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Structural and biological properties of protein hydrolysates from seafood by-products: a review focused on fishery effluents

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Abstract

Enormous amounts of by-products and waste are generated during the processing of seafood, sometimes representing about 65% of the raw material employed. Seafood by-products (SB) include solid waste such as skin, head, viscera, trimmings, and bones; however, liquid wastes (effluents) derived from operations such as washing, thawing, cooking and the production of fishmeal are also produced. SB contain considerable amounts of protein and others biomolecules, which has been regularly processed as food ingredients for animal nutrition. But also, sometimes SB are used as fertilizers or discarded to environment without a previous treatment. Nowadays, there is an increasing interest in adding value to the protein material within SB by improving their properties through hydrolysis and thus releasing of peptides with bioactive properties. Thus, this review aims to present an overview of the potential of SB, focused on effluents, as a source of protein hydrolysates, summarizing their methods of production, bioactive property evaluation and structural characteristics.

Keywords: seafood; effluents; by-products; hydrolysates; bioactive peptides.

Practical Application: This review provides the particularities of biological properties and structural characteristics of protein hydrolysates from solids and specially liquids seafood by-products, focusing mainly in obtaining potential bioactive peptides from fishery effluents, with the aim to enhance their valorization.

1 Introduction

During the processing of seafood considerable amounts of solid and liquid by-products are produced (Food and Agriculture Organization of the United Nations, 2013). Solid by-products represent about 65% of the raw material weight, which includes heads, bones, viscera, among others corporal structures (Food and Agriculture Organization of the United Nations, 2014). Besides, different effluents are produced during processing operations such as washing, thawing, cooking, and during the production of fishmeal (Ferraro et al., 2013). Two examples of fishery effluents that contain an impressive proportion of soluble nutrients, especially proteins, are stickwater and cooking water, which contains approximately 4 to 5% of proteins on a wet basis (Goycoolea et al., 1997; Hung et al., 2014).

Seafood by-products (SB) represent a potential source of functional and bioactive compounds because they contain a high amount of protein-rich material (Afonso & Bórquez, 2002; Fjerbæk Søtoft et al., 2015); however, SB are usually discarded because of their low commercial value, and/or sanitary regulations that prohibit their use in human foods (Jędrejek et al., 2016). In the interest of promoting the use of proteins from SB, several methods have been developed to produce and recover potential bioactive compounds that can contribute to the improvement of human health (Chalamaiah et al., 2012). These methods generally include the hydrolysis of proteins by the addition of exogenous enzymes, followed by ultrafiltration to fractionate hydrolysates and to obtain bioactive peptides within a specific molecular weight range (Picot et al., 2010). Therefore, bioactive peptides derived from SB protein hydrolysates could be considered as potential valuable compounds for improving human health. This review provides an overview of the recovery of protein fractions from solid and liquid SB and waste, focused mainly in obtaining potential bioactive peptides from fishery effluents summarizing their biological properties and structural characteristics.

2 Fishery effluents as an alternative to obtaining valuable protein compounds

The seafood processing plants differ from one another in the raw material employed, source of processing water, the additives used and operational units involved (Cristóvão et al., 2015). Seafood processing operations produce effluents or wastewaters containing salts, soluble organic molecules, colloidal and particle forms, which are considered as potentially polluting substances, with a high content of Chemical Oxygen Demand (COD) mainly from biodegradable matter, such as proteins and lipids (Cristóvão et al., 2015).

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From this perspective, an appropriate effluent treatment process could be applied and thus, an interesting opportunity for fishery effluents valorization could be considered. This is of great interest, aside from the high quantities generated, due to the potential recovery of valuable compounds and unique value-added products, which may result in economic and environmental benefits (Fjerbæk Søtoft et al., 2015; Cristóvão et al., 2015). At this regard, seafood processing effluents, reported as a good source of potential protein-based value-added compounds, are briefly described.

2.1 Cooking juice

The fish canning industry commonly uses tuna or small pelagic fish such as sardine and anchovy as raw material. During canning, several steps are involved, such as washing, thawing and cooking of the raw material, where several effluents are generated. Thus, cooking is an essential operation where an abundant effluent known as "cooking juice" or "cooking wastewater" is made. It has been reported that a fish canning plant yields approximately from 15 to 27 t per day of cooking juice, containing about 4% of water-soluble proteins, including sarcoplasmic proteins and other proteins such as collagen (Jao & Ko, 2002; Hsu et al., 2009). Similarly, during anchovy (*Engraulis japonicus*) processing, approximately 1.5 t for each t of processed species of cooking wastewater is generated; furthermore, this effluent is considered an excellent nutritional resource due to its crude protein (5 g/L) and essential amino acids content (Tang et al., 2015).

2.2 Stickwater

The production of fishmeal involves several steps, such as mincing, cooking, pressing, and drying of whole fish or fish by-products. After the cooking and pressing of raw material, a solid phase and a liquid are obtained. Afterward, the press liquor is centrifuged to remove the oil and an effluent named "stickwater" is derived. Stickwater (SW) represents approximately 60% of the processed fish weight, and proteins (5-9%) are among its major components (Pacheco-Aguilar et al., 2009; Valdez-Hurtado et al., 2018). Nowadays, the fishmeal industry recovers dry matter from stickwater with an additional step of evaporation. The concentrated solids are mixed with the press cake increasing the yield of the fishmeal production. Nevertheless, in some cases fishmeal plants lack appropriate equipment to evaporate and concentrate stickwater, which implies the irregular operation of discharging the stickwater directly to the sea or coastal areas, causing a severe pollution problem (Pacheco-Aguilar et al., 2018).

As mentioned, seafood processing produces large volumes of effluents, which possess an important amount of soluble proteins that could be recovered and concentrated through different processes and could be used as raw material to produce protein hydrolysates. This fact could be considered as a strategy to add value to the fishery industry and contribute to generate economic and environmental benefits by reducing costs related to fishery effluents discharge (Figure 1).

3 Development and isolation of bioactive peptides derived from seafood by-products protein hydrolysates

3.1 Enzymatic hydrolysis

Enzymatic hydrolysis (EH) is a process that allows a better control of the resulting hydrolysate and thereby its properties (Zamora-Sillero et al., 2018). The main variables involved in EH are enzyme-substrate ratio, temperature, pH, time of incubation, the degree of hydrolysis as well as the appropriate selection of

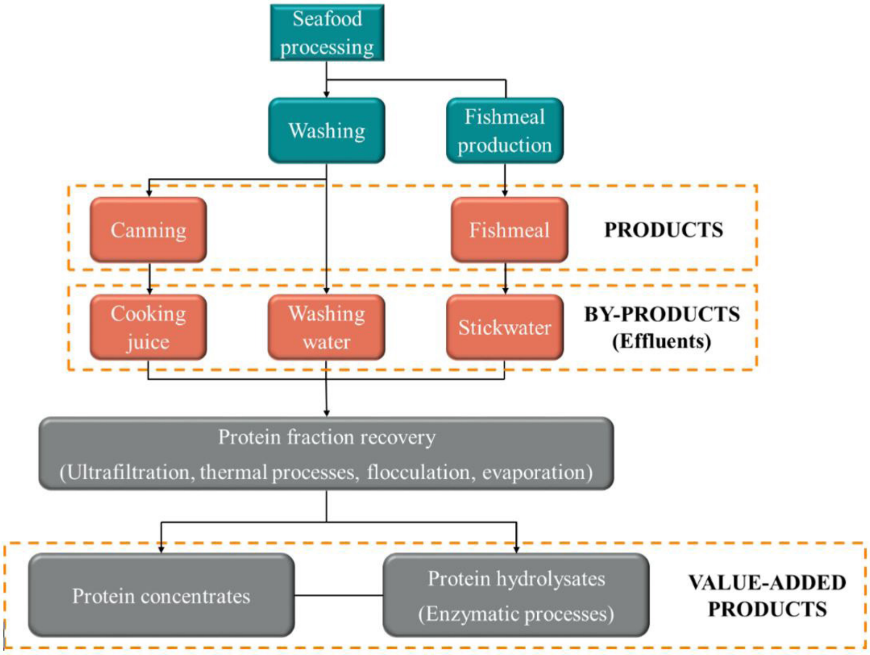

Figure 1. Fishery effluents generation and the process for their valorization and recovery of potential added-value compounds.

the proteolytic enzyme, since its specificity influence the size and composition of peptides which in turn influence the bioactive properties of the resulting hydrolysates (Chalamaiah et al., 2012; Zamora-Sillero et al., 2018).

One of the leading established applications for EH that has attracted the remarkable interest of food researchers worldwide is the production of seafood by-products protein hydrolysates (SBPH) (Chalamaiah et al., 2012); which tuna (Saidi et al., 2014), tilapia (Bernardi et al., 2016; Wachirattanapongmetee et al., 2019) and salmon (Slizyte et al., 2016) are the most studied species. SBPH result from the enzymatic hydrolysis of protein-rich seafood by-products such as bones, heads, viscera, skin, and even effluents, leading to peptides of diverse sizes that exhert bioactive properties (Fernandes, 2016). Compared to solid by-products, information related to the study of fishery effluents as a source of protein hydrolysates, is scarce. For instance, wastewaters from the industrial manufacturing of tuna (Jao & Ko, 2002; Hsu et al., 2009; Hung et al., 2014), and kilka (Mahdabi & Hosseini Shekarabi, 2018) have been considered as a potential source of valuable compounds, such as protein hydrolysates and bioactive peptides (Table 1).

3.2 Membrane technology

Membrane separation technologies (MST) use hydrostatic pressure to force a liquid through a semi-permeable membrane. MST minimize protein denaturation; therefore they are useful to concentrate or fractionate valuable molecules from effluents of seafood processing industries (Bourseau et al., 2009). In this regard, MST have been used in the treatment of seafood processing effluents for the recovery of added-value value compounds such as proteins, production of fish protein concentrates and hydrolysates (Afonso & Bórquez, 2002) and thus, obtaining peptides of specific molecular weight range that could show enhanced bioactive properties (Pan et al., 2016).

4 Bioactive properties of seafood by-products protein hydrolysates

4.1 Antioxidant activity

Oxidative damage can be triggered by the depletion of antioxidants in the organism, due to the formation of free radicals and reactive oxygen species (ROS) by physiological processes such as cellular respiration or even by exogenous molecules (Zamora-Sillero et al., 2018). Oxidative damage is mainly related to deleterious processes, such as lipid peroxidation, enzyme inactivation, protein denaturation, and DNA damage, these processes favor the frequency of several conditions such as cancer (Zamora-Sillero et al., 2018).

In this regard, impressive results related to the production of antioxidant protein hydrolysates and peptides from fishery effluents have been reported. For example, Jao & Ko (2002) reported a high DPPH scavenging activity exhibited by a protein hydrolysate and its fraction obtained from tuna cooking juice. In a study performed by Hsu et al. (2009) found that hydrolysates from tuna cooking juice acted as retarders of lipid peroxidation.

4.2 Antimutagenic activity

Mutations are permanent genetic alterations which may result in heritable changes in the characteristics of the DNA sequence of a living organism, and hence the development of multiple diseases such as cancer. Mutations are mainly induced by external factors (mutagens) or even can be promoted spontaneously from errors in DNA replication, recombination and repair (Słoczyńska et al., 2014). Antimutagenic compounds prevent the interaction between a mutagen and DNA reducing the frequency of induced or spontaneous mutations and therefore delay cancer progression (Słoczyńska et al., 2014). Ames test is one of the most common methods applied to predict the antimutagenic potential of a compound through the induction of reverse mutations in histidine operon of genetically modified *Salmonella typhimurium* strains in the presence of a standard mutagen such as aflatoxin B_1 (AFB_1) (Maron & Ames, 1983). However, the studies on antimutagenic activity derived from SBPH are minimal. Suárez-Jiménez et al. (2015) reported that collagen hydrolysates from jumbo squid by-products exhibited an effective inhibition of the induced mutation with AFB_1 of *S. typhimurium* TA98 and TA100 strains. Additionally, Burgos-Hernández et al. (2016) investigated the antimutagenic potential of several fractions obtained from anchovy viscera against AFB_1 using *S. typhimurium* TA98 and TA100 tester strains. On the other hand, several studies reported the antimutagenic activity of some compounds obtained from seafood products such as white shrimp (López-Saiz et al., 2016), octopus (Cruz-Ramírez et al., 2015), and some other marine resources such as several species of seaweed (Osuna-Ruiz et al., 2016). Nowadays, little is known about the assessment of antimutagenic activity in protein hydrolysates and peptides from fishery effluents.

4.3 Antiproliferative activity

Carcinogenesis involves an accumulation of mutations and increased proliferation of cells (Tanaka, 2013). In order to investigate the potential in vitro antiproliferative activity of compounds such as seafood by-products protein hydrolysates (SBPH), one of the most common methods used is the MTT assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay, which is a method based on the detection of mitochondrial dehydrogenase activity, and thus, measures

Table 1. Bioactive protein hydrolysates derived from liquid seafood by-products.

Wastewater	Method of production	Bioactivity showed	Reference
Kilka stickwater	Alcalase	Antioxidant	Mahdabi and Hosseini Shekarabi (2018)
Tuna cooking juice	Protease XXIII	Antioxidant	Jao & Ko (2002)
Tuna cooking juice	Orientase	Antioxidant	Hsu et al. (2009)
Tuna cooking juice	Protease XXIII and ultrafiltration	Antiproliferative	Hung et al. (2014)

cytotoxicity, proliferation and/or activation of cells (Mosmann, 1983). For instance, Hung et al. (2014) found that the > 2.5 kDa ultrafiltration fraction from tuna cooking juice hydrolysates exhibited high antiproliferative activity against a breast cancer cell line (MCF-7) and it showed no cytotoxic effect on the cell viability of a mammary epithelial cells (MCF-10A).

5 Relationship between structural features and bioactive properties of peptides in seafood by-products protein hydrolysates

The biological activities of peptides in SBPH are mainly related to their structural features, such as amino acid composition, sequence, hydrophobicity, among others. Most of the isolated bioactive peptides from SBPH contain amino acid sequences ranging from 2 to 25 residues; additionally, the main amino acids of these sequences are glycine, hydrophobic amino acids such as proline, leucine, alanine, methionine, and one or more residues of arginine, aspartic acid, glutamic acid, threonine, and tyrosine (Pan et al., 2016; Abdelhedi et al., 2018).

It is considered that hydrophobic amino acids in peptide sequences contribute to scavenging free radicals through the contact with hydrophobic radical species (Pan et al., 2016). Additionally, it has been identified that the aromatic amino acids have an essential radical-scavenging effect by transferring electrons (Pan et al., 2016). Hsu et al. (2009) suggested that the highest antioxidant activity of a fraction from tuna cooking juice, could be attributed to the presence of proline and histidine residues in two characterized peptides, most probably due to their proton-donation ability of the imidazole group from histidine and sensitivity to oxygen from proline.

Additionally, Huang et al. (2011) suggested that peptides with high hydrophobicity can permeate into the hydrophobic core of the cell membrane, which might explain that hydrophobicity plays a vital role in antiproliferative activity. Hung et al. (2014) found that a > 2.5 kDa peptide fraction from hydrolysates of tuna cooking juice composed mainly of hydrophobic amino acids showed antiproliferative activity against a breast cancer cell line. Also, the authors reported that the peptide fraction induced the expression of caspase 3 which activated apoptosis in cancer cells, and induced cell cycle arrest.

6 Conclusion

Several protein hydrolysates and bioactive peptides from seafood by-products with antioxidant, antimutagenic, and antiproliferative activity have been identified from different by-products that are mainly considered as wastes. Also, it has been shown that another essential source of proteins are fishery effluents, and that its recovery could increase their value as they could be a new promising source of bioactive peptides if the appropriate process of isolation is carried out.

It is known that the bioactive properties of these peptides are mainly based on several factors such as amino acid composition, sequence, and primarily the presence of hydrophobic amino acids; this knowledge could contribute to propose possible mechanisms of action. However, more studies about the structure and function of bioactive peptides are needed for better understanding the complete mechanism of action and interactions of these with other components and molecules, since they can be considered valuable compounds that can improve human health.

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