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FOOD TECHNOLOGY

Review: Enzymatic hydrolysates of fish by-products: technological advantages and bioactive properties

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ABSTRACT: Millions of tons of fish are filleted each year, and a significant portion of unwanted carcasses is discarded worldwide. An alternative approach to use these materials is through hydrolysis reactions, which allow for the production of compounds with modified technological properties such as solubility, emulsification capacity, foam formation ability, and viscosity. In addition, they may exhibit different biological activities with beneficial effects such as antioxidant, antihypertensive, anticarcinogenic, lipid profile-lowering, and neuroprotective properties. Thus, these compounds can be included in food formulations as promising adjuncts in treatments, with health benefits to consumers. This study discussed the different enzymatic hydrolyses used in the treatment of fish waste and evaluate the compounds and their effects.
Key words: antioxidant, nutraceuticals, health promoter, enzymes, by-products.

Hidrolisados enzimáticos de subprodutos de peixe: vantagens tecnológicas e propriedades bioativas

RESUMO: Milhões de toneladas de peixes são cortados em filés todos os anos, e uma porção significativa de carcaças indesejadas é descartada em todo o mundo. Uma abordagem alternativa para utilização desses materiais é por meio de reações de hidrólise, que permitem a produção de compostos com propriedades tecnológicas modificadas como solubilidade, capacidade de emulsificação, de formação de espuma e viscosidade. Além disso, podem apresentar diferentes atividades biológicas com efeitos benéficos, como propriedades antioxidantes, anti-hipertensivas, anticarcinogênicas, redutoras do perfil lipídico e neuroprotetoras. Assim, esses compostos podem ser incluídos em formulações de alimentos como coadjuvantes promissores em tratamentos, com benefícios à saúde dos consumidores. Este estudo teve como objetivo discutir as diferentes hidrólises enzimáticas utilizadas no tratamento de resíduos de pescado e avaliar os compostos e seus efeitos.
Palavras-chave: antioxidante, nutracêuticos, promotor de saúde, enzimas, subprodutos.

INTRODUCTION

According to the Food and Agriculture Organization of the United Nations, 89 percent of the 179 million tons of total fish production was used for direct human consumption (FAO, 2022). World fish production is expected to increase by 15% between 2020 and 2030, and carp and other cyprinids stand out among the most cultivated fish species worldwide, accounting for 18% of aquatic animals produced in 2020 (FAO, 2022).

The discarded materials in industrial fish production can reach 60% of the live weight, mainly during the manufacture of fish fillets. These materials include muscles from non-filletable parts, skin, scales, bones, heads, and viscera, which are generally used to make secondary products with less added value and used in animal feed, or not used at all, generating an increase in disposal costs (MARTÍNEZ-ALVAREZ et al., 2015).

To improve the use of by-products, mainly proteins, various techniques for nutrient recovery have been used in the production of food products for human nutrition. Among the available strategies, the use of enzymes capable of producing protein hydrolysates with various lengths of peptide chains enables the incorporation of multiple functionalities into products, enhancing their technological properties through interactions with other molecules. Furthermore, it induces changes in physicochemical properties, such as solubility, emulsification, and foam formation, while acting as an antioxidant, delaying product degradation.

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The search for health-promoting foods has led to the incorporation of these compounds into food formulations to minimize the effects caused by diseases or inappropriate habits (DALIRI et al., 2018). Bioactive compounds from fish by-products have gained prominence due to their physiological effects, bringing benefits to consumers (ROSLAN et al., 2014). Being able to assist as antioxidants, either decreasing or increasing protection against oxidative stress in the physiological system. Moreover, they can confer various health benefits, including anti-hypertensive, immunomodulatory, prebiotic, antithrombotic, and hypocholesterolemic effects.

Thus, this study reviewed and discussed the different types of enzymatic hydrolysis for the conversion of by-products of fish processing into compounds with technological or functional properties, as well as the possible applications and effects.

Technological modifications

Several proteolytic enzymes have the capacity to hydrolyze fish proteins (Figure 1), including alcalase, papain, pepsin, trypsin, chymotrypsin, pancreatin, flavorzyme, pronase, neutrase, bromelain, subtilisin, protamex®, among others (BINGTONG et al., 2020), producing protein hydrolysates with a variety of applications as shown in table 1. These enzymes have different cleavage sites; and therefore, produced different types of hydrolyzed proteins, with differences in the amount and type of bioactive compounds (ULUG et al., 2021).

The hydrolysis reactions lead to the production of different peptide chains, thus the identification, sequencing, and evaluation of their amino acid characteristics and their interactions with the environment is very important (NWACHUKWU & ALUKO, 2019).

The technological properties of proteins are related to their structures, sequence, and composition of amino acids, molecular weights, conformation, and changes in the distribution of their molecular charges. The nature of the charge and the density facilitate interaction with other molecules such as water, ions, lipids, carbohydrates, and vitamins. The pH, temperature, and ionic strength of the environment can affect the formation of bioactive compounds during the manufacture, processing, and storage of the products (GÖRGÜÇ et al., 2020).

In addition to reducing molecular weight, protein breakdown facilitates solubilization, increases the number of exposed groups, and alters the physical and chemical properties of the interactions between the materials. The amphiphilic nature of these molecules, which are able to interact with hydrophobic and hydrophilic groups, leads to an increase in emulsifying capacity, due to the increase in solubility and hydrophobicity. Short periods of enzymatic action produce bioactive compounds of higher molecular weight, which act favorably on the emulsification and gelation process (TAVANO, 2013).

The hydrolysis of proteins from tilapia (*Oreochromis niloticus*) by-products using isoelectric solubilization and pressure-assisted alcalase showed accelerated hydrolysis and high amino acid release.

An increase of 16% in the solubility of fish protein hydrolysates was observed when compared

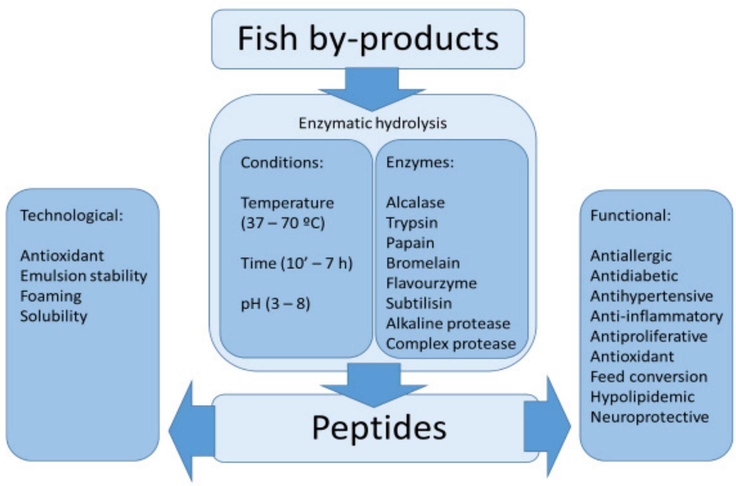

Figure 1 - Enzymatic hydrolysis of fish by-products, conditions, peptides and effects.

Table 1 - Technological and antioxidant effects, conditions of enzymatic hydrolysis of fish by-products.

Effects of hydrolysates	Application conditions (enzyme, E/S ratio, pH, temperature, time)	Raw material used (species) - type of by-product	References
-----Technological-----			
Increased solubility, emulsifying activity, foam formation, and emulsion stability index	Trypsin, E/S 5.5%, pH 8.5, 55 ºC, 40 min Alcalase, E/S 0.25%, pH 8.0, 55 ºC, 30 min	Starry trigger fish *(Abalistes stellaris*) - muscle Bycatch fish - skins, heads, and skeletons	(SRIPOKAR et al, 2019). (ZAMORANO-APODACA et al., 2020)
Increased solubility, emulsifying, foam formation, and emulsion stability.	Alcalase, E/S 3%, pH 8.0, 55 ºC, 10-35 min	Tilapia (*Oreochromis niloticus*) - head, tail and fins	(HEMKER et al., 2020)
Increased solubility and emulsifying properties	Papain, E/S 0.5%, pH 7.0, 50 ºC, 1.5 h	Pugnose ponyfish (*Secutor insidiator*) - *muscle*	(DINAKARKUMAR et al., 2022)
-----Antioxidant-----			
Chelating and radical scavenging	Alcalase, E/S 0.25%, pH 8.0, 55ºC, 30 min	Bycatch fish - skins, heads, and skeletons	(ZAMORANO-APODACA et al., 2020)
Scavenging, reducing power, and lipid peroxidation inhibition	Alcalase, E/S 1%, pH 9.0, 60 ºC, 30 min Bromelain, E/S 0.5%, pH 6.5, 50 ºC, 60 min Flavourzyme, E/S 1%, pH 6.5, 50 ºC, 75 min Protamex®, E/S 2%, pH 6.5, 50 ºC, 60 min	Chinese carp (*Catla catla*) - muscle	(ELAVARASAN et al, 2014).
Lipid peroxidation	Flavourozyme, E/S 2%, pH 7.0, 50 ºC, 2 h	Whitemouth croaker *(Micropogonias furnieri)* - muscle and by-products	(DA ROSA et al., 2014)
Scavenging activity	Papain, E/S 15%, pH 7.0, 50 ºC, 5 h	Bluefin leatherjacket (*Navodon septentrionalis*) - heads	(CHI et al., 2015).
Superoxide and lipid peroxidation,	Alcalase, E/S 2.5%, pH 9.5, 55 ºC, 3.5 h	Miiuy croaker (*Miichthys miiuy*) - swim bladder	ZHAO (2018)
Hydroxyl capture, lipid peroxidation inhibition	Alkaline protease E/S*, pH 8.0, T ºC*, 2 h, + Neutral protease, E/S*, pH 8.0, T ºC*, 3 h.	Anchovy (*) by-products	(WANG et al., 2018)
Scavenging and reducing power	Neutrase + Trypsin, E/S 0.3%, pH 7.5, 50 °C, 2 – 6 h	Round scad (*Decapterus maruadsi)*- by-products	(HU et al., 2019).
Scavenging and reducing power	Alcalase, E/S 3%, pH 8.0, 55 ºC, 10-35 min	Tilapia - head, tail and fins	(HEMKER et al., 2020)
Scavenging and reducing power	Alcalase, E/S 2%, pH 8.0, 50 ºC, 4 h Protamex®, E/S 2%, pH 7.0, 50 ºC, 4 h	Whitemouth croaker and Banded Croaker (*Paralonchurus brasiliensis)* -skin and muscle	(ROCHA et al., 2021)

*Information not provided by the author.

to the control group. Additionally, a remarkable improvement in the Emulsion Stability Index (ESI) was noted in the hydrolysate samples, with values ranging from 19 to 32 minutes, as opposed to the 13 minutes observed in the control group. A significant increase in antioxidant activity was also recorded, as indicated by the reduction in IC50, which represents the concentration required to scavenge 50% of DPPH free radicals. In the control group, the IC50 was 653 µg/mL, whereas in the hydrolyzed materials, it ranged between 304 and 472 µg/mL. Furthermore, it was observed that the hydrolyzed samples exhibited a higher reducing power value (43.5 µg AAE/g) compared to the non-hydrolyzed samples (28 µg AAE/g) (HEMKER et al., 2020)

Pugnose Ponyfish (*Secutor insidiator)* hydrolysates produced by the action of papain exhibited a high protein level (92%). These proteins exhibited solubility exceeding 75% across different pH ranges, in contrast to untreated samples that recorded values below 10%. Furthermore, an Emulsion Activity Index (EAI) of 80 m^2/g was observed at pH 2.0, with an Emulsion Stability Time (ESI) of 70 minutes, establishing them as a promising additive for the food industry (DINAKARKUMAR et al., 2022). Protein hydrolysates from starrytrigger fish (*Abalistes stellaris*) muscle obtained by the action of the tuna liver trypsin enzyme also showed good solubility (minimal 72.8% at pH 5.0 and maximum values 94.0% at pH 3.0), and emulsification capacity (EAI 25 m^2/g for concentration between 1 and 2 % w/v), and provided ESI of 14 minutes (SRIPOKAR et al., 2019).

Hydrolysis with alcalase of acid-soluble collagen derived from mixed by-products of different fish species enabled the separation of 5 peptide fractions with more than 66% of DPPH inhibition. Better results than the inhibition for collagen fractions (30%) showing an antioxidant effect. These fractions also showed technological effects, such as good solubility (reaching approaching 90% at pH between 4 – 10) whereas albumin exhibited a solubility range of 95-100 %. Regarding the EAI, it was observed that the different fractions exhibited slightly lower values (F3 = 130, F2 = 100, F1 = 64 m^2/g) compared to the albumin used as the control (159 m^2/g). However, it is important to note that fraction 3 has a lower molecular weight (5 - 10 kDa) in comparison to the control (66 kDa), and the EAI value was only 18.5% lower. This indicated that this fraction represents a significant alternative as an emulsion ingredient (ZAMORANO-APODACA et al., 2020).

The enzyme alcalase showed effective activity on fish proteins, promoting a high level of hydrolysis in a short reaction period, and the resulting hydrolysates showed technological and nutritional properties (ROSLAN et al., 2014). The emulsifying properties of compounds derived from enzymatic hydrolysis of fish by-products showed changes in their surface properties, with a decrease in surface tension between hydrophobic and hydrophilic groups due to the breakdown of molecules during hydrolysis (CHI et al., 2015).

Antioxidant properties of hydrolysates

The higher consumers' demand for safe food with higher quality, preservation of natural characteristics, less use of preservatives, and low energy values has opened up great interest in the development of new products (LI et al., 2017). Therefore, processing methods that reduce the use of synthetic preservatives, without altering the nutritional values and organoleptic characteristics of the food, as well as reducing costs and making the product competitive in the market are required (AMIT et al., 2017).

Compounds with antioxidant effects have been identified in aquatic organisms such as golden kingfish (*Gnathanodon speciosus*), Round scad (*Decapterus punctatus)*, horsetail (*Scombroid mackerels*), neon goby (*Elacatinus oceanops*), Alaska pollock (*Theragra chalcogramma*), and sardines (*Sardinella aurita*). These compounds can; therefore, be included in the production of foods with health benefits (JEMIL et al., 2014; CHI et al., 2015).

Lipid oxidation is a concern for the food industry, as the compounds can be harmful to consumers' health and reduce the shelf life of the products (HUANG & AHN, 2019). The technological antioxidant effect of protein hydrolysates from fish by-products has been associated with the action of chelating metal ions, removing free radicals, and scavenging singlet oxygen (NWACHUKWU & ALUKO, 2019). The activity of antioxidant compounds in lipoperoxidation systems is affected by several factors including the size of the molecule, chemical properties, and electron transfer.

The antioxidant action of bioactive compounds formed after enzymatic hydrolysis depends on the free amino acids present in the medium, their sequences, and hydrophobicity. Many antioxidant peptides contain hydrophobic amino acids, such as Val and Leu in the N-terminal part, and also Pro, His, and Tyr in their sequences. Tyr contributes to the free radical scavenging activity due to its phenolic side chain, which acts as a potent electron donor (HALIM et al., 2016). The amino acids His, Leu, and Cys also have the ability to remove free radicals. It is worth noting that carboxyl and amino groups can chelate metals (LAPSONGPHON & YONGSAWATDIGUL, 2013).

Hydrophobic amino acids such as Pro, Met, Trp, and Phe can increase the antioxidant activities of peptides, and their presence in bioactive compounds allows the interaction with lipid molecules, donating protons to lipid radicals. Tyr and Phe act as direct radical scavengers, due to the ability of their phenolic groups to donate hydrogen atoms. Conversely, Hys has the ability to donate protons, Met oxidizes sulfoxide, and Cys is a thiol-reduced sulfur donor (NWACHUKWU & ALUKO, 2019).

The peptides from protein hydrolysis with bromelain from Chinese carp (*Catla Catla*) showed antioxidant effects, with high free radical scavenging ability through DPPH assay (77.9 % at 2 mg/mL), while BHA at 200 ppM used as positive control showed 91.5% of radical scavenging and a similar ($P > 0.05$) reducing power (A_{700nm} 0.885 ± 0.06) on the synthetic antioxidant BHT (ELAVARASAN et al., 2014). Bioactive antioxidant and antimicrobial oligopeptides were isolated in anchovy hydrolysates treated with alkaline and neutral proteases. Four fractions were identified by mass spectrometry. The oligopeptide mixture exhibits significantly enhanced biological activities, including a notable superoxide scavenging capability of 16.49 %, a substantial 40 % inhibition of lipid peroxidation, and an effective ferrous ion chelation of 30%. Fraction 1 (Thr-Pro-Ser-Ala-Gly-Lys, 559 Da) demonstrates a remarkably high ferrous ion chelation activity, with a rate of 36.87%. Fraction 3 displays the highest superoxide radical scavenging capacity at 37.85 %, while Fractions 1 and 2 exhibit considerable anti-hydroxyl activity in the range of 30-40 % and approximately 50 % inhibition of lipid peroxidation (WANG et al., 2018). Peptides between 3 and 100 kDa from hydrolysates of red tilapia (*Oreochromis* sp.) scales subjected to the enzyme alcalase showed good antioxidant activity, probably due to the presence of the amino acids Pro, Met, Lys, Phe, Glu, and Asp, which showed the highest concentration (SIERRA et al., 2021). Meat and by-products of whitemouth croaker (*Micropogonias. furnieri*) after hydrolysis with flavorzyme of microbial origin showed lipid peroxidation inhibition of 27.0 and 31.9 %, respectively, with an effect similar ($P > 0.05$) to that of α-tocopherol (25.7 %), demonstrating great potential for use (DA ROSA et al., 2014). Round scad (*Decapterus maruadsi)* by-products were subjected to hydrolysis by the enzymes neutrase and trypsin, leading to the formation of low molecular weight bioactive compounds with good free radical scavenging capacity of DPPH radical (IC_{50} 6.38 mg/mL), hydroxyl radical (IC_{50} 14.45 mg/mL) and reducing power (A_{700}= 0.452) (HU et al., 2019).

Ten bioactive compounds were identified after hydrolysis by alcalase of the swim bladder of miiuy croaker (*Miichthys miiuy*) (SMP), and two compounds (SMP8 and SMP10), exhibited lower values in scavenging activities for hydroxyl radicals (EC_{50} 0.68 and 0.71), DPPH radical (EC_{50} 0.51 and 0.78 mg/mL) and superoxide anion radicals (EC_{50} 0.34 and 0.30 mg/mL) compared to the other compounds. Importantly, theses two compounds were not significantly different from the positive control of ascorbic acid (with respective EC50 values for hydroxyl radicals, DPPH radicals, and superoxide anion radicals being 0.525, 0.012 and 0.099 mg/mL). Futhermore, it was observed that SMP10 also displayed efficiency against lipid peroxidation equivalent to the control (approximately 90%) suggesting the use of these hydrolysates in nutraceutical applications or as antioxidant agents in food (ZHAO et al., 2018).

ROCHA et al. (2021) studied the hydrolysis of muscle and skin from whitemouth croaker and banded croaker (*Paralonchurus brasiliensis)* with alcalase and protamex® and found electron-donating compounds with good free radical stabilization capacity. These compounds also showed the ability to scavenge peroxyl radicals (25 – 30 % DPPH scavenging activity) and a good concentration of sulfhydryl groups (4 – 6 nmol/mg of protein), which had a free radical scavenging effect.

Bioactive compounds and their positive impact on health

Bioactive peptides are characterized by having an average sequence of 20 amino acids and can improve the functional properties of food products (Table 2) and confer health benefits (Table 3), including antihypertensive, antioxidant, immunomodulatory, antimicrobial, prebiotic, antithrombotic and hypocholesterolemic effects (MAHDI et al., 2018). Shrimp (*Litopenaeus vannamei*) head hydrolysates produced through autolysis were administered in diets of Wistar rats and promoted an increase in feed conversion capacity and weight gain, which facilitated the development and weight maintenance of these animals (DA SILVA et al., 2017). VÁZQUEZ et al. (2023) reported excellent yields and nutritional levels of gurnard (*Trigla* spp) by-products (head and skin) subjected to hydrolysis with alcalase.

Chinese sturgeon (*Acipenser sinensis*) hydrolysates obtained with alcalase showed good concentrations of amino acids (42.45 g/100 g of protein), with 27% corresponding to Asn and Gln, and 35% corresponding to Leu, Ile, Arg, Val, and Lys of the total amino acids. This result showed the potential of the hydrolysate for use as a food supplement, or in products with a healthier appeal. Great antioxidant activity was observed for the low molecular weight peptides (<1000 Da) (NOMAN et al., 2022). Similar results were found in viscera of yellowfin tuna (*Thunnus albacares*) hydrolysates obtained by alcalase hydrolysis, which showed high concentrations of essential amino acids and proteins (72.34%), and lower fat content (1.43%), demonstrating the possibility of application in human nutrition (OVISSIPOUR et al., 2012).

Peptides were isolated from the acidified skin of yellowfin tuna through hydrolysis with trypsin. The peptides showed potent antimicrobial activity against gram-positive bacteria such as *Bacillus subtilis*, *Micrococcus luteus*, *Streptococcus* sp. and gram-negative bacteria such as *Aeromonas hydrophila*, *Escherichia coli* and *Vibrio parahaemolyticus* (SEO et al., 2012). Zinc-peptide complexes from silver carp

Table 2 - Bioactive effects and conditions of enzymatic hydrolysis of fish by-products.

Effects of hydrolysates	Application conditions (enzyme, E/S ratio, pH, temperature, time)	Raw material used (species) - type of by-product	References
Antimicrobial activity against *Bacillus cereus* and *Staphylococcus aureus*	Bromelain, E/S 0.5%, pH *, 50 ºC, 10 h	Leatherjacket *(Meuchenias sp.) - muscle*	(SALAMPESSY, 2010).
Antimicrobial activity (*Bacillus subtilis, Micrococcus luteus, Streptococcus sp., Aeromonas hydrophila, Escherichia coli*, and *Vibrio parahaemolyticus)*	Trypsin, E/S 1%, pH *, 37 ºC, 1 h	Yellowfin tuna *(Thunnus albacares)* - skin	(SEO et al., 2012)
Nutritional value, essential amino acids, high antihypertensive activity	Alcalase, E/S 2.5%, pH 7.5, 60 ºC, 2 h	Tilapia (*Oreochromis niloticus*) - head, frames, and tail	(ROSLAN et al., 2014).
Antimicrobial activity (S. aureus and *E. coli*.), greater zinc complexing capacity.	Flavuorozyme, E/S 1%, pH 7.0, 50 °C, 2, 4, 6, 20 h	Silver carp (*Hypophthalmichthys molitrix*) - muscle	(JIANG et al., 2014)
Increased feed conversion capacity and weight gain of Wistar rats' weight.	Autolise, E/S *, pH *, 45 ºC, 3 h	Shrimp (*Litopenaeus vannamei*) - heads	(DA SILVA et al., 2017)
Growth of smooth muscle cells (A7r5 lineage). Reduction of oxidative stress.	Alcalase, E/S 1%, pH 8.0, 58.5 ºC, 55 min	Red tilapia (*Oreochromis* sp.) - scales	(SIERRA et al., 2021).
Reduction in polyphagia, glucose and insulin secretion in diabetic mice. Protective effect on hepatic and renal function, increase in the enzymes SOD, CAT and GPX.	Trypsin,E/S 3000 U/g, pH 8.0, 37 ºC, 4 h	Golden pompano *(Trachinotus ovatus)* - muscle	(WAN et al., 2023).

*Information not provided by the author.

(*Hypophthalmichthys molitrix*) hydrolysates obtained by hydrolysis with the enzyme flavorzyme, exhibited high antibacterial effects against *S. aureus* and *E. coli*. Peptides with a higher number of exposed amino acids had a greater ability to complex zinc, thus increasing their antimicrobial activity (JIANG et al., 2014). The peptide fractions produced through the hydrolysis of Leatherjacket (*Meuchenias* sp.) with bromelain showed minimum inhibitory concentration values of 4.3 mg/mL against *Bacillus cereus* and *Staphylococcus aureus* (SALAMPESSY, 2010). LEE et al. (2017) reported good anti-adipogenic activity of bioactive compounds from fish collagen, which may be a promising therapeutic agent for obesity. Protein hydrolysates from zebra blenny (*Meiacanthus grammistes)* were shown to protect the heart as well as DNA from histopathological damage in rats fed a hypercholesterolemic diet. These protein hydrolysates also increased the activities of the enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT). They have shown promising cardiovascular protection in diseases caused by oxidative stress (KTARI et al., 2017). The inclusion of bioactive compounds from sardine muscle (*Sardinella aurita*) hydrolysates in a hypercholesterolemic diet for rats favored an increase in high-density lipoprotein cholesterol (HDL-C) levels and HDL-C/total cholesterol ratio. A decrease in total cholesterol, triglycerides, and low-density lipoproteins (LDL) levels was also observed, suggesting good hypolipidemic effects in the animals studied. The bioactive compounds from sardines also led to a reduction in lipoperoxidation and an increase in the activity of antioxidant enzymes such as SOD, GPx, and CAT (KHALED et al., 2012).

In model studies with streptozotocin-induced diabetes in rats, golden pompano *(Trachinotus ovatus)* protein hydrolysates produced by hydrolysis with trypsin led to a reduction in the effects of polyphagia, insulin secretion, and glycemic levels, as well as a protective effect on kidney and liver tissues. The authors have also found 25 potential bioactive peptides, with small molecular weights, composed of 2 to 6 amino acids rich in Pro, Arg, Phe, and Asn, and a high concentration of hydrophobic peptides (WAN et al., 2023).

Yellowfin tuna protein hydrolysates produced by hydrolysis with trypsin showed

Table 3 - Health benefits and conditions of enzymatic hydrolysis of fish by-products.

Effects of hydrolysates	Application conditions (enzyme, E/S ratio, pH, temperature, time)	Raw material used (species) - type of by-product	References
Improved memory and learning ability in elderly mice. Acting against neuronal aging.	Complex protease, E/S 3000 U/g protein, pH 8.0, 40 ºC, 3 h	Chum Salmon *(Oncorhynchus keta)* - skin	(PEI et al., 2010)
Antiproliferative effect, prevent uncontrolled cell growth, protecting against the diferente types of cancer. Prostate cancer (DU-145), Liver cancer (HepG2), and Esophageal cancer (Eso-109).	Papain, E/S 1%, pH 6.2, 25 ºC, 6 h Pepsin, E/S 1,000 U/g, pH 3.0, 37 ºC, 5 h	Blackfin tuna (*Thunnus atlanticus*) – muscle Half-fin anchovy *(Setipinna tenuifilis),* - muscle	(HSU et al., 2011) (SONG et al., 2011)
Hypolipidemic effects (increase in HDL, decrease in total cholesterol, triglycerides, and LDL). Reduction in lipoperoxidation and increase in the activity of SOD, GPx, and CAT.	SPHA1, E/S 3, pH 8.5, 50 ºC, 5 h SPHA21, E/S 3, pH 10.0, 50 ºC, 5.5 h PHEE, E/S 3, pH 8.0, 45 °C, 5.5 h	Sardine (*Sardinella aurita*) - muscle	(KHALED et al., 2012)
Antihypertensive effect by inhibiting ACE	Subtilisin + Trypsin, pH 8.0, 50 °C, 4 h	Horse mackerel *(T. mediterraneus),* small-spotted catshark *(S. canicula)* - whole fish	(GARCÍA-MORENO et al., 2015).
Neuroprotective, effects on memory, and protection against neurodegeneration.	Protease N, E/S 0.1%, pH 6.5, 50 °C, 2 h	Lantern fish *(Benthosema pterotum)* - whole fish	(CHAI et al., 2016)
Protection of heart DNA. Increase in the activities of GPx, SOD, and CAT which limit the effects of oxidant molecules in tissues and act in the defense against oxidative cell injury.	Endogenous alkaline protease, E/S 3 U/mg, pH 8.0, 45 °C, 7 h	Zebra blenny *(Meiacanthus grammistes)* - fillets	(KTARI et al., 2017)
Antiallergic, inhibiting hyaluronidase and mast cell degranulation, preventing the release of inflammatory mediators.	Alcalase, E/S 1%, pH 7.0, 50 ºC , 8 h Neutrase, E/S 1%, pH 8.0, 50ºC, 8 h Trypsin, E/S 1%, pH 8.0, 37 ºC, 8 h Pepsin, E/S 1%, pH 2.0, 37 ºC, 8 h	Salmon (*Salmo salar*) - viscera	(WANG, K. et al., 2020).
Improving flow-mediated dilation without altering blood pressure.	Alcalase, E/S: 2%, pH 7.5, 55 ºC, 4h	Tilapia *(Oreochromis niloticus)* - frame, fins, muscle, skin and bones	(OLIVEIRA et al., 2020).
Increased calcium absorption, bone formation stimulation, prevention of bone resorption.	Complex protease, E/S 5%, pH 6.0, 55 ºC, 4 h	Tilapia - skin	(LIU et al., 2022)
Stimulating macrophage activity, enhanced phagocytic capacity, and release of nitric oxide and cytokine secretion.	Trypsin, E/S 3000 U/g, pH 8.0, 37 ºC, 6 h	Yellowfin tuna (*Thunnus albacares*) - trimming	(CAI et al., 2022)
Anti-inflammatory, upregulation of interleukin-10 expression.	Alcalase 1.5%, pH 7.9, 55.8 ºC, 1.5 h	Tilapia - viscera	(RIYADI et al., 2022)

*Information not provided by the author.

immunomodulatory effects, an increased phagocytic capacity in the release of nitric oxide, and cytokine secretion. One fraction showed immune activation, through the nuclear factor kappa B activation (NF-kB) through signaling pathways, which stimulated macrophage activity and increased the release of interleukins and TNF-α (tumor necrosis factor). Therefore, these peptides can be used as immunomodulating agents in functional foods (CAI et al., 2022). Alcalase-hydrolyzed tilapia viscera showed an anti-inflammatory effect in rats exposed to acute lung damage after administration of lipopolysaccharides. A hydrolysate dose of 450 mg/kg of body weight reduced the inflammatory process and increased IL-10 (interleukin-10) expression, stimulating regulatory T lymphocytes and neutrophils (RIYADI et al., 2022). Protein from pepsin-hydrolyzed salmon (*Salmo salar*) viscera showed an anti-allergenic effect, inhibiting hyaluronidase, and mast cell degranulation, preventing the release of inflammatory mediators (WANG et al., 2020).

Blackfin tuna muscle (*Thunnus atlanticus*) was subjected to hydrolysis by papain and protease XXIII, with the formation of bioactive compounds with the following sequences: Leu-Pro-His-Val-Leu-Thr-Pro-Glu-Ala-Gly-Ala-Thr (1206 Da), and Pro-Thr-Ala-Glu-Gly-Gly-Val-Tyr-Met-Val-Thr (1124 Da). These peptides showed inhibition capacity against the proliferation of MCF-7 cells, indicating a good antiproliferative effect (HSU et al., 2011). Antiproliferative effects have also been demonstrated in pepsin hydrolysates from half-fin anchovy (*Setipinna tenuifilis*) on prostate, liver, and esophageal cancer cell lines, due to the presence of hydrophobic amino acids (SONG et al., 2011).

The angiotensin-converting enzyme (ACE) can convert angiotensinogen I into angiotensinogen II, which is a potent vasoconstrictor, thus raising blood pressure. Thus, ACE inhibition is an effective therapeutic alternative for reducing hypertension (MIREMADI et al., 2016). Mediterranean fish by-products such as horse mackerel (*Trachurus mediterraneus*) and small-spotted catshark (*Scyliorhinus canicula*) were evaluated for ACE inhibitory activity, which was associated with the levels of protein hydrolysis. Two proteases, subtilisin, and pancreatic trypsin, were used for protein hydrolysis, and both enzymes led to the production of bioactive compounds with major antihypertensive effects by inhibiting ACE (GARCÍA-MORENO et al., 2015). Tilapia by-products showed nutritional value after hydrolysis with alcalase, due to the presence of essential amino acids (199.15 mg/g) and a high protein content (62.71%). The hydrolysates showed high antihypertensive activity ACE inhibitory activity, (88.26%), which was related to the presence of low molecular weight peptides (ROSLAN et al., 2014). Gelatin hydrolysates from giant catfish skin by the activity of catfish visceral proteases demonstrated ACE inhibitory properties and greater radical scavenging activity, as well as stability over a wide pH range (KETNAWA et al., 2017). Protein hydrolysates from Nile tilapia by-products used as a single-dose food supplement in patients at high risk of cardiovascular disease showed positive effects on macro- and microvascular functions. The single dose of 5 g improved vascular function by improving blood flow-mediated dilation, without altering blood pressure after 60 minutes of intake (OLIVEIRA et al., 2020).

Fifty bioactive compounds were obtained from hydrolyzed tilapia skin, most of them containing the amino acids Gly-Pro, which showed good effects in combating retinoic acid-induced osteoporosis in rats. The studies showed an increase in calcium absorption, as well as stimulation of bone formation, and also helped prevent bone resorption (LIU et al., 2022). Cognitive disorders such as amnesia, learning difficulties, and forgetfulness are common due to aging, which also exposes thousands of people to neurodegenerative diseases such as Alzheimer’s and Parkinson’s, reducing their cognitive ability. Memory and learning capacity was assessed in elderly rats treated with collagen protein hydrolysate from chum salmon (*Oncorhynchus keta*), and compared with the control group of young rats not treated with the hydrolysate. No significant differences were observed between the abilities of the two groups of rats, demonstrating that these peptides can facilitate learning and memory capacity in elderly rats, acting against neuronal aging (PEI et al., 2010). Protein hydrolysates from lantern fish (*Benthosema pterotum)*, such as Phe-Tyr-Tyr, and Asp-Trp, showed neuroprotective properties, with favorable effects on memory, and protection against potential oxidative damage that causes neurodegeneration, such as lipoxidation and the formation of reactive nitrogen species (CHAI et al., 2016).

CONCLUSION

The enzymatic hydrolysis of fish by-products may be a promising alternative for obtaining protein compounds with technological properties and bioactive activities. Several fish species are used worldwide for human consumption, either farmed or fished, with little use of their by-products and carcasses. Thus, studies are required on the beneficial effects of by-products as sources for obtaining raw materials with technological characteristics, providing improvements in the formulation of food products. Peptides derived from fish by-products have antioxidant, anti-hypertensive, cholesterol-reducing, anti-tumor, and neurological protective effects that make them potential assistants in treatments and adjuvants to good health. Therefore, the inclusion of these peptides in food can be a promising approach in the search for functional foods, as these compounds can bring benefits to the consumer, and reduce negative environmental impacts from the disposal of these by-products.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHORS' CONTRIBUTION

The authors contributed to the conception and writing of the manuscript, critically reviewed the manuscript, and approved the final version.

ABBREVIATIONS

ABTS - 2,2′-azino-di-(3-ethylbenzthiazoline sulfonic acid), Ala - Alanine, Asn - Asparagine, Cys - Cysteine, Glu - Glutamate, Gly - Glycine, His - Histidine, Phe - Phenylalanine, Pro - Proline, Met - Methionine, Leu - Leucine, Lys - Lysine, Ser - Serine, Thr - Threonine, Trp - Tryptophan, Tyr - Tyrosine, Val - Valine, ABTS - 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), BHT - 2,6-bis(1,1-dimethylethyl)-4-methylphenol, DPPH - 2,2-diphenyl-1-picrylhydrazyl.

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