane, due to their phospholipid content and thus, they exert selective cytotoxic activity on these cells to healthy cells [107].

In addition to the amino acid sequence, the anti-cancer peptide's function is influenced by net load, amphipathicity, hydrophobicity, structural membrane folding (including secondary structure, dynamics and orientation), oligomerization, and peptide concentration [11]. The cationic amphibious structure predisposes them to interact with the cell membrane anion surfaces [114]. The α helix is a main structural characteristic of this peptide type, with lateral chains of hydrophilic and hydrophobic amino acids, forming clear hydrophilic and hydrophobic surfaces. On the other hand, they concentrate on the N-terminal and the C-terminal to form different hydrophilic and hydrophobic domains. Anti-cancer peptides with a β sheet structure are generally stabilized by disulfide bonds, and these sheets are in β antiparallel formation. Meanwhile [11]. The net charge and positive charge number also influence these peptides activity, since their association with the cancer cell membrane occurs through electrostatic interactions due to its cationic condition and the anion lipopolysaccharide on the external membrane that causes its disturbance [115].

5.1.4 Anticoagulant peptides

Blood clotting is a crucial process for human health, excessive clotting that leads to blocked blood vessels causes strokes, heart attacks, and pulmonary embolism [11]. This makes anticoagulant compounds vital to preserving life quality in modern times. The anticoagulant is a compound that will stop blood clotting by binding to one or more coagulation factors, preventing it from binding to the membrane phospholipids [11]. Heparin is currently the anticoagulant most commonly used, but heparin has several disadvantages, including thrombocytopenia and non-specific plasma binding. In addition, it can cause platelet dysfunction and aggregation [116]. Therefore, there is a marked interest in the search for new anticoagulant compounds with minor collateral risks for the medical treatment of thromboembolic events [11].

Anticoagulant activity is less investigated than other biological activities, and specifically, peptides with this activity isolated from fish-based by-products have not been reported [11]. This way, an oligopeptide from the blue mussel, with a molecular mass of approximately 2,5 kDa has been isolated, showing anticoagulant activity by the prolongation of both thrombin time and activated partial thromboplastin time, by interaction specifically with blood clotting factors IX, X, and II. Nasri et al. [71], in 2012 isolated four anticoagulant peptides from protein hydrolyzates of goby muscle proteins, in which they found that they had Arg in the C-terminal position. Thus, concluding that small peptides with an amino acid charged at the C-end are considered potential thrombin inhibitors and/or other factors involved in the coagulation process [71]. Anticoagulant peptides from yellow-sole fish skeleton have also been isolated [117].

5.1.5 Antimicrobial peptides

The excessive use of conventional antimicrobial products has caused the emergence of resistant strains, which poses a health threat. Therefore, the development of antimicrobials using mechanisms other than traditional antibiotics is needed [11]. In this context, antimicrobial peptides effectively promote toxicity against invading pathogenic microorganisms, and also modulate the immune response in superior organisms [118]. These peptides are produced in all kingdoms, from bacteria to fungi

and plants to mammals. Their unique intrinsic properties make them attractive therapeutic agents, since they show high biological activities associated with low toxicity and high specificity, as well as potentially useful as ingredients of functional or health-promoting foods [119]. These peptides generally contain less than 50 amino acid residues, with a molecular weight less than 10 kDa [120]. Despite their structural diversity, they have common physico-chemical characteristics; they are positively charged (+2 to +9) under physiological conditions due to the presence of lysine, arginine and histidine residues; and contain a substantial portion (50% or more) of hydrophobic residues [118]. These peptides commonly adopt an amphipathic conformation in which positively charged and hydrophobic groups are segregated into opposite faces of a α-helix, a β-leaf, or some other tertiary structure. This gives them the ability to cross the phospholipid membrane. The spectrum of different chemical properties of the amino acid side chain provides a variety of peptide sequences to show a cationic amphibious helical peptide [121]. Having a positive net charge allows them to interact with the anionic phospholipids of the bacterial membrane or other pathogens, and their amphipathicity, i.e., presence of apolar regions (with hydrophobic amino acids), and positive loads regions (cationic amino acids, Arg, Lys or His), facilitates them that, after initial interaction, the polar regions interact with the polar chains of the phospholipids, achieving the insertion of the peptide into the microbial membrane [122]. They are also flexible, which allows their internalization toward the bacterial cytoplasm, and leads to cell death due to ion and metabolic substances loss [123].

The most common mechanisms of action recognized in peptide antimicrobial activity include (i) the barrel model, in which a water-filled channel and an ion channel protein are formed by the interaction of peptides, acting as pores that disrupt the structure of the cell membrane; (ii) toroidal pore, in which less organized pore structures are formed; (iii) carpet models, in which the destabilization of the cell membrane in mycellar structures is caused by the accumulation of peptides above the limit concentration; (iv) molecular electroporation, following the concept that molecular electroporation can be achieved not only by electrical fields externally applied, but also by highly charged molecules that bind to the membrane surface; (v) sinking raft model, product of the induction of the membrane curvature by adsorbed peptides, which is relieved by its aggregation in the bilayer, allowing the aggregate to be translocated into the lumen of the gallbladder by a sinking raft process; and after membrane permeation, intracellular targets activation or blocking occurs [11]. These peptides not only generate toxic effects on microorganisms, but also exert important effects on the host, including immunomodulation, angiogenesis induction, wound healing and gene expression modulation. These effects may complement each other during the control of infectious and inflammatory diseases, and may be highly desirable when considered an optimal combination of an antimicrobial compound and regeneration booster [118]. In recent decades, barbel muscle antimicrobial peptides have been obtained by enzymatic hydrolysis of proteins from aquatic organisms [124]. Mustelus viscera [125], sea cucumber by-products [126], and different fish species [120], among others.

5.2 Commercial peptides obtained from fish sources

Thanks to their potential to produce bioactive compounds, fish parts and their residues have been used to obtain different types of functional inputs that have reached the market in different countries (**Table 7**). It should be noted, however, that few countries in which these products are being marketed. Given that fish, production extends to a much larger number of countries and that waste from that industry is

proportional to production, it is clear that there is a latent possibility of expanding the market for products derived from fish sources.

Commercial name	Source	Functionality	Country
Custom Collagen®	Tilapia	Liver and kidney	US
Hydroiyzed Fish Collagen Type 1	Tilapia	Skin, tendons, and arteries	UK
Amizate®	Atlantic salmon	Muscle anabolism	North America
Protizen®		Stress, weight disorder, sleep trouble	UK
Levenorm®	Sarda	Antihypertensive	Canada
MOLVAL®	Molva	Cholesterol, stress, and cardiovascular health	UK
Norland Hydrolyzed Fish Collagen	Cod	Hair, skin and nails	US
PeptACE®	Sarda	Vascular function and blood pressure	Japan and US
Stabilium®200	Molva dypterygia	Stress, memory, and cognitive function	UK
Seacure®	Hake	Gastrointestinal and bowel function	Canada and US
Seagest™	White fish	Intestinal lining and health	US
Valtyron®	Sardine	Blood pressure	
Vasotensin®	Tuna and verdel	Vascular function and blood pressure	Japan and US
Nutripeptin®	Cod	Weight and blood glucose	US and UK
Liquamen®	Molva	Oxidative stress, glycemic index, and stress	UK

Table 7.
Commercial products obtained by enzymatic Hydroiysis of fish protein by-products [37, 127].

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