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BYPRODUCTS OF AQUACULTURE PROCESSES: DEVELOPMENT AND PROSPECTIVE USES. REVIEW

SUBPRODUCTOS DE LOS PROCESOS DE ACUICULTURA: DESARROLLO Y USOS PROSPECTIVOS. REVISIÓN

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ABSTRACT

Background: Growing aquaculture production around the world generates an important environmental impact because of its waste volume, which reaches nearly 60%. These byproducts have important levels of protein and lipids that can be revaluated to obtain products that are of interest to the pharmaceutical and food industries such as bioactive peptides and functional properties. Recently, technologies have been applied to the isolation and purification of bioactive peptides according to their molecular weight, such as membrane separation techniques and chromatography. Currently, there are commercial products from fish protein hydrolysates that can be used in nutritional and pharmaceutical applications as a source of amino acids with different physiological functions. **Objective:** Give information on aquaculture byproducts, hydrolysis methods, methods of purification, bioactive peptides and functional properties and nutritional supplements. **Methods:** Science Direct, Springer Link, Wiley Online Library, and Scopus were reviewed using the keywords aquaculture products, protein hydrolysis, bioactive peptides, functional properties. For the selection of the articles, the year of publication, the language, the methodology used and the trajectory of the authors were taken into account. **Conclusions:** This review is a brief description of the use of aquaculture byproducts using different types of hydrolysis process and their multiple applications on several industries.

Keywords: aquaculture products, protein hydrolysis, bioactive peptides, functional properties.

RESUMEN

Antecedentes: El crecimiento de la producción acuícola en el mundo genera un importante impacto ambiental debido a su volumen de residuos, que alcanza casi el 60%. Estos subproductos tienen niveles importantes de proteínas y lípidos que pueden reevaluarse para obtener productos que son de interés para las industrias farmacéutica y alimentaria, como péptidos bioactivos y propiedades funcionales. Recientemente, se han aplicado tecnologías para el aislamiento y la purificación de péptidos bioactivos de acuerdo con su peso molecular, así como técnicas de separación de membrana y cromatografía. Actualmente, hay productos comerciales de hidrolizados de proteína de pescado que pueden usarse en aplicaciones nutricionales y farmacéuticas como fuente de aminoácidos con diferentes funciones fisiológicas. **Objetivo:** Proporcionar información sobre subproductos de la acuicultura, métodos de hidrólisis, métodos de purificación, péptidos bioactivos, propiedades funcionales y suplementos nutricionales. **Métodos:** Se revisaron bases de datos

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como Science Direct, Springer Link, Wiley Online Library y Scopus usando palabras clave como productos de acuicultura, hidrólisis de proteínas, péptidos bioactivos, propiedades funcionales. Para la selección de los artículos se tuvo en cuenta el año de publicación, el idioma, la metodología empleada y la trayectoria de sus autores. **Conclusiones:** Esta revisión es una breve descripción del uso de subproductos acuícolas usando diferentes tipos de procesos de hidrólisis y sus múltiples aplicaciones en varias industrias.

Palabras clave: productos acuícolas, hidrólisis de proteínas, péptidos bioactivos, propiedades funcionales.

INTRODUCTION

Worldwide aquaculture production has continuously increased over the last decade and achieved 158 million tons in 2012 with only 86% of that production used for human consumption (1). This growth, has brought about great concern because aquaculture industries discard between 60-70% of their production as waste, which can have negative environmental impacts (2). This waste includes the remains from filleting, such as fins, skin, bones, heads, viscera and scales (3), which, despite being an important source of protein and lipids, are usually discharged without any use or used to obtain products with low added value (4).

Among the current methods used to take advantage of fish wastes are hydrolysis, bioremediation, silage and filtration (5). Hydrolysis improves the quality and functional characteristics of byproducts (6) and has been employed to obtain hydrolyzed proteins with better nutritional characteristics and bioactive compounds (7). However, the kinetics of the reaction of enzymatic hydrolysis of proteins must be studied more deeply (8).

The applications for hydrolyzed proteins from different sources among fish proteins have generated great interest from academic and industrial organizations (9). For this reason, it is necessary to conduct studies that examine enzymatic hydrolysis of proteins and go beyond obtaining the optimum work conditions. This work involves addressing the complexity of these reactions from the perspective of modelling kinetics for which the mathematical models are useful because of their flexibility and predictive capacity, since the necessary factors for obtaining the dimensions of industrial reactors, prediction, description and control of variables of the process, such as temperature, enzyme/substrate ratio, pH, agitation degree, and other factors, should be analyzed properly (10).

The hydrolysates of fish protein, besides having an excellent balance of amino acids and good digestibility as well as fast adsorption, have

some functional properties that have been rarely studied but are important in food formulation. These properties include an emulsifier capacity, oil retention capacity, foaming capacity, and water retention capacity (9). Additionally, these hydrolysates are a very important source of bioactive peptides (8, 11), and these peptides could have antimicrobial (2), antihypertensive, antioxidant, immunomodulation, antithrombotic or anticarcinogenic effects, depending on their sequence, composition and the number of amino acids (frequently 2 - 20) (7, 12). However, production at an industrial level, stability, the mechanisms of action and other important aspects of these peptides have not been widely studied.

Several studies have established the relationship between biological activity and the molecular weight of peptides (11-14). The fractions with molecular weights between 1-4 kDa are particularly interesting for nutritional and/or pharmaceuticals uses (15, 16), thus separation and purification methods, are a key issue for obtaining bioactive peptides (2, 15). The necessity of scientific studies for the generation of products with controlled molecular weights and specific functions (bioactive and/or techno-functional properties) is clear, as well as directed towards techno-economic evaluation of industrial processes and the evaluation of uses in food and nutritional products (14). These topics can be approached through pilot kinetic studies and analyses of aspects related to the thermic and biological stability of fractions with high biological activity.

Currently, there are several technologies for the isolation and purification of bioactive peptides at a pilot scale, such as chromatographic and membrane separation techniques (17, 18). In the latter case, ultra and nanofiltration are better than the traditional techniques, because they can easily be scaled, have lower costs and are environmentally friendly (14). Both techniques has been used widely for fractioning and purifying bioactive peptides hydrolyzed from dairy and soy proteins as well as other vegetable substrates (4).

MATERIALS AND METHODS

A search on databases, Science Direct, Springer Link, Wiley Online Library, and Scopus were conducted to identify articles between 2013 and 2017 (five-year period), which contained keywords such as aquaculture products, protein hydrolysis, bioactive peptides and functional properties of fish protein hydrolysates. Subsequently, from a total of approximately 120 documents found, 94 articles were selected, due that contained novel and relevant information about to take advantage of byproducts from the aquaculture industry. Besides, the included articles should have been published in the last 5 years, on the other hand, the excluded articles were which used a qualitative methodology and were published a language other than English. With respect to the authors, it was reviewed that the authors had previously worked on the topic, that their statistical analyzes were appropriate and what type of references they used to discuss their results.

RESULTS

Aquaculture byproducts

The majority of aquaculture industries produce between 40-60% byproducts depending on the process and the species that is used (19). These waste products are important sources of protein

and lipids that mainly consist of filleting waste (15-20%), skin and fins (1-3%), bones (9-15%), heads (9-12%), viscera (12-18%) and scales (5%) (3). These waste tissues have been considered nutritionally superior compared to vegetable proteins and have a better balance of essential amino acids compared to other animal proteins. Aquaculture byproducts are considered to be rich in fat and minerals in addition to containing amino acids, collagen and jelly, polyunsaturated fatty acids such as EPA and DHA, as well as enzymes such as pepsin, trypsin, chymotrypsin and collagenases, which are extracted mainly from viscera (19). The proximal composition of aquaculture byproducts depends on the type of byproduct, species, age, nutrition and the state of the animal's health (Table 1).

Hydrolysis methods

Hydrolysis can be carried out through chemical or enzymatic methods and breaks proteins to produce peptides of different sizes (20), which directly affects the functional and physicochemical properties because it produces peptides with biological and functional properties that are improved compared to the native protein (7, 20). The processes with added enzymes are used more compared to chemical processes because they have fewer conditions, are easier to control and produce hydrolysates with enhanced functional and biological properties (20).

Table 1. Proximate composition of byproducts for various aquaculture species.

Species	Byproduct	Protein (%)	Fat (%)	Humidity (%)	Ash (%)	References
Argentine hake (<i>Merluccius hubbsi</i>)	Gonad	21.95	10.92	68.72	11.61	(21)
	Liver	16.38	29.71	55.79	1.61	
Tilapia (<i>Oreochromis nilotica</i>)	Skeletons	50.6	30.6	65.3	15.3	(22)
Cape hake (<i>Merluccius capensis</i>)	Byproducts	18.0	1.1	78.5	1.9	(23)
Atlantic salmon (<i>Salmo salar</i>)	Viscera	8 ± 2	44 ± 9	60 ± 8	1 ± 0	(16)
	Heads	13 ± 1	22 ± 2	39 ± 4	4 ± 1	
	Skeletons	15 ± 1	27 ± 1	42 ± 2	4 ± 1	
Black Sea anchovy (<i>Engraulis encrasicolus</i>)	Head	13.39 ± 0.17	10.02 ± 2.38	70.94 ± 1.23	5.00 ± 0.35	(24)
	Frame	16.47 ± 0.38	15.50 ± 0.78	59.72 ± 1.16	7.60 ± 0.55	
	Viscera	12.05 ± 1.44	23.90 ± 4.36	61.50 ± 3.59	2.09 ± 0.22	
Mackerel (<i>Trachurus mediterraneus</i>)	Muscle	21.4	1.0	77.5	1.5	(25)
Sardine (<i>Sardina pilchardus</i>)	Muscle	18.8	1.2	78.1	1.5	
Cuttlefish (<i>Sepia officinalis</i>)	Viscera	17.45 ± 0.25	4.78 ± 0.7	74.99 ± 0.1	1.95 ± 0.0	(26)
Yellowfin tuna (<i>Thunnus albacares</i>)	Skin	32.38	3.22	0.67	62.57	(27)
Blue shark (<i>Prionace glauca</i>)		22.79	0.24	76.03	4.24	
Greenland halibut (<i>Reinhardtius hippoglossoides</i>)		15.95	10.62	55.44	17.63	

Chemical hydrolysis

Protein extraction is an important stage in the production of value-added products from aquaculture residues, which can be achieved with acids and bases, including HCl and NaOH (28). Acid hydrolysis is typically performed at high temperatures (110 to 120°C) for long periods of time (18-96 hours). However, it has been found that the optimal conditions are 6 M HCl for 24 hours to obtain a degree of hydrolysis of 50.45% (29). On the other hand, alkaline hydrolysis has shown that pH contributes to the increase in protein extraction yield, which was demonstrated by Chomnawang and Yongsawatdigul (30), who concluded that the highest protein extraction was achieved in tilapia skeletons when the pH was 12. According to Anal and Noomhorm (29), although these treatments are very effective for the extraction of proteins, it is possible that the proteins are denatured, which causes damage to some amino acids and affects the protein quality.

Chemical hydrolysis has been used by several authors to obtain proteins with bioactive or functional effects in various aquaculture byproducts, including peptides with iron chelating activity of skin from Alaska pollock (*Gadus chalcogrammus*) (31), antioxidant peptides of skin from Amur sturgeon (*Acipenser schrenckii*) (32) and ACE-inhibitory and antimicrobial peptides of skin from black-barred halfbeak (*Hemiramphus far*) (33).

Enzymatic hydrolysis

Enzymatic hydrolysis represents one of the best alternatives for the use of byproducts of the aquaculture industry; the peptide bonds between amino acids is promoted through hydrolysis, which generates smaller molecular peptides with more ionizable amino groups that contribute to the solubility of proteins (8, 16). The hydrolysis process is intended to potentiate some functional characteristics that confer benefits in their use against the original proteins (3, 34). The enzymatic hydrolysis of proteins is carried out in a reactor while controlling variables such as pH, time and temperature, and a protease is used to obtain the rupture of peptide bonds (16). In process development, it is necessary to make some previous considerations, such as the nature and quality of the matrix, the enzyme and the reaction conditions (6, 8, 35, 36).

Numerous commercial proteases have been used for the production of hydrolysates and peptides, including trypsin, chymotrypsin, pepsin, alcalase, flavourzyme, properase E, pronase, collagenases, bromelain and papain (37, 38). The biological activity of the hydrolysates is affected by the free amino acids (size, amount and composition) as well as the amino acid sequence of the protease (37). The enzyme commonly used in the enzymatic hydrolysis process is alcalase, which has been used to obtain peptides with antioxidant activity (20, 38, 39), as well as peptides with anticoagulant activity (40) and calcium-binding peptides (41). The other enzyme of high interest is flavourzyme, which can be used to obtain peptides with antioxidant activity (22), in addition to peptides with antihypertensive activity (42) and peptides binding calcium (43).

Several studies have evaluated the use of aquaculture byproducts as a source of protein to produce hydrolysates. Opheim *et al.* (16), studied the hydrolysis of the viscera, skeleton and head of Atlantic salmon (*Salmo salar*), and they found that the hydrolysates from these byproducts contain high levels of proteins with a high nutritional value and potential bioactive peptides. On the other hand, Silva *et al.* (34), evaluated the viscera and carcass of Nile tilapia (*Oreochromis niloticus*) as a source for protein hydrolysates and found that according to the amino acid composition and lipid profile, these hydrolysates could be used as a protein source in diets for farm animals.

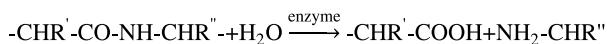
Slizyte *et al.* (44), studied the hydrolysis of thermally defatted salmon backbones yielded fish protein hydrolysates with bioactive properties, and they found correlation between the measured bioactivities, degree of hydrolysis and molecular weight profiles. Wald *et al.* (45), evaluated the antibacterial activity from trout byproducts and the hydrolysates demonstrated inhibitory activity against several Gram-positive and Gram-negative bacteria. The degree of hydrolysis (DH) was found to exert a considerable influence on antibacterial activity.

Mechanism of enzymatic hydrolysis of proteins

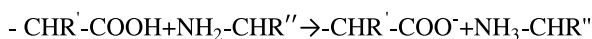
Enzymatic hydrolysis of a protein, which consists of the enzyme-catalyzed cleavage of the peptide bonds that structure the protein chains, consumes one molecule of water in a nucleophilic attack for each broken link. This results in the release of amine and terminal carboxyl groups with

the dependence of the protonation state on the pH of the medium (46).

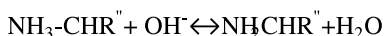
In the hydrolysis of an amide bond at an alkaline pH, the following steps are followed:



The terminal carboxyl group is completely dissociated:



The added base neutralizes the protons:



Methods of hydrolysis control

The degree of hydrolysis (GH) is commonly used to quantify the progress of the reaction, which is defined as the ratio between the number of peptide bonds released and the number of bonds present in the native protein, as shown in (1). In general, the methods for the determination of GH are based on the following: 1) estimation of soluble nitrogen, 2) determination of released α -amino groups, 3) evaluation of the proton released in the hydrolysis, and 4) decreases in the cryoscopic point (47).

$$\text{GH} = \frac{\text{number of peptide bonds hydrolyzed}}{\text{total number of peptide bonds}}$$

Among the methods frequently used for the calculation of GH is the determination of soluble nitrogen after precipitation with trichloroacetic acid (TCA), trinitrobenzenesulfonic acid (TNBS), and orthophenylaldehyde (OPA), which react with free α -amino groups. There is also titration with formaldehyde, osmometric and pH-stat titration, which is the most-used approach for enzymatic hydrolysis due to its speed and simplicity (47).

To carry out the protein hydrolysis reactions, it is necessary to define the appropriate enzyme-substrate ratio, temperature and pH conditions and the time required by the final GH as determinants of the reaction rate. The pH-stat method allows for obtaining a large amount of information over time, so it is possible to estimate the progress of the reaction in fractions of time to expand the analysis landscape and substantially reduce the amount of effort and resources needed for the study. Through the use of this method, it has been established, for example, that the concentration of available hydrolysable bonds is a factor controlling the rate

of hydrolysis, while the hydrolysis time controls the molecular weight of the peptide fractions and the type of hydrolysates in fish protein (15). In addition, it has been possible to analyze and improve the controlled recovery of peptides with biological applications (48, 49).

Kinetic modeling in the enzymatic hydrolysis of proteins

The enzymatic hydrolysis reaction of proteins is highly complex due to (i) the varied and often unknown nature of the substrates, (ii) the formation of new substrates for hydrolysis as the reaction progresses, (iii) inactivation of the enzyme over time (6), iv) effects associated with the reactivity of the linkages related to the enzyme used as well as the accessibility of specific reaction sites and (v) sometimes reactions occur in heterogeneous systems (50), which mainly occurs when muscular fractions and insoluble fat portions are hydrolyzed, as is the case for hydrolysis of some aquaculture products and byproducts.

The analysis of the development of the reaction for the study of kinetic models is conventionally evaluated with the degree of hydrolysis (GH), which includes the concentration of the intact protein and the released peptides. In this approach, Márquez and Fernández (51) and González-Tello *et al.*, (52) developed the structure of a model that considers the effects of inhibition by substrate and product as well as the contribution of enzymatic deactivation phenomena simultaneously through the experimental analysis of kinetics of enzymatic hydrolysis of proteins based on the MM equation. Thus, models of the general form of the equation (1) can be used successfully in the study of protein hydrolysis reactions. This equation models the GH as a function of the reaction time (t) as well as an independent variable along with two kinetic constants (a, b) (see equation 1). In this model, a and b can have different expressions depending on the proposed reaction mechanism, which defines the kinetic parameters of the system (Table 2). The model successfully describes the typical shape of the protein hydrolysis curve over time and was adjusted to the experimental data with high precision, combining kinetic bases, simplicity and predictability for many applications (6, 53).

$$\frac{d(\text{GH})}{dt} = a \text{ EXP}[-b(\text{GH})] \quad (2)$$

Table 2. Different kinetic parameter expressions of a and b for an exponential equation (35).

Reaction mechanism	a	b
No inhibition	$\frac{k_2 e_0}{s_0}$	$\frac{k_3 K_m}{K_2}$
Substrate-inhibition	$\frac{k_2 K_s e_0}{s_0 K_s + s_0^2}$	$\frac{k_3 K_m K_s}{K_2 (K_s + s_0)}$
Product-inhibition	$\frac{k_2 e_0 K_p}{s_0 K_s + p K_m}$	$\frac{k_3 K_m K_p s_0}{K_2 (s_0 K_p + p K_m)}$
Substrate and product-inhibitions	$\frac{k_2 e_0 K_2 K_p}{K_s K_p s_0 + K_p s_0^2 + K_m K_s P}$	$\frac{k_3 K_m K_s K_p s_0}{K_2 (K_s K_p s_0 + K_p s_0^2 + K_m K_s P)}$

Although these approaches provide useful information for understanding the enzymatic hydrolysis of proteins, they do not really clarify what happens with the products over time and also do not help to solve the technical requirements necessary to direct the hydrolysis towards obtaining peptides of specific interest. Therefore, the understanding of peptide formation and the hydrolysis dynamics are of great

Mathematical modeling in enzymatic hydrolysis with protein sources of aquaculture origin

In production processes of aquaculture residue enzymatic hydrolysis, the temperature and pH are generally adjusted to the optimum values of the selected enzyme. The E/S ratio and time are set according to the desired functionalities and final hydrolysate protein recovery (9). The definition of the working conditions that maximize the yield of these reactions is subject to the optimization of the variables for each type of enzyme and substrate that are evaluated, since each fish species has a specific amino acid composition and therefore a specific dynamic of degradation (25).

In his analysis of the kinetic mechanisms implicit in the enzymatic hydrolysis of salmon muscle, Valencia *et al.* (8) concluded that there is a decrease in the rate of hydrolysis in the reaction (typical shape of the hydrolysis curve). It is mainly due to inhibition of the reaction by hydrolysis products. Their results show that the larger peptides have a greater inhibitory effect than the smaller fractions (8). However, the inhibitory effect is not necessarily stronger for low GHs compared to the higher effects because this also depends on the concentration of these peptides (8).

Valencia *et al.* (6), suggest that it is possible to analyze the effects of temperature and enzymes as

well as substrate concentrations on reaction yield and kinetic parameters of the salmon enzymatic hydrolysis through statistical correlation structures. In this analysis, a strategy was developed that combines a conceptual model with the response surface methodology using as the analysis variable the kinetic constants expressed in the proposed deduction (51). The combined model generates quite useful information about the efficiency and extent of hydrolysis reactions that can be used to compare different sources of proteins and enzymes (6).

Valencia *et al.* (53), presented one approach that can be considered the most recent and useful model for the analysis of fish protein hydrolysis kinetics (salmon muscle) at different operating temperatures and even the behavior of a cold start (temperature profiles). The standard operation consists of preheating, isothermal stages and inactivation that makes it the most productive strategy for hydrolyzing enzymatic salmon muscle proteins with alcalase in a discontinuous reactor (53). This model contemplates the effects of inhibition by substrates, inhibition by products, enzyme inactivation and the effects of temperature (according to Arrhenius and van't Hoff) on the catalytic constant (k_{cat}), inactivation constant (k_d), Constant MM (K) and the non-competitive inhibition constant by substrate (K_S), as shown in (4).

$$\frac{dS}{dt} = \frac{K_{cat}(T)E_0 e^{-k_d(T)t} S}{K(T) \left[1 + \left(\frac{P}{K_1} \right) \right] + S \left[1 + \left(\frac{P}{K_{12}} \right) + \left(\frac{S}{K_s(T)} \right) \right]} \quad (4)$$

METHODS OF PURIFICATION

Currently, there are several pilot-scale isolation and purification technologies for bioactive peptides, such as membrane separation techniques, size-exclusion chromatography (gel filtration), ion

exchange chromatography, affinity chromatography, hydrophilic interaction liquid chromatography (HILIC) and reversed phase HPLC (RP-HPLC). These approaches are based on molecular properties such as size, charge, and polarity or hydrophobicity (17, 18, 54, 55). Sometimes is necessary to use diverse methods to achieve a separation of bioactive peptides because several physical, chemical and biological principles are responsible for the interaction between molecules (56).

Chromatography-related technologies are the most common methods for separation of

natural products. The separation efficiency of targeted compounds is highly dependent on their adsorption affinity to the stationary phase (17). The resulting degree of separation or selectivity between constituent solutes in a mixture is thus a subtle interplay between the relative affinity of the molecules for the stationary phase and the degree of diffusive processes that occur during separation. Some authors have evaluated chromatographic techniques to purify and identify bioactive peptides from aquaculture byproducts, as shown in Table 3.

Table 3. Chromatographic techniques for the purification and identification of bioactive peptides from aquaculture byproducts.

Method of purification	Species	Byproduct	Purified bioactive peptides	Reference
Size-exclusion and RP - HPLC	Snakehead fish (<i>Channidae sp</i>)	Muscle	Angiotensin I-converting enzyme (ACE) inhibitory: <i>Leu-Tyr-Pro-Pro-Pro</i> ; <i>Tyr-Ser-Met-Tyr-Pro-Pro</i> .	(57)
	Small-spotted catshark (<i>Scyliorhinus canicula</i>)	Muscle, viscera, skin and frame	Angiotensin I-converting enzyme (ACE) inhibitory: <i>Val-Ala-Met-Pro-Phe</i> .	(58)
	Grass carp (<i>Ctenopharyngodon idella</i>)	Skin	Antioxidant: <i>Pro-Tyr-Ser-Phe-Lys</i> , <i>Gly-Phe-Gly-Pro-Glu-Leu</i> and <i>Val-Gly-Gly-Arg-Pro</i> .	(59)
	Squid (<i>Ommastrephes bartrami</i>)	Viscera	Antioxidant: <i>Trp-Val-Ala-Pro-Leu-Lys</i>	(60)
Size-exclusion, Ion exchange and RP-HPLC	Lizard fish (<i>Saurida elongata</i>)	Muscle	Angiotensin I-converting enzyme (ACE) inhibitory: <i>Arg-Val-Cys-Leu-Pro</i> .	(61)
Ion exchange y and RP-HPLC	Salmon (<i>Salmo sp.</i>)	Fin	Antioxidant: <i>Phe-Leu-Asn-Glu-Phe-Leu-His-Val</i> .	(62)
Ion exchange, size-exclusion and reversed phase HPLC	Bluefin leatherjacket (<i>Navodon septentrionalis</i>)	Head	Antioxidant: <i>Trp-Glu-Gly-Pro-Lys</i> , <i>Gly-Pro-Pro</i> , and <i>Gly-Val-Pro-Leu-Thr</i> .	(63)

Membrane technologies, such as ultra- and nanofiltration, have been used to concentrate, purify and fractionate byproduct protein hydrolysates with the aim of improving their biological and functional properties (17). Also, these approaches offer a good alternative to traditional separation techniques to achieve a more environmentally friendly and cost-effective process (14, 15). The membrane technology is used to obtain fractions of hydrolysates or peptides with a desired molecular size or with a certain functional property and/or activity (18, 37).

Membrane technologies coupled to a bioreactor for enzymatic hydrolysis may improve the conversion rate of substrate, the utilization rate of the enzyme, and the yield of the product by the integration of substrate hydrolysis, enzyme recovery and product separation into a single process compared to the traditional system (14, 64). Several studies refer to the use of ultrafiltration and nanofiltration for purifying fish protein hydrolysates and increasing their specific bioactivity, as shown in Table 4.

Table 4. Membrane technology to purify fish protein hydrolysates.

Method of purification	Molecular weight cut-off (MWCO)	Species	Byproduct	Reference
Ultrafiltration	<1 kDa	Salmon (<i>Salmo salar</i>)	Muscle	(65)
	10 kDa and 3 kDa	Blue mussel (<i>Mytilus edulis</i>)	Muscle	(12)
	<1 kDa	Cod (<i>Gadus morhua</i>)	Frame	(66)
	10 kDa and 5 kDa	Flounder (<i>Paralichthys olivaceus</i>)	Muscle	(67)
	5 kDa and 3 kDa	Cod (<i>Gadus morhua</i>)	MariPep C (specific parts from Cod)	(68)
	10 kDa and 5 kDa	Red tilapia (<i>Oreochromis niloticus</i>)	Head, frames, and tail	(69)
Ultrafiltration and nanofiltration	4 kDa and 1 kDa	Tuna	Dark muscle	(14, 15)
	4 kDa and 1 kDa	Cuttlefish (<i>Sepia officinalis</i>)	Viscera	(26)

Potential applications of aquaculture byproduct hydrolysates

Bioactive peptides

Currently, protein hydrolysates of aquaculture byproducts are one of the most important sources of bioactive peptides (8, 11). These hydrolysates are usually composed of 2 to 20 amino acids, which usually have hydrophobic amino acid residues in addition to proline, lysine and arginine (2, 3).

Some peptides have been recognized as having various biological functions, including angiotensin-converting enzyme (ACE) inhibition, which helps regulate arterial pressure because it catalyzes the conversion of angiotensin-I (the inactive form) to angiotensin-II (a vasoconstrictor) and inactivates bradykinin (a vasodilator) (20, 55). Antioxidant

activity, which has a positive effect on human health, protects the body from the damage caused by free radicals and reactive oxygen species, which cause damage to DNA and generate neurodegenerative, inflammatory, and cardiovascular diseases, as well as diabetes and cancer (62, 70). Anticoagulant activity, as the name implies, is used for therapeutic purposes to prevent the clotting of blood, which prevents the formation of clots or prevents their growth and favors their dissolution (disappearance) should they have already formed (40). Another type of activity is calcium chelating, which is very useful because calcium is necessary for intracellular metabolism, bone growth, blood clotting, nerve conduction, muscle contraction and cardiac function (43). Table 5 shows the bioactivities found in protein hydrolysates of aquaculture byproducts.

Table 5. Bioactivities from protein hydrolysate aquaculture byproducts.

Bioactivity	Species	Byproduct	Reference
Angiotensin I-converting enzyme (ACE) inhibitory	Lizard fish (<i>Saurida elongata</i>)	Muscle	(54, 61)
	Mrigal (<i>Cirrhinus mrigala</i>)	Muscle	(71)
	Cuttlefish (<i>Sepia officinalis</i>)	Muscle	(72)
	Pacific cod (<i>Gadus macrocephalus</i>)	Skin	(73)
	Steelhead (<i>Oncorhynchus mykiss</i>)	Skin	(74)
	Leatherjacket (<i>Meuschenia sp.</i>)	Whole	(75)
	Grass carp (<i>Ctenopharyngodon idella</i>)	Skin	(76)

Bioactivity	Species	Byproduct	Reference
Antioxidant	Silver carp (<i>Hypophthalmichthys molitrix</i>)	Muscle	(77)
	Catshark (<i>Scyliorhinus canicular</i>)	Heads, viscera, frames, skin, trimmings	(78)
	Indian mackerel (<i>Rastrelliger kanagurta</i>)	Backbones	(79)
	Cod (<i>Gadus morhua</i>)	MariPep C (specific parts from Cod)	(80)
	Blue mussel (<i>Mytilus edulis</i>)	Muscle	(12)
	Patin (<i>Pangasius sutchi</i>)	Muscle	(81)
	Grass carp (<i>Ctenopharyngodon idella</i>)	Skin	(59)
	Bluefin leatherjacket (<i>Navodon septentrionalis</i>)	Skin	(82)
	Bluefin leatherjacket (<i>Navodon septentrionalis</i>)	Heads	(63)
Anticoagulant	<i>Hypomesus olidus</i>	Whole	(83)
	Blue mussel (<i>Mytilus edulis</i>)	Muscle	(84)
Calcium chelating	Atlantic salmon (<i>Salmo salar</i> L.)	Bones	(85)
	Nile tilapia (<i>Oreochromis niloticus</i>)	Scale	(39, 43).

Functional properties

There is a potential application for protein hydrolysates from aquaculture byproducts as functional ingredients in different food formulations because they have desirable properties such as protein solubility, foaming capacity, water holding capacity and emulsifying activity (86, 87). Proteins from fish hydrolysates are good emulsifiers due to their amphiphilic nature because they expose more hydrophilic and hydrophobic groups that allow for orientation at the oil-water interface for more efficient adsorption (11). There are some investigations in which functional properties have been evaluated in different species such as solubility with surimi processed (11), interfacial tension with muscle of sardine (*Sardina pilchardus*) and mackerel (*Trachurus mediterraneus*) (25).

Abdollahi *et al.* (88) found the addition of collagen hydrolysate to silver carp (*Hypophthalmichthys molitrix*) protein isolate gel impacted its gel strength and water holding capacity but the effect was dependent of the molecular weight of the hydrolysate. Collagen hydrolysate produced by sequential hydrolysate containing lower molecular weight peptides reduced the breaking force of the gel while it improved its water holding capacity. On the other hand, Jridi *et al.* (89), found cuttlefish skin gelatin hydrolysates increased its solubility with the increase of the degree of hydrolysis but, the increasing of degree of hydrolysis decreases the foaming capacity.

Nutritional protein supplement and commercial products

The growing interest of consumers to meet their nutritional demands with products that pose no risk to their health and come from natural sources makes bioactive peptides a promising product in various markets (90). Hydrolysates from fish proteins have been shown to be a viable resource for nutritional and pharmaceutical applications because they are a source of available amino acids for different physiological functions (91).

Supplementation with protein hydrolysates represents a good complement to diets in states of malnutrition because their bioactive ingredients are easily absorbed due to the high content of di- and tripeptides that are better assimilated by organisms compared to native protein (92). In a study conducted by Landsberg *et al.* (93), fish hydrolysate has anxiolytic properties in dogs, which appear to be manifested by decreased hyperactivity and a reduced cortisol response to stress.

The inclusion of fish byproducts generated by enzymatic hydrolysis represents an advantage because fish proteins contain bioactive peptides that are easily absorbed and can be used for numerous metabolic activities (94). Often, peptides have several bioactivities, are multifunctional and can have more than one effect. Currently, there are products from fish proteins that are commercially produced. In Table 6, some of them are listed with their applications.

Table 6. Commercial products from fish protein hydrolysates

Commercial brand	Manufacturer	Source	Application	Presentation	Country	Information
Custom Collagen®	Custom Collagen	Tilapia	Pharmaceutical and cosmetic use	Powder	United States	www.customcollagen.com
Hydrolyzed Fish Collagen Type 1	Swanson Health Products	Tilapia	Therapeutic	Capsules	United Kingdom	www.swansonvitamins.com
Hydrolyzed Fish Fertilizer	Organic Liquid Fertilizer	NA	Agricultural	Liquid	United States	www.bettervegetablegardening.com
Levenorm®	Ocean Nutrition Canada Ltd.	Sarda	Antihypertensive	NA	Canada	www.trademarkia.com
MOLVAL®	Dielen	Molva	Dietary Supplement	Capsules	France	www.dielen.fr
Norland Hydrolyzed Fish Collagen	Norland Products Incorporated	Cod	Pharmaceutical and Food use	Powder	United States	www.norlandprod.com
PeptACE®	Natural Factors Nutritional Products Ltd.	Sarda	Antihypertensive	Capsules	Canada and United States	www.naturalfactors.com
Protizen®	Copalis Sea Solutions	NA	Anti-stress	Powder	France	www.copalis.fr
Seacure®	Proper Nutrition, Inc.	Hake	Pharmaceutic	Capsules	United States	www.propernutrition.com
Seagest™	Natural Balance	White fish	Dietary Supplement	Capsules	United States	www.naturalbalance.com
Valtyron®	Senmi Ekiu Co., Ltd.	Sardine	Food	Powder	Japan	www.markhound.com
Vasotensin®	Metagenics	Tuna and verdel	Pharmaceutic	Tablets	United States	www.metagenics.com

NA: Information not available.

CONCLUSIONS

This review shows advances in the use of protein from byproducts of the aquaculture industry using chemical and enzymatic processes to obtain products with high added value. In addition, it is possible to conclude that the revaluation of waste from the aquaculture industry is a topic with projection for research and industrial developments around the world due to the number of authors and companies that have studied and evaluated the use of these byproducts.

Enzymatic hydrolysis is the preferred method for the production of bioactive peptides with defined characteristics that are versatile and important compounds that protect the body from different diseases. However, it is advisable to extend the research for its industrial production, mechanism and bioavailability in the gastrointestinal tract, in addition to stability studies.

CONFLICT OF INTEREST

The authors declare that they do not have any conflict of interest.

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AUTHORS CONTRIBUTION

Acquisition of data: Sierra-Lopera L., Sepulveda-Rincón C., Vasquez-Mazo P., Figueroa-Moreno O., Zapata-Montoya J. Writing: Sierra-Lopera L., Sepulveda-Rincón C., Vasquez-Mazo P., Figueroa-Moreno O., Zapata-Montoya J.

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