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REVIEW

Review of fish protein hydrolysates: production methods, antioxidant and antimicrobial activity and nanoencapsulation

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Abstract

Marine products have gained popularity due to their valuable components, especially protein, despite generating significant waste. Protein hydrolysates are widely recognized as the most effective method for transforming these low-value raw materials into high-value products. Fish protein hydrolysate (FPH), sourced from various aquatic wastes such as bones, scales, skin, and others, is rich in protein for value-added products. However, the hydrophobic peptides have limitations like an unpleasant taste and high solubility. Microencapsulation techniques provide a scientific approach to address these limitations and safeguard bioactive peptides. This review examines current research on FPH production methods and their antioxidant and antibacterial activities. Enzymatic hydrolysis using commercial enzymes is identified as the optimal method, and the antioxidant and antibacterial properties of FPH are substantiated. Microencapsulation using nanoliposomes effectively extends the inhibitory activity and enhances antioxidant and antibacterial capacities. Nevertheless, more research is needed to mitigate the bitter taste associated with FPH and enhance sensory attributes.

Keywords Bioactive peptides · Enzymatic hydrolysis · Hydrolysates · Nano-carriers · Nanoliposomes

Introduction

Marine organisms, which account for approximately half of the world's biodiversity, have gained significant recognition in recent years as a promising source of novel bioactive products and a prominent protein source for human consumption. However, a considerable portion of marine catches is typically utilized for the production of inexpensive by-products like fishmeal, fish oil, or animal feed, and in some regions, they remain unused. To optimize the utilization of fish by-products, certain countries employ them in the preparation of oils, protein hydrolysates, protein isolates, fishmeal, silage, and fish sauces (Aspevik et al., 2017; Ramezanzade et al., 2021).

Protein hydrolysates are generated through enzymatic hydrolysis, a technique used to extract proteins from non-consumable raw materials and modify their properties (Golpaigani et al., 2023; Nemati et al., 2019; Ovissipour et al., 2013; Siddik et al., 2021). Peptides, which are essential bioactive compounds found in various aquatic species, consist of short chains of amino acids whose functionality is dictated by their specific composition and sequence. These peptides have various practical applications, including being used as additives in food and medicinal supplements, which contribute to improved consumer health and increased shelf life of food and healthcare products (Mirzapour et al., 2022; Najafian & Babji, 2012). Protein hydrolysates have been found to exhibit diverse bioactive functions, including antioxidant, antimicrobial, antihypertensive, anti-inflammatory, immunomodulatory, and antithrombotic properties (Liu et al., 2022). These properties make protein hydrolysates

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valuable for potential applications in health-promoting foods and other related industries. In contrast, peptides, which can be obtained from protein hydrolysates, have also been recognized for their bioactive functions, such as antioxidant and antimicrobial activities (Liu et al., 2022). These peptides have attracted significant interest in both the scientific community and the food industry due to their potential as functional ingredients in health-promoting foods (Islam et al., 2022; Nirmal et al., 2022; Qin et al., 2022).

Hydrolyzed fish protein has diverse applications and is known for its physiological functions attributed to the presence of bioactive peptides. Growing scientific evidence suggests that hydrolyzed peptides and proteins derived from marine sources possess antioxidant and antimicrobial properties, promoting human health and preventing chronic diseases (Bi et al., 2020; Hasani et al., 2022; Rabiei et al., 2019; Shahosseini et al., 2022; 2023). The antimicrobial activity of these hydrolysates depends on factors such as the extent of hydrolysis, enzyme concentration, and hydrolysis duration (Bahram et al., 2020; Shahosseini et al., 2023). Additionally, the antioxidant capacity of these hydrolysates can be attributed to their ability to scavenge free radicals, function as metal chelators, deoxidizers, or hydrogen donors (Bahram et al., 2020; Golpaigani et al., 2023; Shahosseini et al., 2022; Tkaczewska et al., 2020).

Conversely, due to their short peptide chains, they exhibit high digestibility and can serve as protein supplements in human, animal, and aquatic food applications (Bhaskar et al., 2008; Shahosseini et al., 2022). The bitter taste and high moisture absorption capacity of these compounds necessitate their microencapsulation. Microencapsulation techniques effectively preserve the structural and functional properties of these compounds (Hosseini et al., 2021; Mohan et al., 2015). Various colloidal delivery systems have been employed, including micro-emulsions, nano-emulsions, solid lipid nanoparticles, and liposomes (Hosseini et al., 2021; McClements and Öztürk, 2021).

Liposomes, particularly nanoliposomes, have garnered significant interest as delivery systems due to their composition from natural components such as phospholipids. Nanoliposomes enhance stability, functional properties, cellular uptake, and controlled release of bioactive compounds (Borel et al., 2014; Chotphruethipong et al., 2021; Sharma et al., 2023). Previous studies have explored the use of nanoliposomes for microencapsulation of bioactive peptides, aiming to preserve and enhance the properties of hydrolyzed proteins (Chotphruethipong et al., 2021Pavlović et al., 2022; Sepúlveda et al., 2021; Sharma et al., 2023).

However, achieving high loading and retention of active agents within liposomes and maintaining their structural integrity pose challenges, particularly in the development of stable delivery systems suitable for commercial production conditions (Chotphruethipong et al., 2021; Ramazanzade et al., 2021; Weilin et al., 2012;). Considering the diverse applications of hydrolyzed proteins in various fields, this research aims to present methods for producing hydrolyzed proteins from fish waste, investigate their chemical structure, antibacterial and antioxidant properties, and evaluate the microencapsulation potential of this valuable compound.

Fish protein hydrolysate (FPH)

During the 1960s, extensive research was conducted to find affordable and nutritious protein sources to meet the increasing demands of both humans and animals. Waste materials received significant attention in this regard. In recent years, there has been a global focus on discovering new protein concentrate sources. Seafood offers substantial quantities of high-quality protein that can be consumed by people. Maximizing the utilization of harvested marine products can enhance the nutritional role of these proteins in human diets and contribute to increased fishing yields and aquaculture production (Kristinsson and Rasco, 2000). The global fisheries production has witnessed significant growth, reaching 171 million tons in 2016 (FAO, 2018). Approximately 50% of this production consists of waste or undesired materials that are either processed into fish meal or discarded without proper consideration for the environment. These waste products include fish heads, fins, skin, and intestines, which are rich in biologically valuable fats and proteins that play a crucial role in their decomposition (Bhaskar et al., 2008; Ovissipour et al., 2009; Siddik et al., 2021). According to Das et al. (2021), the average protein content in different fish waste materials is as follows: guts and viscera 9–23%, heads 11–13%, backbones 10–15%, skin 8–12%, spleen and fish roe 14–27%, and trimmings 12–22%. The protein resources of the fisheries industry can be utilized in three sectors: 1) the production of protein products through fish processing, 2) the production of ingredients like surimi from commercial fish, and 3) the production of protein or specific peptide products from fish and its by-products (Kristinsson and Rasco, 2000). Various processes are employed for the recovery and hydrolysis of proteins from marine products and their waste. In the following sections, we will introduce and review these methods.

Chemical hydrolysis

Protein hydrolysis through chemical methods involves the cleavage of peptide bonds using acids or bases. It is an established and commonly used approach for producing hydrolyzed proteins due to its cost-effectiveness and simplicity. Acid hydrolysis is more prevalent than alkaline hydrolysis, involving the reaction of fish proteins with hydrochloric acid or sulfuric acid. Under high temperatures and pressure,

proteins are extensively hydrolyzed. The resulting hydrolyzed protein is neutralized to a pH of 6 or 7, resulting in a paste or dry substance. For example, complete hydrolysis of fish proteins can be achieved by subjecting them to 6N hydrochloric acid at 118 °C for 18 h (Das et al., 2021). Alkaline hydrolysis, on the other hand, utilizes concentrated fish proteins as the initial substrate. It rapidly cleaves peptide bonds, yielding water-soluble polypeptides, followed by slower hydrolysis (Das et al., 2021).

While chemical hydrolysis is a widely used and inexpensive method, it comes with several limitations:

1. Incompatibility with food applications: Chemical hydrolysis can lead to the formation of toxic amino acids such as lysine and alanine, as well as the production of non-absorbable D-form amino acids.
2. Degradation or destruction of certain amino acids: Chemical hydrolysis can result in the degradation or destruction of specific amino acids, including tryptophan, methionine, and cysteine. Additionally, asparagine and glutamine can be converted into aspartic acid and glutamic acid, respectively.
3. Lack of control over the hydrolysis process: Chemical hydrolysis often lacks precise control over the reaction conditions, including temperature, pH, and reaction time.
4. Need for neutralization and salt formation: To stop the hydrolysis reaction, neutralization is typically required. However, this neutralization process often leads to the formation of significant amounts of salt in the final product.
5. Degradation of tryptophan: Tryptophan, an essential amino acid, is particularly susceptible to degradation during chemical hydrolysis.
6. Reduced functional properties due to salt formation: As mentioned earlier, neutralization in chemical hydrolysis can result in salt formation. The presence of salt can affect the functional properties of the hydrolysates, such as solubility, emulsification, and foaming capacity, leading to reduced functionality in various food applications.
7. Limited functionality as a flavor enhancer: Chemical hydrolysis may exhibit limited functionality as a flavor enhancer due to the above-mentioned factors.

These limitations have been documented in previous studies by researchers such as Ovissipour et al. (2009) and Villamil et al. (2017).

Enzymatic hydrolysis

Enzymatic hydrolysis is a method employed to enhance the properties of proteins. The characteristics of the hydrolyzed protein are influenced by factors such as the degree of hydrolysis, peptide structures formed, protein properties, enzyme type, and hydrolysis conditions including temperature and pH (Das et al., 2021; Hou et al., 2017). Compared to acid and alkaline hydrolysis, enzymatic hydrolysis offers several advantages. Enzymes selectively target specific peptide bonds, resulting in controlled degradation of proteins and preservation of amino acids. As a result, enzymatic hydrolysis generally maintains or even increases the nutritional value of proteins. In addition, enzymatic hydrolysis increases protein solubility, which is important for various applications in the food and beverage industry (Das et al., 2021; Dorvaj et al., 2013; Hou et al., 2017). Enzymatic hydrolysis can be achieved through autolytic processes utilizing fish digestive enzymes or through the use of commercial enzymes, which is a cost-effective and relatively straightforward approach. Commercial enzymes offer greater control over the hydrolysis process, resulting in products with specific properties (Kristinsson and Rasco, 2000; Shahidi et al., 1995; Ziyaei and Hosseini, 2021).

The choice of protease enzyme is a crucial factor in enzymatic hydrolysis using commercial enzymes. Various commercial enzymes have been successfully employed for the hydrolysis of fish proteins and other food proteins (Nemati et al., 2019; Ovissipour et al., 2013; Roshan et al., 2015; Shahosseini et al., 2022). It is essential for the enzymes used in fish protein hydrolysis to possess nutritional value, and if they are of microbial origin, the producing organism must be non-pathogenic (Table 1).

Once the appropriate enzyme is selected and added to the substrate, the reaction between the enzyme and protein occurs rapidly, leading to changes in pH due to peptide bond cleavage and the formation of new carboxyl and amino groups. Buffer solutions may be utilized to maintain the desired (Villamil et al., 2017). After the initial rapid hydrolysis, the reaction proceeds at a slower rate until it reach the maximum level of hydrolysis. The hydrolysis step is typically conducted at a constant temperature, and it concludes with the deactivation of the enzyme, which is achieved by subjecting it to temperatures ranging from

Table 1 Types and properties of some commercially enzymes (Aspmo et al., 2005; Ovissipour et al., 2013)

Enzyme name	Source	pH	Temperature
Actividin	Kiwi (fruit)	4–7	15–40
Alcalase 2.4 L	*Bacillus licheniformis*	6–10	55–70
Bromelain	Pineapple (fruit)	5–8	20–65
Flavarzyme	*Aspergillus oryzae*	5–7	50–70
Neutrase 0.8L	*B.amyloliquefaciens*	5.5–7.5	45–55
Papain	Papaya (fruit)	5–9	40–80
Protamex	*Bacillus subtilis*	5.5–7.5	35–60
Promod	*Bacillus spp.*	5–7.5	55–65

75 to 100 °C for 30 min. Enzyme deactivation temperature and pH may vary depending on the specific enzyme used, as each enzyme has an optimal temperature and pH range beyond which it becomes denatured and inactive (Borah et al., 2016; Ziyaei and Hosseini, 2021).

Figure 1 provides an overview of the enzymatic hydrolysis process.

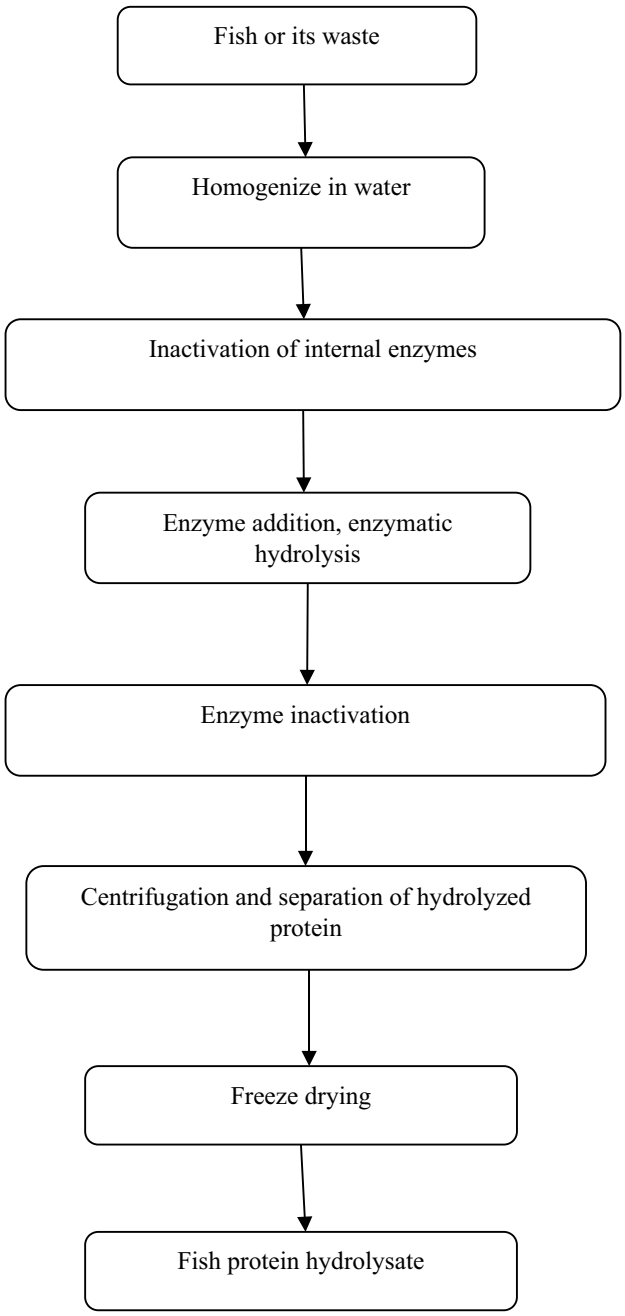

Fig. 1 Schematic diagram of the processesin method of FPH (Borah et al., 2016)

Hydrolyzed protein fractions

The hydrolyzed solution obtained from the enzymatic process is commonly separated into different components using centrifugation. Upon centrifugation, the sludge settles at the bottom, followed by a layer of water, the lipid portion of the protein located between the water layer and the sludge, and finally, the uppermost layer consisting of water and fat. Centrifugation is an effective step for maximal removal of lipids, and the collected fat layer, can be preserved. Multiple centrifugation steps may be necessary to separate soluble proteins from fat and insoluble solids. In commercial operations, the resulting solution is typically spray-dried to transform it into a powder form suitable for incorporation into food products. The insoluble fraction or sludge that precipitates during centrifugation can be utilized as animal feed (Siddik et al., 2021).

Hydrolyzed protein compounds

Protein: Numerous studies have indicated that fish protein hydrolysate (FPH) typically contains a protein content ranging from 70 to 90%. This high protein content can be attributed to the breakdown of proteins into amino acids, protein dissolution, and subsequent removal of insoluble solids through centrifugation (Azizi et al., 2021; Golpaigani et al., 2023; Hamzeh et al., 2018; Nemati et al., 2012; Ovissipour et al., 2009, 2012, 2013; Shahosseini et al., 2022; Wu et al., 2018; Yaghoubzadeh et al., 2020).

Fat: Most studies in this field have reported a fat content in FPH below 5%. The low fat content observed in FPH can be attributed to the disruption of peptide bonds during hydrolysis and the subsequent centrifugation process, which leads to the binding and precipitation of fat with insoluble proteins. Following centrifugation, a separate fat layer is often observed in the supernatant, which can be easily removed and has high nutritional value (Das et al., 2021; Kristinsson and Rasco, 2000; Ovissipour et al., 2009, 2012). Reducing the lipid content in hydrolyzed proteins is crucial for enhancing lipid oxidation stability and ensuring product stability.

Moisture: The moisture content of FPH is typically reported to be less than 10%. The low moisture content is primarily a result of the specific sample type and the high temperatures employed during the evaporation and drying processes, which cause the sample to evaporate (Azizi et al., 2021; Das et al., 2021; Golpaigani et al., 2023; Ovissipour et al., 2009, 2012; Vazquez et al., 2017; Wu et al., 2018; Yaghoubzadeh et al., 2020).

Ash: FPH generally exhibits a relatively high ash content, ranging from 0.45% to 27%. This higher ash content can be attributed to the use of acids and alkalis for pH adjustment during the production process (Azizi et al., 2021; Das et al.,

2021; Golpaigani et al., 2023; Ovissipour et al., 2009, 2012; Vazquez et al., 2017; Wu et al., 2018; Yaghoubzadeh et al., 2020).

Antioxidant properties of hydrolyzed protein

The oxidation of biological molecules within the human body and in food can lead to cell death, tissue damage, and degradation of food quality. Antioxidants are molecules that can counteract this process by trapping free radicals and preventing oxidation (Azizi et al., 2021). In recent years, marine protein sources have gained significant attention as potential suppliers of antioxidant peptides. The first scientific report on the antioxidant activity of hydrolyzed fish protein was published by Shahidi et al. in 1995, and since then, numerous researchers have conducted studies on the production, isolation, and identification of antioxidant peptides from hydrolyzed proteins derived from different fish species (Pezeshk et al., 2019; Tejpal et al., 2017). Table 2 presents a compilation of information regarding the antioxidant properties exhibited by hydrolyzed fish protein. These antioxidant peptides, ranging from 2 to 20 amino acids in length and with a molecular weight below 6000 Da, have been isolated from hydrolyzed proteins of various fish species. The antioxidant activity of these peptides depends on their amino acid sequence, composition, hydrophobicity, and peptide size (Hamzeh et al., 2019; Shahosseini et al., 2022). Smaller peptides, typically containing 2 to 10 amino acids, exhibit higher antioxidant activity compared to longer polypeptides (Halim et al., 2016). Specific amino acids, such as valine, leucine, proline, histidine, and tyrosine, present in the peptide sequence contribute to the antioxidant activity. Tyrosine, in particular, acts as an electron donor and prevents oxidative substances from oxidizing other compounds by capturing electrons (Slizyte et al., 2018).

Hydrolyzed proteins demonstrate antioxidant capacity through several mechanisms. Firstly, they can neutralize free radicals by donating electrons or hydrogen atoms, thus stabilizing the radicals and preventing them from causing oxidative damage. Secondly, hydrolyzed proteins can chelate metal ions like iron and copper, inhibiting their ability to catalyze the production of reactive oxygen species (ROS) through Fenton and Haber–Weiss reactions. Thirdly, hydrolyzed proteins can directly deactivate molecular oxygen, reducing the formation of ROS. Additionally, hydrolyzed proteins can donate hydrogen atoms to ROS or other reactive species, interrupting the chain reaction of lipid peroxidation and preventing the propagation of oxidative damage. Lastly, hydrolyzed proteins can form protective layers around oil droplets, acting as a physical barrier to prevent the penetration of oxidized fat initiators and maintaining the stability and quality of lipid-containing systems (Halim et al., 2016; Liao et al., 2020; Shahidi and Zhong, 2015). Methods for evaluating antioxidant activity can be categorized into two groups based on the chemical reactions involved. The first category includes methods based on hydrogen atom transfer (HAT), which measure the ability of antioxidants to react with and neutralize peroxyl radicals. Examples of such methods are the oxygen radical absorption capacity (ORAC), total radical scavenging antioxidant parameter (TRAP), and beta-carotene decolorization assays. The second category comprises methods based on electron transfer (ET), which assess the capacity of antioxidants to reduce oxidants. Trolox equivalent antioxidant capacity (TEAC), ferric reducing antioxidant power (FRAP), and DPPH radical scavenging capacity are examples of methods based on electron transfer (Halim et al., 2016; Shahidi and Zhong, 2015).

Antibacterial properties of hydrolyzed proteins

Antimicrobial peptides are small molecules consisting of 1 to 50 amino acids and with a molecular weight below 10 kDa. These peptides are typically amphipathic, rich in cysteine residues, and possess a positive charge in their active form, allowing them to exert antimicrobial effects against bacterial cells, yeast, fungi, viruses, and even tumor cells (Bi et al., 2020; Cheung et al., 2015). Marine antimicrobial peptides exhibit distinct structural characteristics compared to peptides derived from non-aquatic sources. They often possess unique structures, broad-spectrum antimicrobial activity, low sedimentation rates, and high specificity. These peptides demonstrate antibacterial properties against both Gram-negative and Gram-positive bacteria and hold potential for the development of antibiotics and antibacterial agents in the food industry (Cheung et al., 2015; Pezeshk et al., 2019).

Mechanisms of action of peptides on bacterial cell membranes have been proposed, involving initial binding to the surface of the cytoplasmic membrane followed by pore formation. The formation of pores disrupts the integrity and permeability of the membrane, leading to impairment of cell respiration, interference with the electrochemical gradient, and influx of water and ions, ultimately resulting in cell swelling and lysis (Perez Espitia et al., 2012).

Two models, namely the Barrel-stave model and the carpet model, have been proposed to explain the pore formation process. In the Barrel-stave model (Fig. 2a), a channel is formed by the association of bipolar alpha helices, with the hydrophobic surface of the peptide interacting with the lipid core of the membrane and the hydrophilic surface turning inward, leading to pore formation. An increase in peptide concentration on the membrane surface enlarges the pores, resulting in cell death and release of cellular contents (Perez Espitia et al., 2012). On the other hand, in the carpet model (Fig. 2b), a high concentration of peptides contacts the outer surface of the membrane, allowing penetration. The

Table 2 Antioxidant activity of protein hydrolysates from different types of fish

Source	Part of source	Enzyme type	Molecular weight or peptide Sequence	Antioxidant activity	References
Anchovy sprat (*Clupeonella engrauliformis*)	Whole part	Alcalase Bromelain Flavourzyme Papain Promod Protamex	-	DPPH: 11.2- 15.6 µmol TE g – 1 protein ABTS: 684- 976 µmol TE g – 1 protein Reducing power: 3.6–7.6 µmol TE g – 1 protein Ferrous chelation: 19.9- 52.5%	Ovissipour et al. (2013)
Decapterus maruadsi	Muscle	Alcalase	III: < 5 kDa II: 5–10kDa I: > 10 kDa HEKVC HDHPVC	DPPH 39.36% (FPH) I: 47.23% II: 36.27 III: 50.54 EC50 0.0677 (mM) EC50 0.0310 (mM)	Jiang et al. (2014)
Thunnus albacores	Skin	Pepsin	< 3 kDA	DPPH 435.97- 457.67 (mg BHT Eq/mL)	Blanco et al. (2017)
Tilapia	Whole waste	Pepsin	< 18 kDA	0.5–2.5 mg DPPH: 29.48–73.62% 20- 40 mg/ ml FRAP: 0.7- 55.49	Tejpal et al. (2017)
Cuttlefish (*Sepia Pharaonis*)	Muscle	Alcalase	-	DPPH: 5.2 µmol TE/g sample Reducing power: 0.4 Total antioxidant capacity: 0.6 mg ascorbic acid equivalent/g	Hamzeh et al. (2018)
Yellowfin tuna (*Thunnus albacores*)	Viscera	Protamex	FPH < 3, 3–10, 10–30, and 30 kDa <	DPPH, IC50: 1.8–3.63 ABTS, IC50: 1.54–1.84 1–4 mg Reducing power: 0.08–1.3	Pezeshk et al. 2019
Klunzinger's mullet (*Liza klunzingeri*)	Muscle	Papain	-	ABTS, IC50: 0.60–0.12 mg/mL DPPH, IC50:3.18–2.08 mg/mL hydroxyl radicals, IC50: 4.13–2.07 mg/mL	Rabiei et al. (2019)
Carp (*Cyprinus carpio*)	Skin gelatin	Alcalase Protamex Flavourzyme Neutrase	-	DPPH: 0.43- 2.11 µmol TE/ mg protein FRAP: 3.60–4.20 µmol TE/ mg protein	Tkaczewska et al. (2020)
Rainbow Trout (*Oncorhynchus mykiss*)	Skin	Alcalase	-	DPPH: 18.5–50% FRAP: 0.2–0.5	Yaghoubzadeh et al. (2020)
Lizard fish (*Saurida tumbil*)	Muscle	Alcalase Flavourzyme	-	DPPH: 37.92% ABTS: 77.34% Hydroxyl: 21.79% Ferrous ion: 26.95%	Bahram et al. (2020)
Whitefish of Caspian Sea (*Caspian kutum*)	Viscera	Alcalase Flavourzyme	-	DPPH: 19–75% FRAP: 0.32–0.78	Azizi et al. (2021)

Table 2 (continued)

Source	Part of source	Enzyme type	Molecular weight or peptide Sequence	Antioxidant activity	References
Liza abu	Muscle	Alcalase FlavAlcalase + Flavourzyme ourzyme	< 3, 3–10, 10–30 kDa	DPPH: 38.51- 84.46% ABTS: 31.79- 80.11% FRAP: 0.18–0.34 Metal chelation: 51.41- 89.94%	Shahosseini et al. (2022)
Indian mackerel (*Rastrelliger kanagurta*)	skin and head	Alcalase Flavourzyme	-	DPPH: 30.43- 90.42% ABTS: FRAP: 0.17–0.37 31.56- 91%	Hasani et al. (2022)
Rainbow trout	Roe	Alcalase Pepsin	-	DPPH: 37.72- 87.39%	Golpaigani et al. (2023)

peptides align on the membrane surface resembling a carpet, and through a series of interactions, including hydrophilic and hydrophobic interactions, they induce the formation of hydrophobic pores and disrupt the membrane structure, ultimately leading to membrane destruction (Perez Espitia et al., 2012).

Various methods are employed to assess the antimicrobial activity of hydrolyzed proteins and peptides. One commonly used method is the agar diffusion method, which measures the ability of antibiotics to inhibit bacterial growth. The agar diffusion method is frequently employed to determine the minimum inhibitory concentration (MIC) in solid media (Najafian and Babji, 2012). The MIC value represents the lowest concentration of the antimicrobial agent that significantly inhibits the growth of the tested microorganism and is typically expressed in mg/ml or mg/l (Li et al., 2017).

Table 3 provides a compilation of the antimicrobial activity of hydrolyzed fish protein.

Restrictions on the use of bioactive peptides

Sensory characteristics and bitter taste of bioactive peptides

The widespread application of biopeptides in the food industry is constrained by several factors, including sensory drawbacks such as undesirable color and taste. Additionally, the activity of these peptides can be influenced by other food components like carbohydrates and fats, thereby impacting product stability during processing and storage (Li-Chan, 2013). The challenge of consumer acceptance regarding the bitter taste associated with hydrophobic peptides, which are bioactive peptides generated during the hydrolysis process of proteins. Hydrophobic peptides contain nonpolar amino acids, such as phenylalanine, leucine, and valine, which contribute to their unique structural characteristics (Liu et al., 2016; 2022). However, the bitter taste of hydrophobic peptides can be a significant obstacle in the development and acceptance of products containing these peptides. In general, bitterness tends to escalate with increasing degrees of hydrolysis. The perception of bitterness in a peptide is influenced by various factors, including its amino acid composition and sequence, three-dimensional structure, and molecular weight. These attributes collectively contribute to determining the threshold at which the peptide is perceived as having a bitter taste (Li-Chan, 2013, Liu et al., 2022).

Solubility properties

Bioactive compounds possess high water solubility, making them suitable for incorporation into water-based food products such as beverages, sauces, desserts, and soups. However, their limited solubility in the lipid phase hinders their

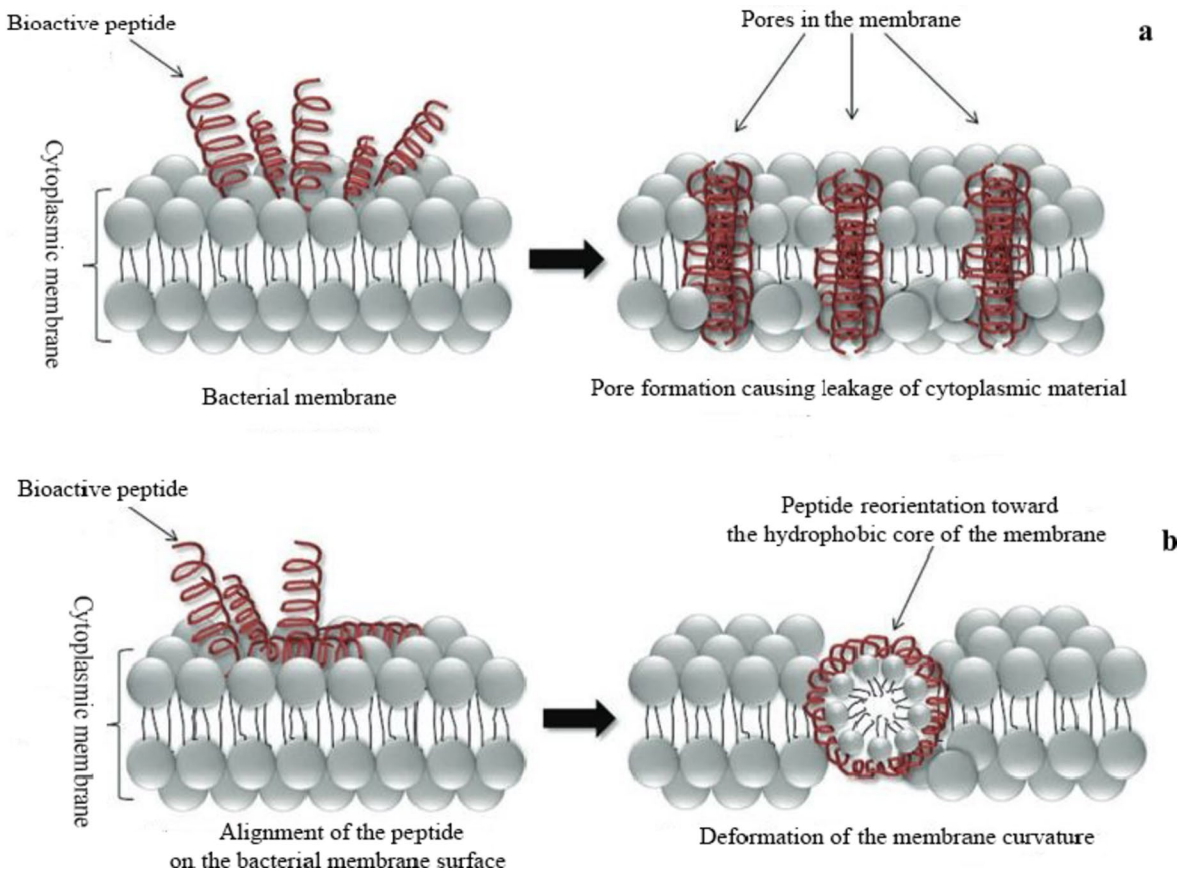

Fig. 2 Barrel-stave model: **a** carpet model, **b**. (Perez Espitia et al., 2012)

mass transfer within lipid-based food systems. To overcome this challenge and utilize these compounds in lipid-based food formulations like mayonnaise, margarine, and butter, appropriate micro-coating techniques should be employed (Hosseini et al., 2021).

Chemical and physical instability

Proteins and peptides derived from protein hydrolysis are prone to chemical instability due to variations in factors like pH, ionic strength, temperature, light, oxygen, and reactive compounds. To safeguard these compounds from such environmental factors, micro-coating techniques play a crucial role (Mosquera et al., 2014).

The presence of metal ions, including calcium, copper, and iron, can induce protein precipitation and polysaccharide gelatinization. Micro-coating methods effectively mitigate these undesired physical changes within the system by separating reactive species and providing protection to bioactive compounds (Burey et al., 2008).

In general, for addressing concerns such as bitter taste reduction, moisture attraction prevention in bioactive proteins, preservation of bioactivity, and protection of hydrolyzed protein components, optimal loading conditions are employed. Advanced technologies like nano and microencapsulation have proven successful in ensuring the stability of bioactive peptides in food applications (Mohan et al., 2015; Sharma et al., 2023).

Microencapsulation of protein compounds and bioactive peptides

Microencapsulation involves the encapsulation of solid, liquid, or gas compositions within capsules made of various carriers. In the food industry, the growing demand for healthier foods and the utilization of micronutrients and bioactive compounds have led to the widespread application of microencapsulation technology for the enrichment and preservation of compounds in food formulations (Eskandari et al., 2021). One category of these bioactive compounds is proteins and peptides derived from enzymatic hydrolysis. Bioactive peptides, in particular, possess distinct structures and properties compared to other compounds like vitamins and polyphenols. Consequently, it is crucial to separate them from the food matrix due to their unique physicochemical properties. Recent studies have

Table 3 Antimicrobial activity of protein hydrolysates from different types of fish

Source	Part of source	Enzyme type	Molecular weight or peptide Sequence	Tested bacterial strain	Antimicrobial activity	References
Atlantic mackerel (*Scomber scombrus*)	By-products	Protamex	SIFIQRFTT RKSGDPLGR AKPGDGAGSGPR GLPG-PLGPAGPK	*Listeria innocua (+)* *Escherichia coli (-)*	Inhibited tested bacterial strains	Ennaas et al. (2015)
Rainbow trout (*Oncorhynch us mykiss*)	By-products	Pepsin		Several gram positive and gram negative bacteria	The highest antibacterial activity detected was against the *Flavobacterium psychrophilum* and *Renibacterium salmoninarum*, MIC: 2-5 mg/ml,	Wald et al. (2016)
African catfish (*Clarias gariepin*)	By-products	Pepsin Trypsin Protease	4.1 KD	*Staphylococcus aureus (+)* *Aeromonas sobria* (-) *Aeromonas hydrophila* (-) *E. coli* (-)	It showed antimicrobial activity against both Gram + and -MIC: 105 to 420 µg/mL.	Li et al. (2017)
Nile Tilapia (*Oreochromis niloticus*)	Whole waste	Papain	-	*E. coli* (-) *S. aureus* (+) *Listeria monocytogenes* (+)	8 and 10 mg /mL FPH was potentially active against gram +, Smaller zones of inhibition against gram -	Srikanya et al. (2018)
Argentine croaker (*Umbrina canosai*)	Whole fish	Alcalase Protamex	-	Several gram positive and gram negative bacteria	In all the evaluated microorganisms, only 7 were inhibited, most being Gram + e. Alcalase had more antibacterial activity	Da Rocha et al. (2018)
Yellowfin tuna (*Thunnus albacores*)	Viscera	Protamex	FPH < 3, 3–10, 10–30, and 30 kDa <	*E. coli (-)* *Pseudomonas spp (-)* *S. aureus (+)* *L. monocytogenes (+)*	3 kDa had significantly the highest percentage of bacteria inhibition activity against both Gram + and -	Pezeshk et al. (2019)
Pacific chub mackerel (*Scomber japonicus)*	Fin waste	Papain Pepsin Trypsin Protease	-	*E. coli* (-) *Salmonella spp* (-)	All hydrolysates have antibacterial activity Best activity against both strains was shown by Trypsin	Ediriweera et al. (2019)
Turbot (*Scopthalmus maximus*)	Viscera	Pepsin Trypsin	GITDLRGMLKRLKKMK	*E. coli* (-) *Salmonella typhimurium* (-) *Hafnia alvei (-)* *S. aureus* (+) *L. monocytogenes* (+) *Bacillus subtilis* (+)	Antibacterial activity against both Gram + and—bacteria by damaging the cell membrane integrity	Bi et al. (2020)

Table 3 (continued)

Source	Part of source	Enzyme type	Molecular weight or peptide Sequence	Tested bacterial strain	Antimicrobial activity	References
Lizard fish (*Saurida tumbil*)	Muscle	Alcalase	-	*E. coli* (-) *Pseudomonas spp* (-) *S. aureus* (+) *L. monocytogenes* (+) *Streptococcus iniae* (+) *Bacillus cereus* (+)	FPH showed no antibacterial activity against the studied bacterial strains	Bahram et al. (2020)
Liza abu	By-products	Alcalase Flavourzyme	-	*E. coli* (-) *S. aureus* (+)	It showed antimicrobial activity against both Gram + and -MIC: 156 to 1006 µg/mL.	Shahosseini et al. (2023)

focused on various aspects of microencapsulation of bioactive peptides derived from fish (Table 4). The reasons for micro-coating these compounds include addressing issues such as bitter taste and high hygroscopicity (Mohan et al., 2015; Sharma et al., 2023).

Nanocarrier systems can be classified into two categories: biopolymers (proteins and polysaccharides) and lipid-based carriers (nanoemulsions, microemulsions, nanoliposomes, and solid lipid nanoparticles) (McClements and Öztürk, 2021; Otchere et al., 2023). Lipid-based nanoencapsulation systems offer several advantages, including the ability to encapsulate substances with diverse solubilities, the utilization of natural compounds, and the potential for targeted delivery (Fathi et al., 2012).

The encapsulation of FPH and fish oils in double emulsions has been reported to provide several benefits, including taste masking, protection against oxidation, and improved bioavailability for human health (Paulo & Santos, 2018). Double emulsions, specifically water-in-oil-in-water (W1/O/W2) emulsions, are prepared by dispersing W1/O emulsion droplets within an aqueous phase (W2) (Sapei et al., 2012). This emulsion structure involves encapsulating the oil droplets within an inner matrix and surrounding them with a protective coating.When encapsulating PUFA-rich oils, such as x-3 PUFA, it is necessary to treat the oil-in-water emulsions before forming the W1/O/W2 double emulsions (Jamshidi et al., 2019; 2020). Studies have shown that W1/O/W2 double emulsions have potential applications as delivery systems for bioactive lipids and as a means to trap and protect both hydrophilic and lipophilic compounds (McClements et al., 2007; Jamshidi et al., 2019; 2020). The emulsion structure acts as a protective barrier, preventing direct interaction between the encapsulated peptides and other food components, such as enzymes or pH conditions, which may degrade or alter the activity of the peptides. This protective effect enhances the functional properties of the peptides, including antioxidant, antimicrobial, or bioactive effects (Jamshidi et al., 2019; 2020). When utilizing W/O/W double emulsions for peptide encapsulation in food applications, several factors need to be considered. These factors include emulsion composition, processing conditions, and choice of surfactants, all of which can influence encapsulation efficiency, release profile, and overall stability of the encapsulated peptides (Carneiro et al., 2013). Careful consideration of these factors is essential to optimize the encapsulation process and achieve desired outcomes in terms of peptide functionality and delivery in food systems (Jamshidi et al., 2020; Poyato et al., 2013; Sapei et al., 2012). Thermodynamic instability is the main challenge of using W1/O/W2 emulsions in food applications.

Among lipid nanocarriers, nanoliposomes have emerged as one of the most significant systems for microencapsulating bioactive compounds such as hydrolyzed proteins. They

Table 4 Encapsulation of protein hydrolysates from different types of fish with different nanostructured materials

Source	Part of source	Enzyme type	Nano-delivery system	Major outcome(s)	References
Whitemouth croaker (*Micropogonias furnieri*)	Muscle and by-product	Flavourzyme	Phosphatidylcholine	The capsules maintained the antioxidant activity of the FPH	Da Rosa et al. (2014)
Sea bream (*Sparus aurata*)	Fish scale	Esperase	Nanoliposomes	Retention of the ACE-inhibitory and antioxidant activity after encapsulation procedure	Mosquera et al., 2014
Atlantic salmon (*Salmo salar*)	Muscle	Trypsin	Chitosan-coated liposomes	Liposomes with chitosan coating show great promise as a potential delivery system for FPH	Li et al. (2015)
Rainbow trout (Onchorhynchus mykiss)	Skin gelatin	Alcalase	Nanoliposomal vesicles	-Liposomal nanovesicles exhibited the antioxidant activity of the peptidic fraction -Encapsulated peptide with liposomome can be a good source for nutraceutical and functional foods	Hosseini et al. (2017)
Rainbow trout (Onchorhynchus mykiss)	Skin gelatin	Alcalase	Chitosan-coated nanoliposome	-Chitosan-coated nanoliposomes maintained the antioxidant activity of FPH -Prolonged peptide release behavior	Ramezanzade et al. (2017)
Common Kilka (*Clupeonella cultriventris*)	Whole fish	Alcalase	Chitosan-coated nanoliposome (NP)	-Sustained in vitro release of bioactive peptide was achieved by nano-sized vehicles -Efficacy of NPs in retaining antioxidant activity was affirmed by DPPH/FRAPS assays	Hosseini et al. (2018)
Common carp (Cyprinus carpio)	Whole waste	Alcalase	Chitosan-coated liposomes	Encapsulation of FPH in the liposomal system could be a useful approach for direct application of peptides with antioxidant potential in food products	Hasani et al. (2019)
Shrimp	Waste	Neutrase	Chitosan-coated nanoliposome	Coating bioactive peptides with combined coating is a suitable technique to protect and increase the efficiency of peptides	Reyhani Poul and Yeganeh (2021)
Common Kilka (*Clupeonella cultriventris*)	Whole fish	Alcalase	Chitosan-sodium tripolyphosphate-liposomes	Hybrid system maintained the antioxidant activity and stability of FPH	Ramezanzade et al. (2021)
Salmon	Salmon Frame	Papain	Liposomes	Encapsulation of liposomes with 1% plastein1 could be an efficient delivery system for improving the sensory characteristics by lowering the bitterness of protein hydrolysates	Sharma et al. 2023

[a]Plastein is a reaction, which has been used for lowering the bitterness of protein hydrolysate

share a similar structure to W/O/W double emulsions (Reyhani Poul and Yeganeh, 2021; Sharma et al., 2023).

Nanoliposome

Liposomes (Fig. 3) are spherical particles composed of polar lipids such as phosphatidylcholine and phosphatidylethanolamine, or mixtures of polar lipids with cholesterol or ergosterol. When exposed to water (a polar solvent), lipids form bilayer membranes, giving rise to the characteristic structure of liposomes. Due to their composition, liposomes are widely utilized as delivery systems for microencapsulation, protection, and controlled release of bioactive compounds in the food industry (Mozafari et al., 2008; Reyhani Poul and Yeganeh, 2021). Liposomes are flexible carriers capable of transporting hydrophilic substances within the vesicles and hydrophobic substances within the bilayer membrane (Mozafari et al., 2008). Challenges associated with liposomes include poor stability under certain physical conditions, rapid dissolution, manufacturing complexities, and limited loading capacity. However, extensive research has been conducted to enhance techniques and processing methods, incorporating stabilizing and modifying compounds to overcome these limitations (Krobthong et al., 2021). Nanoliposomes, known for their compatibility, biodegradability, and absence of toxic compounds, can effectively mask the unpleasant odor of hydrolyzed collagen obtained from fish skin (Chothphruethipong et al., 2020). The membrane structure of liposomes closely resembles biological cell membranes, making them suitable for targeted delivery of bioactive ingredients through membrane interactions and serving as a model for biological membranes (Sharma et al., 2023). Nanoliposomes enable targeted delivery of substances with varying solubilities, including nutritional compounds like peptides and vitamins, as well as undesirable components such as odoriferous omega-3 fatty acids, thereby improving bioavailability (Mozafari et al., 2008; Reyhani Poul and Yeganeh, 2021).

The application of nanoliposomes as carriers for hydrolyzed fish proteins enhances their antioxidant and antibacterial properties and improves skin permeability. Moreover, liposome nanoencapsulation proves to be an effective technique for reducing the bitter taste associated with hydrolyzed fish proteins (Sharma et al., 2023).

All in all, in recent times, the utilization of seafood waste has evolved from being considered a disposable material to being recognized as a valuable source of biologically active compounds with various potential applications. One approach to effectively utilize marine protein waste is through enzymatic hydrolysis, which converts proteins into bioactive peptides. Bioactive peptides are released during the hydrolysis process and are composed of sequences of primary amino acids. Aquatic hydrolyzed proteins and the peptides derived from them possess antioxidant properties, as they can neutralize free radicals. Additionally, these compounds exhibit antibacterial properties, as antimicrobial peptides can act against a broad spectrum of microorganisms, including bacteria (both Gram-positive and Gram-negative), fungi, and viruses. Despite the numerous advantages of bioactive peptides, their application in the food industry is limited due to factors such as sensory characteristics, bitter taste, physical and chemical instability, and high solubility. Therefore, the use of nanoencapsulation techniques becomes essential to enhance the stability and targeted release of these compounds. Various colloidal delivery systems, including microemulsions, nanoemulsions, solid fat nanoparticles, and liposomes, have been employed for this purpose. Liposomes, in particular, are commonly employed as delivery systems for the microencapsulation, protection, and controlled release of hydrophilic bioactive compounds in the development of food formulations and ingredients. However, further research is required to investigate the use of different nanocarriers to preserve and improve the quality of hydrolyzed marine proteins, evaluate the impact of incorporating nanoencapsulated hydrolyzed proteins on the shelf life and sensory properties of food products, and assess their stability, diffusion, and absorption in digestive environments.

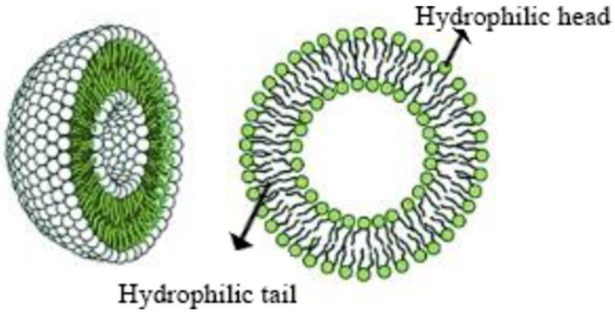

Fig. 3 Design of liposome (Divyasree et al., 2022)

Data availability Data available on request from the authors.

Declarations

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

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