VITAE, REVISTA DE LA FACULTAD DE CIENCIAS FARMACÉUTICAS Y ALIMENTARIAS
ISSN 0121-4004 / ISSNe 2145-2660. Volumen 25 número 3, año 2018
Universidad de Antioquia, Medellín, Colombia. Págs. 128-140
DOI: http://dx.doi.org/10.17533/udea.vitae.v25n3a03

BYPRODUCTS OF AQUACULTURE PROCESSES: DEVELOPMENT AND PROSPECTIVE USES. REVIEW

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

Leidy Maritza SIERRA LOPERA[1*]PhD candidate, Cindy Tatiana SEPÚLVEDA RINCÓN[1] PhD candidate, Priscilla VÁSQUEZ MAZO[1] PhD candidate, Omar Alfredo FIGUEROA MORENO[2] PhD candidate, José Edgar ZAPATA MONTOYA[1] PhD[1]

Received: June 07 of 2018. Approved: November 29 of 2018

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 revaluarse 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

[1] Facultad de Ciencias Farmacéuticas y Alimentarias. Universidad de Antioquia. Medellín, Colombia

[2] Facultad de Ingeniería. Universidad de la Guajira, Km 5 Vía Maicao, Riohacha, Colombia.

[*] Autor de correspondencia: maritza.sierra@udea.edu.co

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 anti-carcinogenic 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 exclused articules 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 (*Merluccius hubbsi*)	Gonad	21.95	10.92	68.72	11.61	(21)
	Liver	16.38	29.71	55.79	1.61	
Tilapia (*Oreochromis nilotica)*	Skeletons	50.6	30.6	65.3	15.3	(22)
Cape hake (*Merluccius capensis*)	Byproducts	18.0	1.1	78.5	1.9	(23)
Atlantic salmon (*Salmo salar*)	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 (*Engraulis encrasicholus*)	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 (*Trachurus mediterraneus*)	Muscle	21.4	1.0	77.5	1.5	(25)
Sardine (*Sardina pilchardus*)	Muscle	18.8	1.2	78.1	1.5	
Cuttlefish (*Sepia officinalis*)	Viscera	17.45 ± 0.25	4.78 ± 0.7	74.99 ± 0.1	1.95 ± 0.0	(26)
Yellowfin tuna (*Thunnus albacares*)	Skin	32.38	3.22	0.67	62.57	(27)
Blue shark (*Prionace glauca*)		22.79	0.24	76.03	4.24	
Greenland halibut (*Reinhardtius hippoglossoides*)		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 a 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:

$$\text{-CHR}'\text{-CO-NH-CHR}''\text{-}+H_2O \xrightarrow{\text{enzyme}} \text{-CHR}'\text{-COOH}+NH_2\text{-CHR}''$$

The terminal carboxyl group is completely dissociated:

$$\text{- CHR}'\text{-COOH}+NH_2\text{-CHR}'' \rightarrow \text{-CHR}'\text{-COO}^-+NH_3\text{-CHR}''$$

The added base neutralizes the protons:

$$NH_3\text{-CHR}''+ OH^- \leftrightarrow NH_2CHR''+H_2O$$

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).

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

Among the methods frequently used for the calculation of GH is the determination of soluble nitrogen after precipitation with tricloacetic 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).

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

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	$\frac{k_2 e_0 K_2 K_p}{{}_\circ K_s K_p s_0 + K_p s_0^2 + K_m K_s P}$	$\frac{k_3 K_m K_s K_p s_0}{{}_\circ K_2(K_s K_p s_0 + K_p s_0^2 + K_m K_s P)}$

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 (kcat), inactivation constant (kd), Constant MM (K) and the non-competitive inhibition constant by substrate (KS), as shown in (4).

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

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 (*Channidae sp*)	Muscle	Angiotensin I-converting enzyme (ACE) inhibitory: *Leu-Tyr-Pro-Pro-Pro; Tyr-Ser-Met-Tyr-Pro-Pro.*	(57)
	Small-spotted catshark (*Scyliorhinus canicula*)	Muscle, viscera, skin and frame	Angiotensin I-converting enzyme (ACE) inhibitory*: Val-Ala-Met-Pro-Phe.*	(58)
	Grass carp (*Ctenopharyngodon idella*)	Skin	Antioxidant: *Pro-Tyr-Ser-Phe-Lys, Gly-Phe-Gly-Pro-Glu-Leu* and *Val-Gly-Gly-Arg-Pro*.	(59)
	Squid (*Ommastrephes bartrami*)	Viscera	Antioxidant: *Trp-Val-Ala-Pro-Leu-Lys*	(60)
Size-exclusion, Ion exchange and RP–HPLC	Lizard fish (*Saurida elongata*)	Muscle	Angiotensin I-converting enzyme (ACE) inhibitory*: Arg-Val-Cys-Leu-Pro.*	(61)
Ion exchange y and RP-HPLC	Salmon (*Salmo sp.*)	Fin	Antioxidant: *Phe-Leu-Asn-Glu-Phe-Leu-His-Val.*	(62)
Ion exchange, size-exclusion and reversed phase HPLC	Bluefin leatherjacket (*Navodon septentrionalis*)	Head	Antioxidant: *Trp-Glu-Gly-Pro-Lys, Gly-Pro-Pro*, and *Gly-Val-Pro-Leu-Thr.*	(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 (*Salmo salar*)	Muscle	(65)
	10 kDa and 3 kDa	Blue mussel (*Mytilus edulis*)	Muscle	(12)
	<1 kDa	Cod (*Gadus morhua*)	Frame	(66)
	10 kDa and 5 kDa	Flounder (*Paralichthys olivaceus*)	Muscle	(67)
	5 kDa and 3 kDa	Cod (*Gadus morhua*)	MariPep C (specific parts from Cod)	(68)
	10 kDa and 5 kDa	Red tilapia (*Oreochromis niloticus*)	Head, frames, and tail	(69)
Ultrafiltration and nanofiltration	4 kDa and 1 kDa	Tuna	Dark muscle	(14, 15)
	4 kDa and 1 kDa	Cuttlefish (*Sepia officinalis*)	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 (*Saurida elongata*)	Muscle	(54, 61)
	Mrigal *(Cirrhinus mrigala)*	Muscle	(71)
	Cuttlefish (*Sepia officinalis*)	Muscle	(72)
	Pacific cod (*Gadus macrocephalus*)	Skin	(73)
	Steelhead (*Oncorhynchus mykiss*)	Skin	(74)
	Leatherjacket (*Meuchenia sp*.)	Whole	(75)
	Grass carp (*Ctenopharyngodon idella*)	Skin	(76)

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

ACKNOWLEDGMENTS

The authors acknowledge the funding to University of Antioquia, through their strategy Sostenibilidad.

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.

REFERENCES

1. FAO. The State of World Fisheries and Aquaculture. 2014. Rome. 223pp. (also available at www.fao.org/3/a-i3720e/index.html).
2. Saadi S, Saari N, Anwar F, Abdul Hamid A, Ghazali HM. Recent advances in food biopeptides: production, biological functionalities and therapeutic applications. Biotechnol Adv. 2015; 33(1):80-116.
3. Martínez-Álvarez O, Chamorro S, Brenes A. Protein hydrolysates from animal processing byproducts as a source of bioactive molecules with interest in animal feeding: a review. Food Res Int. 2015; 73:204-12.
4. Abejón R, Belleville MP, Sánchez-Marcano J, Garea A, Irabien A. Optimal design of industrial scale continuous process for fractionation by membrane technologies of protein hydrolysate derived from fish wastes. Sep Purif. Technol. 2018; 197:137-46.
5. Wisuthiphaet N, Kongruang S, Chamcheun C. Production of fish protein hydrolysates by acid and enzymatic hydrolysis. J Med Biol Eng. 2015; 4(6):466-70.
6. Valencia P, Espinoza K, Ceballos A, Pinto M, Almonacid S. Novel modeling methodology for the characterization of enzymatic hydrolysis of proteins. Process Biochem. 2015; 50(4):589-97.
7. Shahidi F, Ambigaipalan P. Novel functional food ingredients from marine sources. Curr Opin Food Sci. 2015; 2:123-9.
8. Valencia P, Pinto M, Almonacid S. Identification of the key mechanisms involved in the hydrolysis of fish protein by Alcalase. Process Biochem. 2014; 49(2):258-64.
9. He S, Franco C, Zhang W. Functions, applications and production of protein hydrolysates from fish processing co-products (FPCP). Food Res Int. 2013; 50(1):289-97.
10. Pérez-Galvez R, Garcia-Moreno P, Huong N, M. Guadix E, Guadix A, Bergé J-P. Multiobjective optimization of a pilot plant to process fish discards and byproducts on board. Clean Techn Environ Policy. 2016; 18(3):935-48.
11. Liu Y, Li X, Chen Z, Yu J, Wang F, Wang J. Characterization of structural and functional properties of fish protein hydrolysates from surimi processing byproducts. Food Chem. 2014; 151:459-65.
12. Wang B, Li L, Chi CF, Ma JH, Luo HY, Xu YF. Purification and characterisation of a novel antioxidant peptide derived from blue mussel (*Mytilus edulis*) protein hydrolysate. Food Chem. 2013; 138(2-3):1713-9.
13. Picot L, Ravallec R, Fouchereau-Peron M, Vandanjon L, Jaouen P, Chaplain-Derouiniot M, *et al*. Impact of ultrafiltration and nanofiltration of an industrial fish protein hydrolysate on its bioactive properties. J. Sci. Food Agric. 2010; 90(11):1819-26.
14. Saidi S, Deratani A, Ben Amar R, Belleville MP. Fractionation of a tuna dark muscle hydrolysate by a two-step membrane process. Sep. Purif Technol. 2013; 108:28-36.
15. Saidi S, Deratani A, Belleville MP, Amar RB. Production and fractionation of tuna by-product protein hydrolysate by ultrafiltration and nanofiltration: Impact on interesting peptides fractions and nutritional properties. Food Res. Int. 2014; 65, (Part C): 453-61.
16. Opheim M, Šližytė R, Sterten H, Provan F, Larssen E, Kjos NP. Hydrolysis of Atlantic salmon (*Salmo salar*) rest raw materials-effect of raw material and processing on composition, nutritional value, and potential bioactive peptides in the hydrolysates. Process Biochem. 2015; 50 (8): 1247-57.
17. Ren Q, Xing H, Bao Z, Su B, Yang Q, Yang Y, *et al*. Recent advances in separation of bioactive natural products. Chin. J. Chem. Eng. 2013; 21 (9): 937-52.
18. Li-Chan ECY. Bioactive peptides and protein hydrolysates: research trends and challenges for application as nutraceuticals and functional food ingredients. Curr Opin Food Sci. 2015; (1): 28-37.
19. Ghaly AE, Ramakrishnan VV, Brooks MS, Budge SM, Dave D. Fish processing wastes as a potential source of proteins, amino acids and oils: a critical review. J Microb Biochem Techno. 2013; 5(4): 107-29.
20. Benjakul S, Yarnpakdee S, Senphan T, Halldorsdottir SM, Kristinsson HG. Fish protein hydrolysates: production, bioactivities, and applications. Antioxidants and functional components in aquatic foods. London, England: John Wiley & Sons; 2014. p. 237-81.
21. Leonarduzzi E, Rodrigues KA, Macchi GJ. Proximate composition and energy density in relation to Argentine hake females (*Merluccius hubbsi*) morphometrics and condition indices. Fish Res. 2014; 160: 33-40.
22. Chuesiang P, Sanguandeekul R. Protein hydrolysate from tilapia frame: antioxidant and angiotensin I converting enzyme inhibitor properties. Int J Food Sci Technol. 2015; 50 (6): 1436-44.
23. Pires C, Teixeira B, Cardoso C, Mendes R, Nunes ML, Batista I. Cape hake protein hydrolysates prepared from alkaline solubilised proteins pre-treated with citric acid and calcium ions: Functional properties and ACE inhibitory activity. Process Biochem. 2015; 50 (6): 1006-15.
24. Gencbay G, Turhan S. Proximate composition and nutritional profile of the black sea anchovy (*Engraulis encrasicholus*) whole fish, fillets, and byproducts. J Aquat Food Prod T. 2016; 25 (6): 864-74.
25. Morales-Medina R, Tamm F, Guadix AM, Guadix EM, Drusch S. Functional and antioxidant properties of hydrolysates of sardine (*S. pilchardus*) and horse mackerel (*T. mediterraneus*) for the microencapsulation of fish oil by spray-drying. Food Chem. 2016; 194: 1208-16.
26. Soufi-Kechaou E, Derouiniot-Chaplin M, Ben Amar R, Jaouen P, Berge JP. Recovery of valuable marine compounds from cuttlefish by-product hydrolysates: combination of enzyme bioreactor and membrane technologies. C. R. Chimie. 2017; 20(9-10): 975-85.
27. Sousa SC, Vazquez JA, Pérez-Martín RI, Carvalho AP, Gomes AM. Valorization of byproducts from commercial fish species: extraction and chemical properties of skin gelatins. Molecules. 2017; 22 (1545): 1-12.
28. Lynch SA, Álvarez C, O'Neill EE, Keenan DF, Mullen AM. Optimization of protein recovery from bovine lung by pH shift process using response surface methodology. J. Sci. of Food Agric. 2018; 98 (5): 1951-60.
29. Anal AK, Noomhorm A, Vongsawasdi P. Protein hydrolysates and bioactive peptides from seafood and crustacean waste: Their extraction, bioactive properties and industrial perspectives. Marine Proteins and Peptides: Biological Activities and Applications. 2013:709-35.
30. Chomnawang C, Yongsawatdigul J. Protein recovery of tilapia frame byproducts by pH-shift method. J Aquat Food Prod T. 2013; 22(2): 112-20.
31. Guo L, Hou H, Li B, Zhang Z, Wang S, Zhao X. Preparation, isolation and identification of iron-chelating peptides derived from Alaska pollock skin. Process Biochem. 2013; 48 (5-6): 988-93.
32. Nikoo M, Benjakul S, Xu X. Antioxidant and cryoprotective effects of *Amur sturgeon* skin gelatin hydrolysate in unwashed fish mince. Food chem. 2015; 181: 295-303.
33. Abdelhedi O, Nasri R, Mora L, Toldrá F, Nasri M, Jridi M. Collagenous proteins from black-barred halfbeak skin as a source of gelatin and bioactive peptides. Food Hydrocoll. 2017; 70: 123-33.
34. Silva JFX, Ribeiro K, Silva JF, Cahú TB, Bezerra RS. Utilization of tilapia processing waste for the production of fish protein hydrolysate. Anim. Feed Sci. Technol. 2014; 196: 96-106.
35. Qi W, He Z. Enzymatic hydrolysis of protein: mechanism and kinetic model. Front. Chem. China. 2006; 1 (3): 308-14.
36. Qu W, Ma H, Zhao W, Pan Z. ACE-inhibitory peptides production from defatted wheat germ protein by continuous coupling of enzymatic hydrolysis and membrane separation:

modeling and experimental studies. Chem Eng J. 2013; 226: 139-45.
37. Wang C, Chang T, Shi L, Yang H, Cui M, Tambalu L. Seafood processing byproducts: collagen and gelatin. New York, United Stated: Se-Kwon Kim, 2014:p.207-42.
38. Najafian L, Babji AS. Production of bioactive peptides using enzymatic hydrolysis and identification antioxidative peptides from patin (*Pangasius sutchi*) sarcoplasmic protein hydolysate. J Funct Foods. 2014; 9: 280-89.
39. Chen D, Mu X, Huang H, Nie R, Liu Z, Zeng M. Isolation of a calcium-binding peptide from tilapia scale protein hydrolysate and its calcium bioavailability in rats. J Funct Foods. 2014; 6: 575-84.
40. Ren Y, Wu H, Lai F, Yang M, Li X, Tang Y. Isolation and identification of a novel anticoagulant peptide from enzymatic hydrolysates of scorpion (*Buthus martensii* Karsch) protein. Food Res. Int. 2014; 64: 931-38.
41. Wang L, Ding Y, Zhang X, Li Y, Wang R, Luo X, *et al*. Isolation of a novel calcium-binding peptide from wheat germ protein hydrolysates and the prediction for its mechanism of combination. Food Chem. 2018; 239: 416-26.
42. Zarei M, Forghani B, Ebrahimpour A, Abdul-Hamid A, Anwar F, Saari N. In vitro and in vivo antihypertensive activity of palm kernel cake protein hydrolysates: Sequencing and characterization of potent bioactive peptides. Ind Crop Prod. 2015; 76:112-20.
43. Nie R, Liu Y, Liu Z. The calcium-binding activity of fish scale protein hydrolysates. J Agric Chem Environ. 2014; 03(01):11-5.
44. Slizyte R, Rommi K, Mozuraityte R, Eck P, Five K, Rustad T. Bioactivities of fish protein hydrolysates from defatted salmon backbones. Biotechnol Rep. 2016; 11:99-109.
45. Wald M, Schwarz K, Rehbein H, Bubmann B, Beermann C. Detection of antibacterial activity of an enzymatic hydrolysate generated by processing rainbow trout byproducts with trout pepsin. Food Chem. 2016; 205:221-8.
46. Alder-Nissen J. Enzymatic hydrolysis of food proteins. Barking, UK. Elsevier Applied Science Publishers; 1986. 110 p.
47. Rutherfurd S. Methodology for determining degree of hydrolysis of proteins in hydrolysates: a review. J AOAC Int. 2010; 93(5):1515-22.
48. Benhabiles MS, Abdi N, Drouiche N, Lounici H, Pauss A, Goosen MFA, *et al*. Fish protein hydrolysate production from sardine solid waste by crude pepsin enzymatic hydrolysis in a bioreactor coupled to an ultrafiltration unit. Mater Sci Eng C. 2012; 32(4):922-8.
49. Vandanjon L, Grignon M, Courois E, Bourseau P, Jaouen P. Fractionating white fish fillet hydrolysates by ultrafiltration and nanofiltration. J Food Eng. 2009; 95(1):36-44.
50. Fernandez A, Riera F. β-Lactoglobulin tryptic digestion: a model approach for peptide release. Biochem Eng J. 2013; 70:88-96.
51. Márquez MC, Fernández V. Enzymic hydrolysis of vegetable proteins: mechanism and kinetics. Process Biochem. 1993; 28(7):481–90.
52. Gonzalez-Tello P, Camacho F, Jurado E, Paez M, Guadix E. Enzymatic hydrolysis of whey proteins: I. Kinetic models. Biotechnol Bioeng. 1994; 44(4):523-8.
53. Valencia P, Pinto M, Almonacid S. Analysis of the operational strategies for the enzymatic hydrolysis of food proteins in batch reactor. J Food Eng. 2016; 176:121-7.
54. Lan X, Liao D, Wu S, Wang F, Sun J, Tong Z. Rapid purification and characterization of angiotensin converting enzyme inhibitory peptides from lizard fish protein hydrolysates with magnetic affinity separation. Food Chem. 2015; 182:136-42.
55. Soares de Castro RJ, Sato HH. Biologically active peptides: processes for their generation, purification and identification and applications as natural additives in the food and pharmaceutical industries. Food Res Int. 2015; 74:185-98.
56. Agyei D, Ongkudon CM, Wei CY, Chan AS, Danquah MK. Bioprocess challenges to the isolation and purification of bioactive peptides. Food Bioprod Process. 2016; 98:244-56.
57. Ghassem M, Babji AS, Said M, Mahmoodani F, Arihara K. Angiotensin I-converting enzyme inhibitory peptides from snakehead fish sarcoplasmic protein hydrolysate. J Food Biochem. 2014; 38(2):140-9.
58. García-Moreno PJ, Espejo-Carpio FJ, Guadix A, Guadix EM. Production and identification of angiotensin I-converting enzyme (ACE) inhibitory peptides from Mediterranean fish discards. J Funct Foods. 2015; 18:95-105.
59. Cai L, Wu X, Zhang Y, Li X, Ma S, Li J. Purification and characterization of three antioxidant peptides from protein hydrolysate of grass carp (*Ctenopharyngodon idella*) skin. J Funct Foods. 2015; 16:234-42.
60. Song R, Zhang K, Wei R. In vitro antioxidative activities of squid (*Ommastrephes bartrami*) viscera autolysates and identification of active peptides. Process Biochem. 2016; 51(10):1674-82.
61. Wu S, Feng X, Lan X, Xu Y, Liao D. Purification and identification of Angiotensin-I Converting Enzyme (ACE) inhibitory peptide from lizard fish (*Saurida elongata*) hydrolysate. J Funct Foods. 2015; 13:295-9.
62. Ahn CB, Kim JG, Je JY. Purification and antioxidant properties of octapeptide from salmon byproduct protein hydrolysate by gastrointestinal digestion. Food Chem. 2014; 147:78-83.
63. Chi CF, Wang B, Wang YM, Zhang B, Deng SG. Isolation and characterization of three antioxidant peptides from protein hydrolysate of bluefin leatherjacket (*Navodon septentrionalis*) heads. J Funct Foods. 2015; 12:1-10.
64. Qu W, Ma H, Li W, Pan Z, Owusu J, Venkitasamy C. Performance of coupled enzymatic hydrolysis and membrane separation bioreactor for antihypertensive peptides production from *Porphyra yezoensis* protein. Process Biochem. 2015; 50(2):245-52.
65. Girgih AT, Udenigwe CC, Hasan FM, Gill TA, Aluko RE. Antioxidant properties of Salmon (*Salmo salar*) protein hydrolysate and peptide fractions isolated by reverse-phase HPLC. Food Res Int. 2013; 52(1):315-22.
66. Girgih AT, He R, Hasan FM, Udenigwe CC, Gill TA, Aluko RE. Evaluation of the in vitro antioxidant properties of a cod (*Gadus morhua*) protein hydrolysate and peptide fractions. Food Chem. 2015; 173:652-9.
67. Ko JY, Kang N, Lee JH, Kim JS, Kim WS, Park SJ, *et al*. Angiotensin I-converting enzyme inhibitory peptides from an enzymatic hydrolysate of flounder fish (*Paralichthys olivaceus*) muscle as a potent anti-hypertensive agent. Process Biochem. 2016; 51(4):535-41.
68. Habebullah SF, Andersen LL, Otte J, Nielsen HH, Jessen F, Jacobsen C. Antioxidant activity of cod (*Gadus morhua*) protein hydrolysates: Fractionation and characterisation of peptide fractions: fractionation and characterisation of peptide fractions. Food Chem. 2016; 204:409-19.
69. Roslan J, Mustapa SM, Md. Yunos KF, Abdullah N. Assessment on multilayer ultrafiltration membrane for fractionation of tilapia by-product protein hydrolysate with angiotensin I-converting enzyme (ACE) inhibitory activity. Sep Purif Techn. 2017; 173:250-7.
70. Ghribi AM, Sila A, Przybylski R, Nedjar-Arroume N, Makhlouf I, Blecker C, *et al* Purification and identification of novel antioxidant peptides from enzymatic hydrolysate of chickpea (*Cicer arietinum* L.) protein concentrate. J Funct Foods. 2015; 12:516-25.
71. Elavarasan K, Shamasundar B, Badii F, Howell N. Angiotensin I-converting enzyme (ACE) inhibitory activity and structural properties of oven-and freeze-dried protein hydrolysate from fresh water fish (*Cirrhinus mrigala*). Food Chem. 2016; 206:210-6.
72. Balti R, Bougatef A, Sila A, Guillochon D, Dhulster P, Nedjar-Arroume N. Nine novel angiotensin I-converting enzyme (ACE)

inhibitory peptides from cuttlefish (*Sepia officinalis*) muscle protein hydrolysates and antihypertensive effect of the potent active peptide in spontaneously hypertensive rats. Food Chem. 2015; 170:519-25.

73. Ngo D-H, Vo T-S, Ryu B, Kim S-K. Angiotensin-I-converting enzyme (ACE) inhibitory peptides from Pacific cod skin gelatin using ultrafiltration membranes. Process Biochem. 2016; 51(10):1622-8.
74. Cheung IW, Li-Chan EC. Enzymatic production of protein hydrolysates from steelhead (*Oncorhynchus mykiss*) skin gelatin as inhibitors of dipeptidyl-peptidase IV and angiotensin-I converting enzyme. J Funct Foods. 2017; 28:254-64.
75. Salampessy J, Reddy N, Phillips M, Kailasapathy K. Isolation and characterization of nutraceutically potential ACE-Inhibitory peptides from leatherjacket (*Meuchenia* sp.) protein hydrolysates. LWT-Food Sci Technol. 2017; 80:430-6.
76. Yi J, De Gobba C, Skibsted LH, Otte J. Angiotensin-I converting enzyme inhibitory and antioxidant activity of bioactive peptides produced by enzymatic hydrolysis of skin from grass carp (*Ctenopharyngodon idella*). Int J Food Prop. 2017; 20(5):1129-44.
77. Malaypally SP, Liceaga AM, Kim KH, Ferruzzi M, Martin FS, Goforth RR. Influence of molecular weight on intracellular antioxidant activity of invasive silver carp (*Hypophthalmichthys molitrix*) protein hydrolysates. J Funct Foods. 2014; 18:1158-66.
78. Vázquez JA, Blanco M, Massa AE, Amado IR, Pérez-Martín RI. Production of fish protein hydrolysates from *Scyliorhinus canicula* discards with antihypertensive and antioxidant activities by enzymatic hydrolysis and mathematical optimization using response surface methodology. Mar drugs. 2017; 15(10):306.
79. Sheriff SA, Sundaram B, Ramamoorthy B, Ponnusamy P. Synthesis and in vitro antioxidant functions of protein hydrolysate from backbones of *Rastrelliger kanagurta* by proteolytic enzymes. Saudi J Biol Sci. 2014; 21(1):19-26.
80. Sabeena KH, Lystbaek L, Hauch H, Jacobsen C, Jakobsen G, Johansson I, *et al*. Antioxidant activity of cod (*Gadus morhua*) protein hydrolysates: in vitro assays and evaluation in 5% fish oil-in-water emulsion. Food Chem. 2014; 149:326-34.
81. Najafian L, Babji AS. Isolation, purification and identification of three novel antioxidative peptides from patin (*Pangasius sutchi*) myofibrillar protein hydrolysates. LWT-Food Sci Technol. 2015; 60(1):452-61.
82. Chi CF, Wang B, Hu FY, Wang YM, Zhang B, Deng SG, *et al*. Purification and identification of three novel antioxidant peptides from protein hydrolysate of bluefin leatherjacket (*Navodon septentrionalis*) skin. Food Res Int. 2015; 73:124-9.
83. Gou M, Wang L, Liu X. Anticoagulant activity of a natural protein purified from Hypomesus olidus. Nat Prod Res. 2017; 31(10):1168-71.
84. Qiao M, Tu M, Wang Z, Mao F, Chen H, Qin L, *et al*. Identification and antithrombotic activity of peptides from blue mussel (*Mytilus edulis*) protein. Int J Mol Sci. 2018; 19(1):138.
85. Liu WY, Lu J, Gao F, Gu RZ, Lin F, Ren D-F, *et al*. Preparation, characterization and identification of calcium-chelating Atlantic salmon (*Salmo salar* L.) ossein oligopeptides. Eur Food Res Technol. 2015; 241(6):851-60.
86. Noman A, Xu Y, Al-Bukhaiti WQ, Abed SM, Ali AH, Ramadhan AH, *et al*. Influence of enzymatic hydrolysis conditions on the degree of hydrolysis and functional properties of protein hydrolysate obtained from Chinese sturgeon (*Acipenser sinensis*) by using papain enzyme. Process Biochem. 2018.
87. Meneses AL, Alencar TK, de Freitas S, Caetano ME, de Almeida CA, Gadelha TS, *et al*. Functional protein hydrolysate from goat byproducts: optimization and characterization studies. Food Biosci. 2017; 20:19-27.
88. Abdollahi M, Rezaei M, Jafarpour A, Undeland I. Sequential extraction of gel-forming proteins, collagen and collagen hydrolysate from gutted silver carp (*Hypophthalmichthys molitrix*), a biorefinery approach. Food Chem. 2018; 242:568-78.
89. Jridi M, Lassoued I, Nasri R, Ayadi MA, Nasri M, Souissi N. Characterization and potential use of cuttlefish skin gelatin hydrolysates prepared by different microbial proteases. Biomed Res Int. 2014; 2014:14.
90. Chakrabarti S, Wu J. Bioactive peptides on endothelial function. Food Sci Hum Wellness. 2016; 5(1):1-7.
91. Khosravi S, Rahimnejad S, Herault M, Fournier V, Lee C-R, Dio Bui HT, *et al*. Effects of protein hydrolysates supplementation in low fish meal diets on growth performance, innate immunity and disease resistance of red sea bream *Pagrus major*. Fish Shellfish Immun. 2015; 45(2):858-68.
92. Nesse KO, Nagalakshmi AP, Marimuthu P, Singh M, Bhetariya PJ, Ho M, *et al*. Safety evaluation of fish protein hydrolysate supplementation in malnourished children. Regul Toxicol Pharm. 2014; 69(1):1-6.
93. Landsberg GM, Mougeot I, Kelly S, Milgram NW. Assessment of noise-induced fear and anxiety in dogs: modification by a novel fish hydrolysate supplemented diet. J Vet Behav. 2015; 10(5):391-8.
94. Khosravi S, Bui HTD, Rahimnejad S, Herault M, Fournier V, Kim S-S, *et al*. Dietary supplementation of marine protein hydrolysates in fish-meal based diets for red sea bream (*Pagrus major*) and olive flounder (*Paralichthys olivaceus*). Aquaculture. 2015; 435:371-6.