

MARINE-DERIVED BIOACTIVE PEPTIDES AND THEIR HEALTH-PROMOTING PROPERTIES

Abstract

Bioactive peptides are chains of acids that have the ability to produce specific effects, on the human body. These peptides can be obtained from sources, such, as animal, plant and microbial proteins. Among these marine derived bioactive peptides possess a significant place in literature. In years a lot of research has been conducted on producing, purifying and characterizing peptides because of their potential to promote good health. This chapter aims to explore the methods used in generating marine derived peptides and the techniques employed for their purification and characterization. Moreover it will delve into the range of health benefits associated with consuming bioactive peptides.

Keywords: Bioactive peptides, marine peptides, bioactive properties, human health

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I. INTRODUCTION

The scientific community acknowledges oceans as a major supplier of bioactive compounds. The vast expanse of oceans, which occupy 70% of the Earth's surface is a rich reservoir of bioactive compounds that can be listed as follows: Polyunsaturated fatty acids (PUFA), gelatin, collagen, minerals, antioxidants, polysaccharides, peptides. These compounds have structures and hold great promise as potential therapeutic agents.

In times there has been a surge of interest, in investigating the health advantages of bioactive peptides derived from marine resources. These small protein fragments, usually consisting of 2-20 amino acids. In certain investigations, longer peptides have been observed. For instance, Lunasin, a soy-based peptide, consists of 43 amino acid residues. Peptides that are small, in size have a chance of easily passing through the intestinal wall without any obstacles allowing them to effectively demonstrate their biological and functional effects. Bioactive peptides have shown potential in promoting wellbeing and preventing health issues [1].

Bioactive peptides obtained from marine resources possess qualities such as antioxidant properties, antimicrobial activity, blood pressure regulation capabilities, anti-diabetic, anti-obesity, anti-inflammatory effects and immune system modulation among others [2]. Bioactive peptides offer benefits over conventional drugs, including their lower toxicity and minimal side effects, in humans. Given their range of functionalities, they are considered candidates for application in industries such, as food production, pharmaceuticals and nutraceuticals [3]. The BIOPEP-UWM, which is a database containing extensive data on bioactive peptides, presently comprises 4,699 bioactive peptides that have been identified.

Bioactive peptides, while initially inactive within the structure of a parent protein, exhibit a wide range of biological activities after being destroyed via conventional or unconventional methods. These activities are determined by the specific amino acid composition and sequence of the peptides [4].

The crude protein hydrolysate comprises a blend of peptides and unbound amino acids. Obtaining these peptides involves several important steps. Firstly, we should select the protein source and employ various enzymatic, chemical or microbial techniques to produce the peptides. Then it becomes crucial to purify and refine the desired peptides from a mixture of proteins and other substances. In this purification process innovative technologies, like chromatography, ultrafiltration and electro dialysis play a role.

After the purification process, it is crucial to examine the peptides to determine their safety, stability and biological effects. To accurately understand the structure and functions of these peptides advanced analytical techniques such, as mass spectrometry, nuclear magnetic resonance (NMR) spectroscopy and bioassays are utilized.

II. PRODUCTION, PURIFICATION AND CHARACTERIZATION OF BIOACTIVE PEPTIDES

In recent years, it has been known that marine proteins offer a rich resource of bioactive peptides [5]. Over the past few decades, numerous researchers have isolated and

reported various kinds of bioactive peptides derived from marine organisms [2]. Bioactive peptides can be found encased in food proteins and they are found passively within the structure. This non-active structure should be destroyed via hydrolysis methods for the emergences of bioactive properties [6]. Conventional methods for the production of bioactive peptides are enzymatic hydrolysis, fermentation and chemical hydrolysis. Due, to the nature of enzymes their low yield, high cost and the limited availability of food grade enzymes, the researches shifted their attention to searching for alternatives [7]. Several innovative processing techniques have been arising as substitutes for these conventional methods in the production of bioactive peptides (Figure 1).

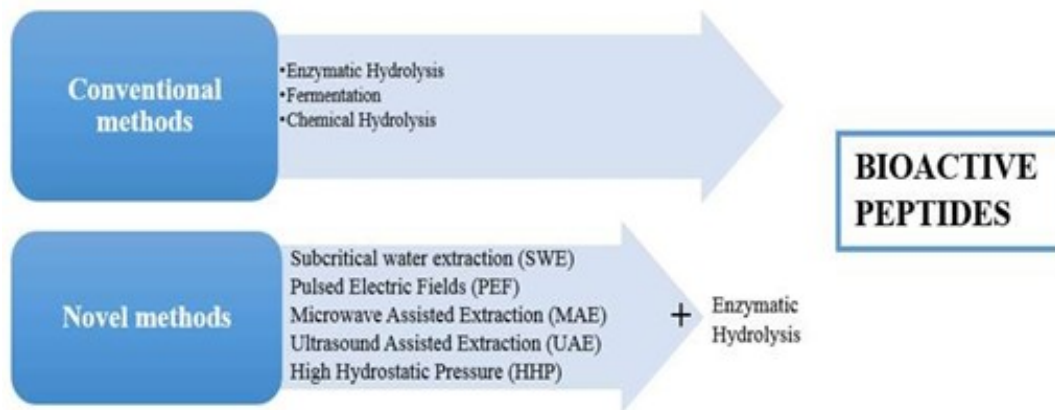


Figure 1: Main Methods for Production of Bioactive Peptides

1. Production: Chemical hydrolysis methods are renowned for their simplicity, rapidity, and long-standing reputation, establishing them as a conventional approach for peptide synthesis. The production of protein hydrolysates involves subjecting them to chemical hydrolysis, either in acidic or alkaline environments, with these processes operating at elevated temperatures (around 120°C) and pressures (approximately 100 kPa). Within this framework, alkali-based methodologies frequently yield derivatives of lysinoalanine, a well-documented toxic compound formed when essential amino acids like tryptophan or cysteine undergo hydrolysis. Furthermore, the neutralization step in chemical hydrolysis procedures leads to a substantial salt generation. Additionally, chemical techniques are susceptible to amino acid degradation due to high temperatures, fluctuating pH levels, and, in certain instances, pressure. Despite their drawbacks, such as limited sensitivity, specificity, suboptimal nutritional attributes, and constrained functionalities, the chemical liberation of antioxidant peptides remains a more economically viable option for industrial peptide production [8].

The breakdown of protein molecules, through enzymatic hydrolysis is the main method used to produce bioactive peptides (Figure 2). Enzymatic hydrolysis is known for its easily manageable conditions making it a precise method, for breaking peptide bonds. One of its advantages is the absence of side reactions or any loss in value. Moreover it offers enhanced protein recovery and purification capabilities for peptides. These characteristics have garnered increasing interest in producing hydrolysate, with functional and bioactive properties. Usually the process of hydrolysis occurs in a container called a

reactor. In this reactor various factors such as temperature, pH level, agitation and duration can be controlled. Firstly, pH and temperature had to be adjusted for the protease which is used for hydrolysis. Once protease is added, the interaction between the enzyme and substrate leads to alterations in the solution's pH as a result of cleavage of peptide bonds to form new amino or carboxyl groups able to release [9].

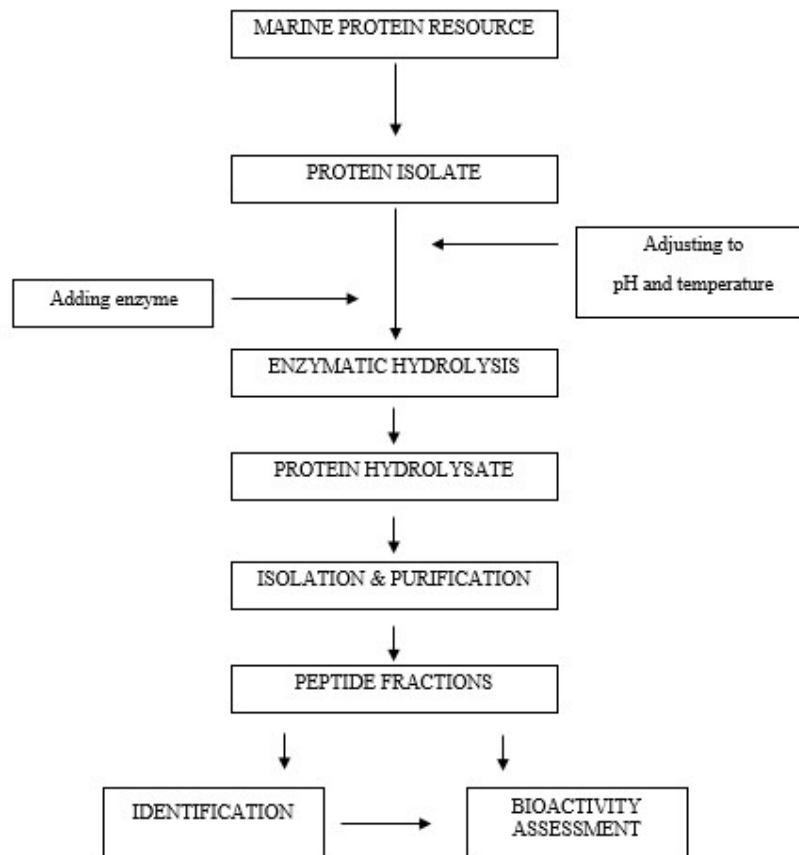


Figure 2: Flow Chart of Bioactive Peptide Production by using Proteases

Fermentation, an age-old technique for preserving food, enables the creation of bioactive peptides. Fermented goods are typically derived from microorganisms through both spontaneous and controlled fermentation processes. In this context, lactic acid bacteria assume a vital role in generating fermented products, as they contribute essential nutritional and technological characteristics. These attributes encompass improvements in texture and the development of flavors, both of which are pivotal aspects of the fermentation process. When compared to enzymatic methods, the cost of producing peptides via fermentation is relatively lower. Nevertheless, the industrial application of fermentation for peptide production encounters challenges, such as issues with peptide yield and the lack of precision in peptide formation [7].

HHP (High Hydrostatic Pressure) represents an environmentally conscious and non-thermal approach conducted under iso static pressures spanning from 100 to 1000

MPa. The HHP technique functions as a batch-based system in which water acts as a medium for transmitting the pressure. Over the past few decades, this processing technique has been extensively studied for its ability to inhibit microbial activity while preserving the organoleptic and nutritional attributes of food products. Therefore, the main benefit of this approach is that it causes minimal interference with biologically active mixtures by employing lower or moderate temperatures [7]. A method called high pressure processing was used along, with hydrolysis to enhance the breakdown of proteins. High pressure processing causes proteins to unfold, which makes it easier for enzymes to break them down and speed up the process of protein hydrolysis. To enhance proteolysis a combination of pressure processing and enzymatic hydrolysis (referred to as HHP assisted hydrolysis) was utilized. The application of pressure causes the protein structure to unfold thereby exposing sites where enzymes can facilitate the hydrolysis of proteins. In a research, the aim was to enhance the characteristics of fish gelatin through the application of hydrostatic pressure (HHP) and ultrasonication (US). The rate at which the pressure was increased was 340 MPa, per minute. This was done until it reached a pressure of 400 MPa. The time taken to release the pressure for each treatment was, than 20 seconds. The researchers were report that both the HHP and US processing methods had an impact, on increasing the gel strength of the samples compared to those that were not treated. The outcomes derived from FTIR analysis showed that the applications induced modifications to the configuration of acids. Both treatments yielded improvements in gel characteristics such as gel strength gelling temperature and melting temperature for fish gelatin. Ultimately after assessing parameters it was determined that the optimal combination, for fish gelatin was achieved with a pressurization level of 400 MPa at 10 °C for a duration of 15 minutes [10].

Ultrasonic Assisted Extraction (UAE) is a technology that utilizes ultrasonic waves with frequencies, above 20 kHz to produce peptides. This innovative non-thermal physical technique relies on the transmission of waves through a medium. Acoustic waves, which can be classified based on their similarity to frequencies to humans fall within the range of 20 Hz to 20 kHz. Frequencies below this range are referred to as infrasound whereas those, above it are called ultrasound [11]. The constraints associated with enzyme hydrolysis have been surmount by the utilization of ultrasound, resulting in enhanced protein conversion rates and a reduction in the time required for hydrolysis. [12]. The benefits of ultrasound technology such, as enhanced energy and mass transfer, time and temperature requirements, improved process control, selective extraction capabilities and quicker initiation have made it a favored pretreatment method, for generating bioactive peptides. Ultrasound pretreatment has been found to enhance the production of peptides (ranging from 200 to 3000 Da) that are rich, in hydrophobic amino acids and possess antioxidant properties [13].

Liquid water, on Earth stands out as a green technology for extracting bioactive compounds when compared to conventional methods that employ organic solvents. Subcritical water (SCW), which possesses toxic and nonflammable properties has gained significant attention due to its ability to extract substances without leaving any solvent residue. Subcritical water refers to water maintained at temperatures that encompass the range of 100°C to 374°C and pressures below 22 MPa (below the critical point of water). Subcritical water hydrolysis has been widely used to extract molecules, such, as protein hydrolysates in the form of peptides or amino acids. Numerous studies have utilized water

to produce peptides, from animal and plant sources, including fish [14]. SCW exhibits the ability to generate hydrolysates, with weight from proteins. When squid muscle was treated with SCW at a temperature of 220°C it resulted in the production of peptides, with antioxidant properties. However when higher temperatures were used the peptides underwent decomposition. Transformed into organic compounds [15].

A microwave operates by utilizing waves that fall within the range of 300 MHz to 300 GHz. These waves transmit energy through interactions, in the material through processes known as rotation and ionic conduction. Recently scientists have been using microwave assisted (MAE) hydrolysis to extract bioactive peptides from various sources such as rainbow trout frames [16], algae [17], sea cucumber [18]. A group of researchers has explored the anti-oxidative properties of brown algae, employing MAE with diverse proportions of algae-to-water, compression levels and extraction durations. According to their report, MAE technology can be employed to obtain bioactive peptides. Additionally mechanical disruption techniques have proven valuable, in breaking down the siliceous skeletons of certain hard sponges [19]. In general this technique facilitates selective and expedited compound extraction compared to conventional methods. It yields even better results while consuming less energy and solvent volume. As a result it reduces costs. Possesses a more environmentally friendly nature compared to traditional extraction processes [20].

- 2. Purification:** Prior to evaluating the bio-properties of the objective bioactive peptides, the purification of these peptides is essential. The assessment of the bio-properties of the objective bioactive peptides extracted from marine protein hydrolysate should only occur after their purification. Purification techniques of bioactive peptides involve various methods to isolate and separate the desired bioactive peptides from complex mixtures. These techniques are listed as follows: Ultrafiltration, gel filtration, ion change column chromatography, high performance liquid chromatography (HPLC), reverse-phase chromatography high performance liquid chromatography (RP-HPLC). However, there are advantages and disadvantages, to each purification method [21].

Ultrafiltration (UF) is a membrane-based method is commonly used to separate peptides of varying weights by utilizing membranes. The best fractions are identified through a comparison of the ultrafiltration-purified hydrolysates to the primary hydrolysates. Various molecular weight cutoffs (MWCO) of ultrafiltration membranes have been employed to fractionate marine protein hydrolysates. These membranes have a molecular weight cutoff (MWCO) ranging from 1 to 10 kDa. Furthermore, the purity of hydrolysates after UF will experience a substantial increase, with a range of 1.51 to 525 times, over against the unprocessed hydrolysates. [22].

UF has advantages, including its scalability, independence, from chemical agents and compatibility with other processes. In a research investigation, soybean peptides exhibiting inhibitory activity underwent isolated through the utilization of ultrafiltration membranes classified based on their different molecular weight categories [27]. Conversely, DPP-IV inhibitory peptides demonstrated potency at molecular weights ranging from 5–10 kDa to >10 kDa. Additionally, antimicrobial properties were observed in chia seed hydrolysate derived peptides with weights, below 3 kDa [23]. Size exclusion chromatography, also termed gel filtration chromatography (GFC), has served as a

method for more than forty years to separate, desalt and estimate the molecular weight of peptides and proteins.

GFC is considered one of the gentlest techniques in chromatography as it separates molecules based on their size differences. The mechanism behind its separation involves filtering molecules separated by their scale; tiny molecules penetrate the gel's pores. Move further despite being larger ones have reduced retention times. Differing from chromatographic methods like ion exchange, GFC does not involve molecule binding to the chromatography medium. As a result variations in buffer composition do not directly impact resolution. This presents a favorable aspect for GFC, as it allows for the modification of elution parameters to suit the characteristics of the sample and the demands of subsequent purification, analysis, or preservation, all without undermining the effectiveness of the dividing procedure. Biomolecules that are responsive to alterations in pH, variations in ion levels of metal and coenzyme presence, as well as challenging environmental circumstances, are especially appropriate for GFC. The final separation outcome remains unaffected when one employs it immediately following ion exchange chromatography. Moreover GFC offers selectivity and resolution levels—making it an important step, within purification strategies. There are types of GFC media, each, with unique characteristics. For instance, Superdex Increase or Superdex is specifically created to achieve resolution shorter run times and increased recovery. In a research, conducted a purification process to isolate trypsin inhibitor from the roe of Yellowfin Tuna (*Thunnus Albacores*) [24]. Column chromatography techniques were employed, including Sephacry S 200, Sephadex G 50, and DEAE cellulose. Eventually they determined that the trypsin inhibitor had a molecular weight of approximately 7×10^4 Da. Furthermore, experts widely recommend Sephadex for separating various groups. It is commonly used in processes such as deionization, and buffer interchange. In the realm of organism purification, it finds extensive application. While it can pose challenges in terms of time and expense, GFC's outstanding specificity capacity to deliver sharp results render it valuable for various separation and purification applications [25].

In times, the use of ion exchange chromatography (IEX) techniques has gained significant importance in separating, detecting and determining the structure of proteins, peptides and small nucleotides. The groups within IEX media possess charges that draw in molecules bearing an opposing charge. Subsequently, the medium releases these bound molecules as a charged molecule is introduced at an increasing concentration. Functional groups within proteins can bear either positive or negative charges [26]. Effective separation of proteins can be achieved by altering the pH level or ionic concentration of the phase. Operators utilize IEX to either catch chosen proteins or eliminate large-scale contaminants from significant quantities. It can function as a purification step, removing impurities. Additionally, IEX takes on the role of the final stage in the pursuit of high-resolution purification, ensuring that the end product is of the utmost quality. Ion exchange, being an adsorption technique offers the flexibility to be employed in both negative capture modes. The choice between these modes depends on the pH or conductivity of the sample. In chromatography the target compound is adsorbed while the contaminant remains unretained. Conversely in chromatography it is the contaminant that gets adsorbed while the target remains unretained. Additionally there exists an array of ion exchange (IEX) media available, allowing for selecting a suitable medium based on specific requirements such, as target compound, sample characteristics and desired

resolution. One major benefit of using IEX is its ability to separate masses, which can lead to time savings and improved accuracy compared to GFC. However it's important to note that IEX can be expensive and complex and may not be suitable for biomolecules that are sensitive to factors such as pH and metal ions. Future research in the field of IEX could focus on discovering a material with high resolution capabilities, which would replace the costly media currently used. While achieving this goal may pose challenges we can expect adoption of IEX, for biological separation in the coming years [25].

- 3. Identification:** The examination of the sequence underlying the detected biological effects has not garnered significant attention, primarily concentrating on identifying the most probable sequence. Initially, peptide identification relied on Edman degradation. However, in recent times, chromatographic procedures, particularly LC-MS/MS techniques, have attained greater acknowledgment because of their accuracy and sensitivity. These methods are now considered the standard for analyzing bioactive compounds [27].

Mass spectrometry has become a crucial analytical tool in characterizing bioactive molecules, thanks to advancements in ionization techniques and mass analyzers. With these developments, even complex samples can now be effectively analyzed. This includes a wide range of bioactive molecules, from small metabolites to large macromolecular assemblies. This can be attributed to the convergence of advancements that enable the analysis of small quantities of challenging compounds like peptides in complex mixtures along, with the rapid expansion of genomic databases that can be searched using mass spectrometry data. Various platforms with distinct capabilities are employed in proteomic studies to analyze samples [28]. These platforms include matrix assisted laser desorption ionization time of flight (MALDI-TOF), electrospray ionization liquid chromatography/mass spectrometry (ESI-LC/MS), FAB-MS (fast atom bombardment mass spectrometry), ion trap instruments and Fourier transform mass spectrometer (FT-MS) systems. The accuracy of these approaches hinges on the precise determination of the molecular mass-to-charge ratio and chemical structure of peptides, achieved through high-resolution and highly sensitive techniques [5].

Utilizing a combination of 1D and 2D NMR techniques along with mass spectrometry, the isolated peptides undergo a thorough characterization and elucidation through spectroscopic analyses [29]. NMR spectroscopy stands out as one of the most effective analytical tools available today for determining the structure of bioactive compounds. The principle behind NMR is based on nuclear spin, which means that when a molecule is placed in a magnetic field, its momentum aligns either in the same or opposite direction to the field. This alignment creates two distinct states separated by an energy gap and results in resonance frequency. The difference in resonance frequency depends on both the chemical environment of the nucleus within a molecule and the strength of the magnetic field [27].

III. EVALUATION OF MARINE-DERIVED BIOACTIVE PEPTIDES FOR HEALTH-PROMOTION

Bioactive peptides derived from marine sources exhibit advantageous attributes such as antioxidant properties, antimicrobial activity, blood pressure regulation, anti-diabetic and

anti-obesity effects, anti-inflammatory effects, and modulation of the immune system (Figure 2) [30].

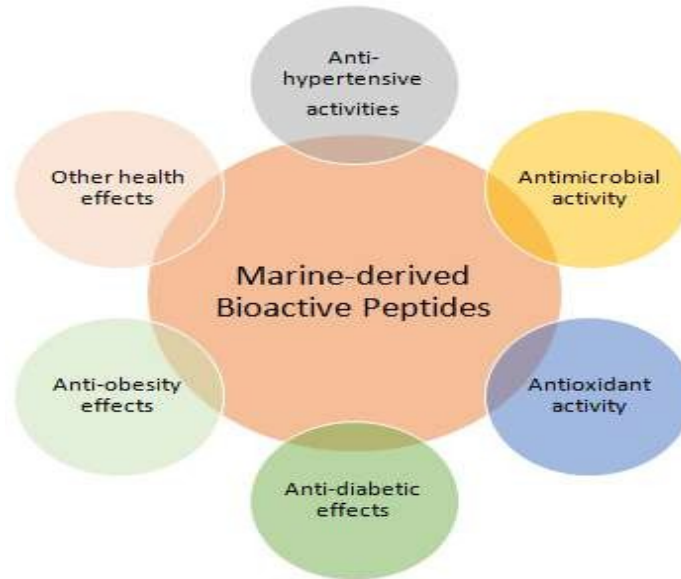


Figure 2: The Primary Health-Enhancing Characteristics of Bioactive Peptides extracted from Marine Sources

- 1. Blood Pressure-Lowering Effect:** Cardiovascular diseases (CVDs), often referred to as heart diseases or disorders related to the heart and circulatory system, impact the heart and blood vessels [31]. The World Health Organization (WHO) reports that CVDs are responsible, for the loss of 17.9 million lives [32]. High-blood pressure (hypertension) is the one of the main risk factor for CVDs. Hypertension drugs, treatments that inhibit the angiotensin I converting enzyme (ACE; EC 3.4.15.1) are commonly employed to manage blood pressure levels in the renin-angiotensin system. ACE is present in several organs such as the lungs, blood vessel walls, brain and kidney [33]. ACE, also known as dipeptidyl-carboxypeptidase, is an enzyme that breaks down peptides into dipeptide units by cleaving them at the C terminus. One of its significant roles is converting angiotensin-I (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu) into angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe), an active vasoconstrictor that elevates blood pressure. Additionally, ACE degrades and inactivates Bradykinin, which normally promotes arterial relaxation and lowers blood pressure. These processes collectively work together to directly elevate blood pressure (Figure 3). ACE inhibitors, as hypertension drugs, are employed to suppress these responses, causing vasodilation and ultimately lowering blood pressure [8]. Lisinopril, captopril, and enapril are frequently prescribed as synthetic ACE inhibitors to effectively manage hypertension. On the hand it should be acknowledged that synthetic medications, such, as ACE inhibitors can sometimes bring about side effects. Some typical side effects associated with ACE inhibitors are feelings of dizziness, persistent coughing, increased levels of potassium, in the body and allergic reactions [34].

In recent times, there has been increasing interest in exploring natural marine

products as potential alternatives to synthetic drugs. Due to their various beneficial effects, natural marine products have captured the attention of numerous researchers [33, 35, 36]. ACE-inhibitory compounds have been extracted from seaweed [37], fish gelatin [38], algae [39], rainbow trout [40], jawless vertebrate lamprey [41], and Atlantic sea cucumber [42]. The majority of reported peptides function as competitive inhibitors of ACE, where they compete with the substrate by binding to the active site of ACE. Conversely, in the non-competitive inhibition regulatory mode, the inhibitor attaches to a distinct site, leading to a change in ACE's shape. Preventing substrate binding at the active site, this alteration results in the formation of enzyme-reactant and enzyme-inhibitor complexes that cannot interact simultaneously. [33].

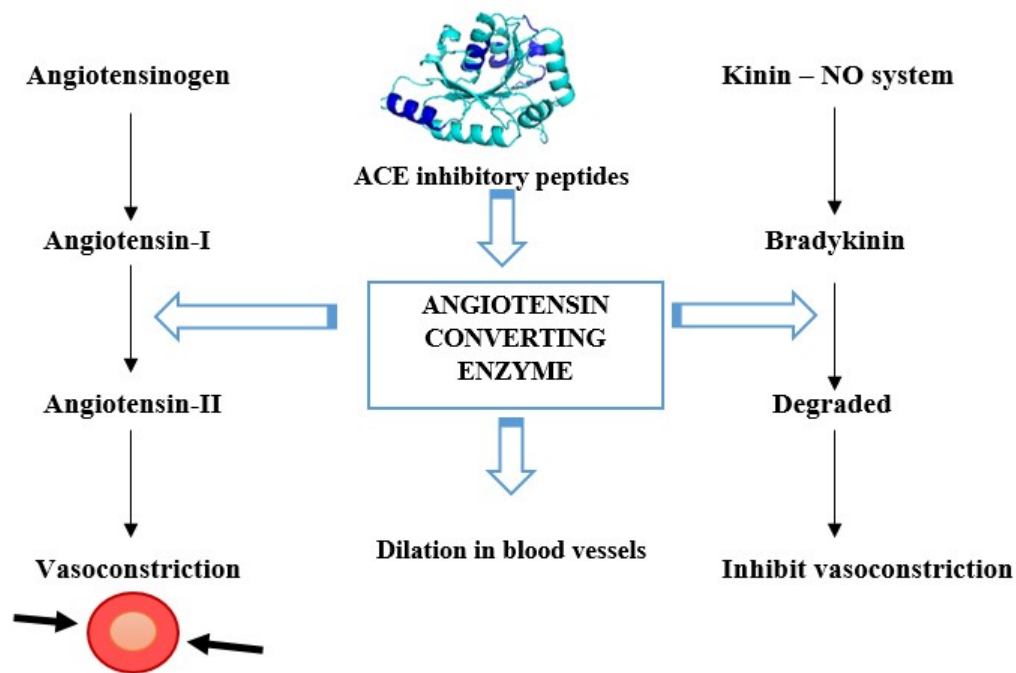


Figure 3: Basic Mechanism of ACE Inhibition Effect of Bioactive Peptides

The quantification of the effectiveness of peptides derived from marine organisms relies on their half maximal inhibitory concentration (IC_{50}). This measure indicates the concentration of ACE inhibitor required to achieve a 50% reduction in ACE activity. Additionally, the inhibition mode of ACE inhibitory peptides is commonly assessed using Lineweaver–Burk plots [43]. The widely used spectrophotometric assay for ACE activity measurement was initially developed by Cushman and Cheung [44]. To put it briefly, this method consists of extracting hippuric acid (HA), which is generated from the substrate hip–his–leu (HHL) as a result of ACE activity, using ethyl acetate. Subsequently, HA is quantified using a spectrophotometric assay. Several modifications to this method have been reported. Among these are the replacement of ethyl acetate extraction with the specific binding of His–Leu through 2,4,6-trinitrobenzene sulphonate (TNBS) or the alteration of HA through a specific reaction with benzene sulfonyl chloride [43]. Despite its value, the spectrophotometric assay is distinguished by its intricacy, time-intensive procedures, and constraints in the examination of minute sample quantities. As an

alternative, reverse phase HPLC has been employed to determine ACE activity by measuring the extracted HA content through a UV monitor [45]. Using an HPLC method with the C18 column, ACE-inhibitory activity can be directly determined without the need for HA extraction. This approach achieves complete baseline separation of HA from HHL with enhanced sensitivity [46].

Fresh tilapia (*Oreochromis niloticus*) was filleted, deskinning, minced, defatted with isopropanol, and then lyophilized to prepare the fish samples. The researchers added crude proteinases to the mixture at a concentration of 5 U/g and conducted hydrolysis at 60 °C for different periods (0, 2, 4, 8 h). The researchers conducted ACE inhibitory activity tests on the hydrolysates using an HPLC after the hydrolysis process. As a result of the research, with an increase in hydrolysis time, the ACE inhibitory activity of the hydrolysate rose. Furthermore, the hydrolysis process for 8 hours resulted in the highest ACE inhibition activity, displaying an IC_{50} of 0.54 mg/ml. Changes in the amino acid composition lead to variations in the functionality of the peptides formed during hydrolysis. Categorized by a molecular weight below 5 kDa, the fraction displayed the highest ACE inhibitory activity, achieving a specific inhibitory activity of 626.71% per mg of peptide. On the other hand, the fraction above 30 kDa in molecular weight exhibited the lowest ACE inhibitory activity. Binding with the active site of ACE is typically more challenging for the longer chain peptides. This leads to a decrease in inhibitory activity. This might, in part, explain the reduced inhibitory activity noticed in the fraction above 30 kDa in molecular weight. Conversely, despite the lower peptide yield, the fraction with $MW < 5$ kDa exhibited a nearly 4-fold increase in specific inhibitory activity compared to the crude hydrolysate. As a result, sequential ultrafiltration emerged as an effective method for concentrating ACE inhibitory peptides in the hydrolysates [47].

To attain high-quality protein hydrolysates from *Tetrademus obliquus*, a green microalgae, the investigators employed a combination of chemical and mechanical methods for protein extraction. These techniques involved buffered suspension and ultrasonication, grinding with glass beads, employing an alkaline process, implementing the HCl-Bligh and Dyer method, utilizing methyl tert-butyl ether, Hexane/ethanol, and osmotic shock. After pretreatment, the mixtures were hydrolyzed by Alcalase and purified with extraction on C18 solid-phase cartridges. They subjected purified fragments to bioactivity assays, and the most active fragments were determined using reverse-phase Nano HPLC-MS/MS. As for the outcomes, GPDRPKFLGPF and WYGPDRPKFL, among other peptide fragments, displayed an ACE inhibitory activity percentage of 80%. Commonly, the ACE inhibitory effect is associated with the occurrence of hydrophobic and aromatic elements at both the molecule's start and end. In both peptides we observed an amount of these particular components, which led to their impressive effectiveness, with IC_{50} values of 5.73 and 0.82 $\mu\text{mol L}^{-1}$ respectively [48]. The waste fish and by-products from fishing fleets in North-West Spain, including entire fish, heads, scales, were enzymatically hydrolyzed using Alcalase. The resulting hydrolysates were then lyophilized for 72 hours, and bioactivity assessments were performed on the dried samples. The researchers reported that in their findings, all fish protein hydrolysates (FPH) demonstrated significant ACE inhibitory activity when tested at 5 mg/mL, with values falling between 61.20% and 85.95%. Notably, FPH derived from fish heads generally exhibited lower ACE inhibitory activity, ranging from 60.77% to 79.35% [49].

Rainbow trout viscera protein hydrolysate was obtained using Alcalase enzyme under conditions involving a temperature of 60°C, a pH of 8.5, and a duration of 6 hours. The internal organs and their digests underwent *in vitro* digestion including oral phase (salivary fluid), gastric phase (pepsin solution), and intestinal phase. After digestion, soluble fractions were analyzed via Fast Protein Liquid Chromatography using AKTA purifier and thirty-six fractions with a molecular weight (MW) less than 10 kDa from the digested samples were automatically collected. Viscera exhibited the highest IC₅₀ value ($P < 0.05$), indicating the lowest ACE inhibitory activity. However, the IC₅₀ value of digested viscera was significantly ($p < 0.05$) lower, approximately 53.7% less than that of viscera, suggesting that potential antihypertensive peptides could be formed during the gastrointestinal digestion of viscera. While no significant differences were observed between the IC₅₀ values of non-digested (H) and digested hydrolysate (HD), it can be inferred that ACE inhibitory peptides in H may be resistant to SGID (Simulated Gastrointestinal Digestion) or that other peptides with comparable activity might have been generated during the digestion process [50].

In another research, rainbow trout samples underwent hydrolysis through the application of papain and bromelain at a concentration of 0.05% w/w for 60 minutes at 50°C. Subsequently, the protein hydrolysate was subjected to purification using a 3 kDa cut-off ultrafiltration membrane. After that, the peptide fragments were tested for its bioactive properties. ACE inhibitory activity was reported as $55.6 \pm 0.2\%$ at 1035 µg/mL concentration and an IC₅₀ value of 0.81 ± 0.0016 mg/mL [40].

The tridecapeptide IRLIIVLMPILMA, which inhibits renin and originates from the papain hydrolysate of *Palmaria palmata*, exerts its effects while passing through the gastrointestinal (GI) system. After undergoing *in vitro* gastrointestinal (GI) digestion, the degradation products were detected through mass spectrometry analysis, revealing the presence of the recognized renin and angiotensin I converting enzyme inhibitory dipeptide. Spontaneously hypertensive rats (SHRs) were employed in *in vivo* animal experiments to confirm the antihypertensive effects of both IRLIIVLMPILMA and the seaweed protein hydrolysate, the source of this peptide. Following a duration of 24 hours, the group of spontaneously hypertensive rats (SHR) that received the protein hydrolysate from *P. Palmata* experienced a decrease of 34 mm Hg in their systolic blood pressure (SBP). Their SBP dropped from 187 (± 0.25) mm Hg to 153 (± 0.64) mm Hg. In comparison, the group that was given the tridecapeptide IRLIIVLMPILMA showed a blood pressure decrease of 33 mm Hg. They went from an initial reading of 187 (± 0.95) mm Hg to 154 (± 0.94) mm Hg SBP at the beginning of the experiment [51].

The researchers selected Alcalase from among five protease types to hydrolyze Skipjack tuna (*Katsuwonus pelamis*) muscle for the production of bioactive peptides with potent angiotensin-I-converting enzyme (ACE) inhibitory (ACEi) attributes. The choice aimed to create peptides with high ACE inhibitory potential. Optimal hydrolytic settings were determined via single-variable and response surface studies. Utilizing alcalase under these optimized conditions using 2.3% enzyme dose, a temperature of 56.2°C and a pH of 9.4. The outcomes revealed a remarkably potent ACE inhibiting protein hydrolysate (TMPH) derived from skipjack tuna muscle, with the TMPH demonstrating an ACE inhibition activity of 72.71% at a concentration of 1.0 mg/mL. Subsequently, employing ultrafiltration and chromatography techniques to obtain six novel ACE inhibiting peptides

from TMPH; Ser Pro (SP), Val Asp Arg Tyr Phe (VDRYF), Val His Gly Val Val (VHGVV), Tyr Glu (YE), Phe Glu Met (FEM) and Phe Trp Arg Val (FWRV). These peptides had mass value of 202.2 Da, 698.9 Da, 509.7 Da, 310.4 Da, 425.6 Da and 606.8 Da respectively. It is worth noting that they reported significant ACE inhibition activity for SP and VDRYF, with IC_{50} values of approximately 0.06 ± 0.01 mg/mL and 0.28 ± 0.03 mg/mL, in that order. [52].

- 2. Anti-Diabetic Activity:** Diabetes mellitus (DM) is a metabolic disease related to high level of blood sugar and it has become a notable issue, in public health worldwide. The global incidence of DM has seen an increase over the years. From 1980 to 2014 the number of individuals affected by this condition increased dramatically from 108 million to a 422 million. Low and middle income countries experienced a more rapid rise, in prevalence compared to high income nations. In 2019, DM and diabetes-related kidney problems lead to about 2 million deaths [53]. When blood sugar levels are high and the body can't effectively derive energy from the food we eat, it becomes problematic. The pancreas plays a vital role in regulating blood sugar levels, typically between 4-6 mM, by releasing insulin and glucagon [54].

DM is mainly categorized into two types and they can be listed as Type 1 diabetes (T1DM) and Type 2 diabetes (T2DM). Also, there is one more type of diabetes called gestational diabetes, which affects females during pregnancy Briefly, when the levels of glucose in the bloodstream increase, it signals the pancreatic β -cells to secrete insulin, allowing the body's cells to take in and make use of glucose. In T1DM, these cells are destroyed, resulting in a deficiency of insulin production. On the other hand, T2DM is marked by inadequate insulin production by the body or reduced responsiveness of cells to insulin referred to as insulin resistance (Figure 4) [55].

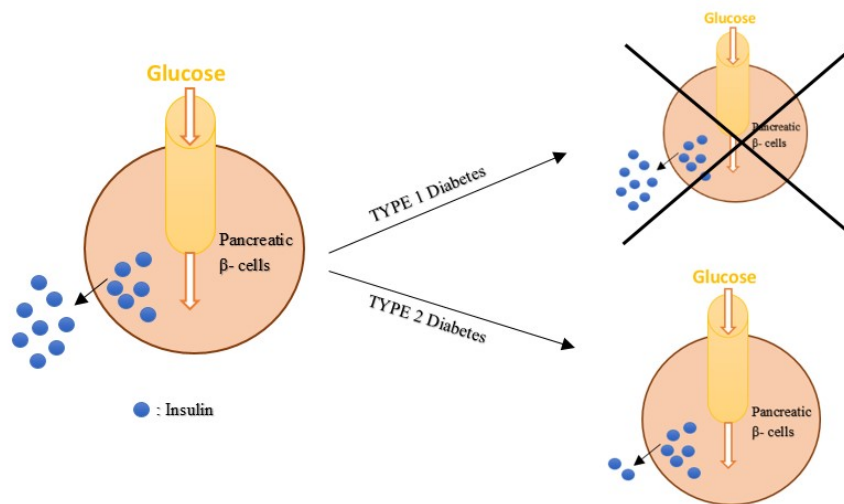


Figure 4: Simplified Scheme of the Pathogenic Influences on β -cells in T1DM and T2DM

T1DM is a long term condition that occurs when the immune system mistakenly attacks and destroys the beta cells in the pancreas. These beta cells play a role in producing insulin a hormone that helps regulate blood sugar levels by allowing glucose to

enter the body's cells for energy. When beta cells fail to secrete insulin it leads to an insufficiency of this hormone resulting in levels of glucose in the bloodstream (hyperglycemia). The exact cause of this immune system attack on beta cells is not fully understood. It is believed to be influenced by both genetic and environmental factors [56]. Type 1 diabetes is typically diagnosed during childhood or early adulthood [57]. Requires lifelong insulin therapy to effectively manage blood sugar levels. Individuals with T1DM must regularly monitor their blood glucose levels, administer insulin through injections or an insulin pump. Carefully manage their diet and physical activity to maintain stable blood sugar levels. Since insulin plays a role in glucose metabolism individuals with T1DM cannot naturally produce sufficient amounts of this hormone. Without treatment persistently high blood sugar levels can lead to significant health complications over time. Therefore it is crucial for those with T1DM to manage their condition in order to prevent potential complications such, as heart disease, kidney damage, nerve problems and vision impairments [58].

According to global data, around 415 million individuals are presently affected by T2DM. Projections suggest that without prompt interventions, this number could surge to 642 million by the year 2040 [59]. T2DM is a condition that affects the body's metabolism and causes high blood sugar levels. It occurs when the body either doesn't produce insulin or becomes resistant, to its effects. Insulin, a hormone produced by the pancreas plays a role in regulating blood sugar levels by allowing glucose to enter cells and provide energy. In T2DM the body's cells don't respond properly to insulin leading to what's known as insulin resistance. As a result the pancreas may try to compensate by producing insulin. However over time it may struggle to keep up with the demand resulting in insulin production. Lifestyle factors like being overweight or sedentary and having a diet often contribute to T2DM. Additionally, genetic factors and family history can also play a role in its development. Unlike Type 1 that typically appears during childhood or early adulthood and requires insulin therapy from a stage, T2DM can sometimes be managed through lifestyle modifications. For the management of T2DM, there are various strategies are employed, including the regular use of different anti-diabetic medications, lifestyle changes, and regular physical exercises [60].

At present, there is no known medication that can fully cure T2DM. The current drugs mainly function by managing blood sugar levels enhancing the body's response, to insulin and reducing the harm caused by hyperglycemia. Consequently, there exists a desire to discover or create more potent medications or treatments for tackling T2DM [61]. Bioactive peptides have the ability to improve the balance of glucose in the body and increase sensitivity to insulin [62, 63]. The researchers have shown significant interest in exploring new bioactive peptides, particularly from marine sources, due to the increasing population of individuals suffering from diabetes and the scarcity of effective anti-diabetic medications [64]. In recent years, anti-diabetic peptides have been obtained from various marine organisms, such as oyster [65], red seaweed (*Porphyra* spp.) [66], silver carp swim bladder [67], Atlantic salmon skin (*Salmo salar*) [68], Tilapia (*Oreochromis niloticus*) by-products [69], dogfish and shark [70]. The management of T2DM can involve the inhibition of carbohydrate-hydrolyzing enzymes, such as α -amylases and α -glucosidases, found within the gastrointestinal system [71,72]. An alternative approach to managing T2DM is that lower glucose level, like inhibitors of dipeptidyl peptidase IV (DPP-IV) [73, 74, 75] (Figure 5). Because of their chemical

structure, peptides have the capacity to interact with amino acids present in the catalytic site of the enzymes, thereby inhibiting their function [76].

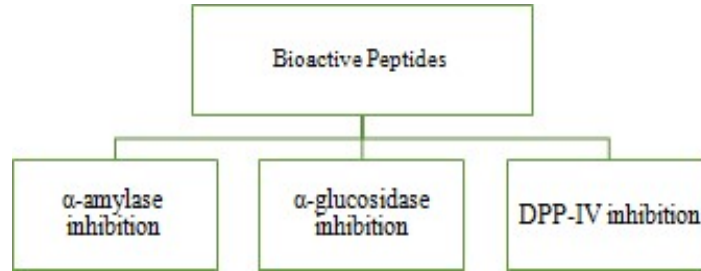


Figure 5: Bioactive Peptides Exhibit Anti-Diabetic Effects for T2DM based on Inhibition

Pancreatic and salivary α -amylase inhibitors are renowned for their capacity to impede the enzymatic breakdown of complex starch molecules into smaller sugar molecules known as oligosaccharides. This critical role they play helps prevent the absorption of starch glucose components by the human body. Additionally, inhibitors of α -glucosidase block the breakdown of di, tri and oligosaccharides into glucose within the small intestine [77]. By inhibiting both enzymes, the digestion rate of carbohydrates decreases, leading to reduced absorption and transportation of glucose into the circulation and combining the inhibition of α -amylase activity with α -glucosidase is considered an effective approach to managing diabetes [78].

Dipeptidyl peptidase IV (DPP-IV/CD26; EC.3.4.14.5) is a protein found in cell membranes that participates in a wide range of biological processes. This serine protease, consisting of 766 amino acids has the ability to break down dipeptides located at the beginning of proline or alanine residue. It is also known as CD26, a marker on the surface of lymphocytes or as a binding protein for adenosine deaminase (ADA). DPP-IV is found in various organs such as the pancreas, sweat glands, salivary and mammary glands, thymus, lymph nodes, intestines and biliary tract, kidney, liver, placenta, uterus, prostate, brain, blood cells and skin. It attaches itself to the plasma membrane. The enzyme DPP-IV plays roles in different physiological processes including degradation of incretion hormones like GLP-1 and GIP. The hormones GLP-1 and GIP which are commonly referred to as hormones are discharged into the bloodstream by specific cells, in the duodenum (known as K cells) and the mucosa of the intestine (known as L cells) correspondingly. This release occurs when food is consumed. These hormones called incretins help increase insulin secretion from the β -cells in the islets of Langerhans when we eat. They do this in a way that depends on the level of glucose. This is very important, for keeping our blood sugar levels balanced. After a meal the levels of GLP-1 and GIP hormones in the body increase by two to three times. These hormones collectively contribute to around 60% of the insulin released after eating. However it is worth noting that both GIP and GLP 1 are broken down by an enzyme called DPP-IV since these hormones are naturally occurring substances in the gut. Unfortunately due to the action of DPP IV enzyme both GIP and GLP 1 have short durations (less, than 7 and 2 minutes respectively) [79].

A substance that inhibits DPP-IV boosts the effectiveness of both added and

naturally occurring GLP-1 and GIP hormones by stopping their initial breakdown. Consequently this leads to the prevention of their deactivation. In individuals who have T2DM, researchers have noticed a decrease in the body's response to incretins. This results in insulin production higher levels of glucagon after meals and an increase, in glucose levels following meals. DPP-IV inhibitors increase the duration of action. Enhance the levels of active incretins in the bloodstream. As a result, when the levels of incretins rise it causes a decrease in glucagon production. This leads to an increase in insulin release a delay in emptying and a reduction, in blood sugar levels. As a result, DPP-IV inhibitors can enhance glucose tolerance by augmenting the incretin effect in patients with T2DM [73].

Vildagliptin, saxagliptin, sitagliptin and alogliptin have been approved as medications for diabetes treatment in both the United States and Europe. Although most artificial DPP-IV inhibitors are generally well tolerated there have been reports of potential side effects such, as nasal congestion, headaches and urinary tract infections [80].

The search for natural alternatives to pharmaceutical drugs has expanded to include the study of peptides derived from marine resources. Red seaweed laver (*Porphyra* species) samples are hydrolyzed by proteases (alcalase, neutrase, pepsin and trypsin) separately under their optimal conditions. The hydrolysis was carried out using enzyme/substrate ratio of 1:100 (w/w) and duration of 4 h. After hydrolysis, protein hydrolysates were separated using consecutive chromatographic techniques, and 10 fragments were obtained from RP-HPLC. Among these fragments D₂ inhibited α -amylase activity (88.67 ± 1.05 %) significantly at 1mg/ml protein concentration. In mass spectrometry analysis (ESI-Q-TOF-MS) identified 8 peptides in this fraction. In this analysis, were discovered two peptides; Gly- Gly-Ser-Lys and Glu-Leu-Ser. To confirm their potential, for inhibiting α -amylase, was synthesized these peptides chemically. The synthesized peptides showed activity with IC₅₀ values of 2.58 ± 0.08 mM for Gly-Gly-Ser-Lys and 2.62 ± 0.05 mM, for Glu-Leu-Ser. The α -amylase inhibitory drug, sold commercially (acarbose) showed a much stronger effect in inhibiting α -amylase compared to the synthesized peptides, when tested under the same reaction conditions. The IC₅₀ value for acarbose was measured at 0.45 ± 0.01 mM, which was significantly lower ($P < 0.05$). The peptides that have been identified may not be as effective in inhibiting as acarbose, but this finding implies that the peptides obtained from pepsin digestion of laver protein, could have potential benefits for health and could be used in functional foods and pharmaceuticals to manage diabetes. Acarbose, which is a sugar analog, works through competitive inhibition. However, the peptides analyzed in this study have different characteristics and would exhibit diverse inhibition behaviors [66].

Boarfish (*Capros aper*) protein hydrolysate was experienced at 50°C, pH 7.0 with Alcalase 2.4L and Flavourzyme 500. After 3.75 h incubation time, simulated gastrointestinal digestion of hydrolysate was done. DPP-IV inhibitor activity was tested on the digested hydrolysate. The hydrolysate underwent a process called semi preparative reverse phase high performance liquid chromatography. Fractions were collected between 6 and 12 minutes, 12-15 minutes, 15-21 minutes, 21-25 minutes, 25-29 minutes, 29-33 minutes and 33-45 minutes using a fraction collector. The RP HPLC fractions that showed the highest activity, namely F28 and F29, were subjected to additional separation

using an ACQUITY UPLC system. Subsequently, these fractions were analyzed utilizing a microTOF Q II mass spectrometer. In result, the most potent DPP-IV inhibitory peptide (IPVDM) had IC_{50} value of $21.72 \pm 1.08 \mu\text{M}$ [59].

Salmon skin collagen was underwent to simulated gastrointestinal digestion. After hydrolysis, the digests was lyophilized. DPP-IV inhibitor activity were measured on the digests and MS analysis were done. The findings showed that the effectiveness of collagen in inhibiting DPP-IV increased significantly (from $11.57 \pm 1.84\%$ to $53.63 \pm 3.14\%$, $P < 0.05$) after undergoing simulated digestion in a laboratory setting. As a result, the cleavage sites of enzymes and the pretreatments applied to proteins can lead to the formation of peptides with varying sequences. While the amino acid in the second position of the peptide sequence is not Pro (P), having a Phe (F) at the C terminal of peptide sequences is a characteristic feature of DPP IV inhibitory peptides. Furthermore the presence of the amino acid Threonine (T), at the beginning and Leucine (L) in the position were also taken into account as contributors to the inhibition. As a result the two discovered peptides TKLPAVF and YLNF which were identified during this study could be regarded as peptides, with a level of DPP IV inhibition [81].

Sardinella (Sardinella sindensis) was hydrolyzed with using alcalase (pH 8, 50°C), pepsin (pH 2.5, 37°C), and papain (pH 6.3, 65°C). Enzyme: substrate ratio and hydrolysis time was set as %2.5 (w/w) and 3 h, respectively. As a source of compounds, 250 $\mu\text{g}/\text{mL}$ of pistachio green hulls extract (PGH) was added to the hydrolysates. After hydrolysis, ultrafiltration was experienced with 10 kDa and 2 kDa MW cut-off membrane for purification. After all, Peptide profiles were evaluated by HPLC and the fragments were assessed for α -glucosidase, α -amylase and DPP-IV inhibition assays. The papain and alcalase hydrolysates did not exhibit any effect. The pepsin hydrolysate showed a mild inhibitory effect. Upon fractionation the best glucosidase inhibitory activities were observed in Alcalase 2–10. The interaction, between the extract and peptides had a negative effect. Based on the results it seems that this interaction may have reduced the availability of groups, in the inhibitor required for interacting with α -glucosidase. For the α -amylase inhibitory effects of the hydrolysates and fractions, the inhibitory effects of pepsin hydrolysate were found to be lower compared to papain and alcalase hydrolysates with the latter two exhibiting activities ($p > 0.05$). However through the process of ultrafiltration the inhibitory effects of pepsin hydrolysate were increased. When it comes to papain hydrolysate fractionation did not have an impact, on α -amylase inhibition. On the hand fractionating the alcalase hydrolysate resulted in the alcalase₁₀ fraction showing the inhibitory effect. The hydrolysate and fractions had an effect compared to acarbose (with an IC_{50} value of 29.28 mg/mL) which is the standard inhibitor. Overall the hydrolysates and fractions showed effects, against α amylase, than α glucosidase. In DPP-IV activity assay, the hydrolysis process using papain resulted in a hydrolysate that displayed the effect. There was no difference observed between the effects of hydrolysates produced with pepsin and Alcalase. Upon fractionation it was found that the low molecular weight fractions exhibited inhibitory effects compared to the high molecular weight fractions. Amongst the fractions it was observed that fraction <2 of pepsin had the inhibitory effect. Despite having a proline content the Alcalase hydrolysate demonstrated an inhibitory effect, on DPP-IV compared to both the papain hydrolysate and pepsin 2-10 fraction. The pepsin <2 fraction, which had the proline level showed to be the inhibitor of DPP-IV. The activity of DPP- IV inhibitors in hydrolysates is

influenced by both proline content and peptide size. Smaller fractions with a proline content were able to inhibit DPP-IV enzyme activity. Both papain hydrolysate and pepsin 2-10 demonstrated proline content with no difference in their inhibition of DPP-IV enzyme activity. Diprotin A was used as a control. Exhibited an IC_{50} value of 4 $\mu\text{g/mL}$. Peptides that display strong inhibitory effects, on DPP-IV typically contain hydrophobic amino acids and/or proline within their sequence. Some bioactive peptides containing alanine and proline residues have been found to inhibit DPP-IV activity [82].

- 3. Anti-Obesity Activity:** Obesity poses a multifaceted challenge to address. It is a significant and long lasting health condition that can adversely impact various bodily systems. The prevalence of obesity is rapidly escalating worldwide. In years there has been an increase, in the number of people worldwide who are struggling with obesity, which poses a big challenge to public health [83]. According to the WHO, the prevalence of obesity has nearly tripled from 1975 to 2016. In 2016 approximately 1.9 billion adults were identified as being overweight and out of that number more than 650 million were classified as obese. In 2016 nearly four, out of ten adults, aged 18 and above were dealing with being overweight while around one, in eight individuals were classified as obese [84]. Being obese can heighten the chances of developing several health issues, such as diabetes, heart disease, osteoarthritis and specific forms of cancer [83].

Cellular accumulation arises from a disruption in the delicate equilibrium between the energy ingested and the energy expended. This leads to the development of obesity. A crucial enzyme known as lipase assumes a pivotal role in the intricate process of lipid metabolism within human body. Lipase (EC 3.1.1.3) is an enzyme that specifically targets the ester bonds found in triglycerides. Its role is to break down these bonds, resulting in the release of free fatty acids, mono and di glyceride molecules. In the context of obesity management, certain inhibitors like "orlistat," a widely available medication, can slow down the absorption of free fatty acids into both the systemic circulation and adipocytes for obesity management [85]. Inhibiting this enzyme has proven to be a method for preventing obesity making it an important approach for evaluating obesity treatments, in laboratory settings [86]. Obesity at the cellular level is marked by a rise in the number of fat cells that develop from fibroblastic preadipocytes in adipose tissues [87]. Another approach to combat obesity involves restraining the growth, maturation and storage of preadipocytes or promoting the breakdown of stored fat [88].

Currently, the prevalent methods used to tackle overweight and obesity in today's society include approaches such, as weight management programs, specific diets like calorie control and intermittent fasting devices for weight loss medications, for reducing weight, surgery and liposuction procedures. However there are weight loss strategies, like surgery or liposuction that come with surgical risks, including the potential for serious complications and even death. While it is true that the combination of over the counter dietary supplements and temporary interventions, along with a consistent routine of healthy eating and physical activity, can assist in achieving weight loss, it is important to acknowledge that certain medications for weight loss have been withdrawn from the market due to negative side effects For instance, the use of aminorex and sibutramine has been linked to cases of heart problems, high blood pressure in the lungs and several other non-deadly cardiovascular events [89].

It is widely accepted that consuming diets in carbohydrates and/or fats along, with a lifestyle are the primary lifestyle factors associated with disrupted lipid metabolism. This disruption often leads to levels of triglycerides (TG) LDL cholesterol and imbalanced glucose regulation [90]. Investigating the capabilities of peptides derived from natural sources to hinder pancreatic lipase shows potential as a therapeutic approach for combating obesity. Pancreatic lipase inhibitory peptides have been derived from marine organisms such as crucian carp (*Carassius carassius*) [86], jellyfish [91], green and brown seaweed [92], and yellow cat fish [93]. Fish muscle from Crucian carp (*Carassius carassius*) was optimized with the aid of response surface methodology. Fish muscle was hydrolyzed with neutral protease, alkaline protease, papain, and protamex (100 U/mL) for 14 h. The researchers carried out an evaluation to determine the ability of fish protein hydrolysate to inhibit pancreatic lipase. When using alkaline protease, the hydrolysates derived from fish water soluble protein showed a higher rate of inhibition on porcine pancreas lipase compared to neutral protease, papain and protamex. The results showed that, certain model terms were important, while the lack of fit terms did not have much significance. The optimal conditions for hydrolysis using alkaline protease were determined to be an initial pH of 11, a temperature of 39 °C, an enzyme dosage of 122 U/mL and a hydrolysis duration of 10 hours. It was observed that under these specific conditions, the activity of porcine pancreas lipase amounted $53.04\% \pm 1.32\%$ [86].

Peptides were produced from fresh Atlantic sea cucumber (*Cucumaria frondosa*) using the enzymes alcalase (A) and trypsin (T). The enzyme concentrations were set at 3000 U/g for Alcalase and 2000 U/g for trypsin, with a ratio of 1:3 (w/v). The mixture of reactions was left to incubate with gentle stirring at a temperature of 25 °C for a duration of 2 hours. The peptides were separated into different fractions using ultrafiltration membranes with varying cut off sizes (10 kDa, 5 kDa, 3 kDa and 2 kDa). This separation process resulted in obtaining 14 distinct peptide fractions (A<10, A10 5, A<5, A5 3, A<3, A3 2, A<2, T<10, T10 5, T<5, T5 3, T<3, T3 2, T<2). After that, the focus shifted towards the peptides found in the fractions below 2kDa (A<2 and T<2) because of their strong inhibitory effects on multiple enzymes. To identify these peptides accurately and precisely analyze them using LC MS/MS combined with Peaks software. The activity of peptides in inhibiting lipase was assessed by measuring the production of free fatty acids from olive oil at a temperature of 37 °C and a pH level of 7.5. In results, the peptides strongly inhibited pancreatic lipase (5.3–17.0%), ACE (52.2–78.8%) and α -amylase (16.3–27.2%). Tripeptides with a significant presence of R residues were created and showed strong effectiveness in inhibiting lipase enzymes [94].

- 4. Antioxidant Activity:** Oxidation is a process found in all living organisms, where the production of free radicals and other reactive oxygen species (ROS) plays a key role in signaling. However an excess of radicals can contribute to various human diseases, including heart conditions, strokes, arteriosclerosis, diabetes and cancer. The heart, lungs and brain are particularly susceptible, to the effects of free radicals as they rely heavily on oxygen [95]. Antioxidants are substances that play a role in reducing the harmful effects caused by reactive species like reactive oxygen and nitrogen. In the context of food antioxidants are compounds that can slow down hinder or even prevent oxidation processes. However there are worries regarding the risks and unfavorable opinions linked to synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). These substances might have impacts on vital organs, like the

liver, spleen, lungs and other internal organs in humans [25]. As a result there is a growing demand for natural antioxidants, in recent years. Natural antioxidants commonly employed in foods include vitamin C, vitamin E and polyphenols. In the case of vitamin C and vitamin E specifically the antioxidant molecules are either recharged by accepting electrons, from types of antioxidants or they undergo recycling for tissue repair purposes [96].

Since Prokorný's report in 1991, where it was mentioned that specific hydrolysates of peptides and proteins can help reduce the speed of autoxidation and peroxide levels in fats a significant amount of research has been focused on investigating antioxidant peptides, with enhanced antioxidant properties [97]. The antioxidant properties of an antioxidant peptide are influenced by factors such as the composition of amino acids the sequence of acids in the peptides the weight of the peptide chain as well as the amino acid residues, at both ends (N terminal and C terminal) and their hydrophilic characteristics [95].

It has been reported that, there is a link between the way amino acids in peptides are structured and their antioxidant activity. Specifically, when the second L-histidine, in Pro-His-His is replaced with D-histidine it significantly reduces the peptides antioxidant activity. The relationship between the properties of peptides and their amino acid structures was found to be meaningful. In a research performed an experiment where they created peptides without Tyr, Tyr-Phe, Tyr-Phe-Tyr and Tyr-Phe Tyr-Pro. These peptides were derived from the antioxidant peptide called Tyr-Phe-Tyr-Glu-Leu, which was originally extracted from casein. Different sequences showed varying levels of activity; Glu-Leu (EL) demonstrated the activity, followed by Tyr-Phe-Tyr-Pro-Glu-Leu (YFYPEL) Phe-Tyr- Pro-Glu-Leu (FYPEL), Tyr-Pro-Glu-Leu (YPEL) and Pro-Glu-Leu (PEL). This indicates that the EL sequence is particularly important for its activity [98]. Furthermore a relationship between weight and antioxidant activity has been established. Peptides that have low molecular weights have a tendency to move through biological membranes more easily reaching the desired location [99].

Marine-derived peptides have shown increased levels of antioxidant activities. In years scientists have discovered multiple anti oxidative peptides derived from marine organisms such as Antarctic Krill (*Euphausia superba*) [100], marine algae (*Gracilariopsis lemaneiformis*) [101], and marine by-products [102, 103, 104].

Two peptides possessing antioxidant properties were extracted from skin protein hydrolysates of horse mackerel (*Magalaspis cordyla*) and croaker (*Otolithes ruber*) using a series of fractionations, such as ion exchange chromatography and gel filtration chromatography. The peptide sequence in the skin protein hydrolysate of horse mackerel was determined to be Asn-His-Arg-Tyr-Asp-Arg (856 Da) through electron spray ionization mass spectrometry (ESI MS/MS) while that of croaker was identified as Gly-Asn-Arg- Gly-Phe-Ala-Cys-Arg-His-Ala (1101.5 Da). The antioxidant activity of these peptides was assessed using electron spin resonance (ESR) spectrometry with 1 diphenyl 2 picryl hydrazyl (DPPH·) and hydroxyl (OH·) scavenging assays. Both peptides demonstrated efficacy in inhibiting peroxidation of polyunsaturated fatty acids (PUFAs) compared to α tocopherol, a natural antioxidant. These findings indicate that the two isolated peptides, from horse mackerel and croaker skin protein hydrolysates possess

antioxidative properties and hold promise as potential food additives or pharmaceutical agents [105].

The peptides Leu-His-Tyr, Leu-Ala-Arg-Leu-Gly-Gly-Glu, Gly-Ala-His-Gly-Ala-Trp-Ala, Pro-His-Tyr-Leu and Gly-Ala-Leu-Ala-Ala-His were derived from the waste produced during the processing of sardinelle (*Sardinella aurita*). These peptides were obtained by utilizing an enzyme extract, from sardine (*Sardina pilchardus*). Among these peptides the first tripeptide showed the ability to scavenge DPPH radicals. It is likely that the presence of the amino acid sequence His-Tyr contributes significantly to the activity of these peptides [106].

- 5. Anti-Microbial Activity:** The excessive and inappropriate use of antibiotics worldwide has caused an increase in microbial resistance [107]. This poses challenges to human health as we are now witnessing the re-emergence of infectious diseases and a rapid rise in the number of pathogenic bacteria that are resistant to commonly available antibiotics. This situation threatens to bring us to a time before antibiotics were discovered. To address this issue scientists are prioritizing the search for antibacterial molecules that can effectively combat resistance. One promising avenue of research is exploring occurring cationic antimicrobial peptides (AMPs) which have caught the attention of scientists as potential alternatives to conventional antibiotics. These AMPs have been found in organisms and play a crucial role in their defense mechanisms against infections. The number of AMPs that have proven effective against bacteria continues to grow. They are small peptides (<60 amino acids) with broad spectrum activity against microorganisms such, as Gram positive and Gram negative bacteria, fungi, viruses and parasites [108]. An additional advantage is their likelihood of developing resistance. Furthermore AMPs may serve functions beyond their antibiotic properties. In reality certain peptides possess properties that can combat cancer. They also have the ability to enhance the system through promoting the release of cytokines facilitating chemo taxis and antigen presentation initiating angiogenesis and inflammatory responses as well as inducing adaptive immune responses [109].

Currently, antimicrobial peptides (AMPs) are an area of research for potential drug candidates. This is because, they have a range of activity and importantly they may be able to combat antimicrobial resistance. AMPs work by targeting the lipid components in the invading pathogens membranes, which disrupts this structure and creates a barrier against the development of resistance. Due to this fact scientific research has focused on exploring the world of AMPs with, over 2000 reported in antimicrobial peptide databases [110].

Despite the potential of AMPs they do have some drawbacks. These include stability under certain pH conditions the ability to disrupt the cellular membrane of eukaryotic organisms which can lead to hemolytic side effects high production costs and technical challenges in manufacturing them. Additionally there is a lack of data regarding their toxicity, pharmacodynamics and pharmacokinetics properties. Furthermore their activity can be reduced when exposed to cations, like calcium and iron or specific serum conditions [111].

Antimicrobial peptides can be categorized into classes; (i) those consisting of α -

helices (ii) small proteins and β -sheet structures, (iii) peptides containing thio-ether rings, (iv) peptides, with an abundance of one or two specific amino acids (v) lipopeptides and (vi) macrocyclic cystine knot peptides. This classification is based on research findings [112].

Marine-derived antimicrobial peptides have been obtained from many resources such as fish species [113,114], black tiger shrimp [115], and marine invertebrates [112]. A peptide called XL Asp P1, which has properties was discovered in the skin of *Xenopus laevis*. It has shown to be highly effective, against both Gram positive and Gram negative bacteria, well as having promising inhibitory effects, on breast cancer cells. Researchers were able to understand how this unique peptide works by using transmission electron microscopy. The antimicrobial activity is attributed to its ability to damage the cell membrane [116].

- 6. Other Health Effects:** Inflammation refers to the body's response, to types of injury such as trauma, infection, lack of blood flow, toxins or autoimmune conditions. It involves an interaction between cells and substances in any part of our body. Typically inflammation plays a role in fighting infections and promoting wound healing. There are two types of inflammation; acute and chronic. Acute inflammation is the response against microorganisms, damaged tissue, or tiny particles.. It is a process that self-limits over time. Acute inflammation is mediated by substances like fatty acid signaling molecules and blood vessel-affecting amines that promote the movement of plasma and immune cells to the area. However if phagocytes fail to remove harmful agents when the immune system is unable to repair tissue during inflammation it can progress into chronic inflammation. Chronic inflammation involves the release of cytokines and growth factors that attract advanced immune cells like leukocytes, lymphocytes and fibroblasts. Unfortunately during inflammation these cells can cause damage, to tissues. Numerous research studies have been conducted to investigate the connection, between inflammation and conditions such, as heart attacks, Alzheimer's disease and cancer [117]. Despite the importance of inflammation there have been efforts to develop therapies that specifically target the inflammatory aspect of cardiovascular and malignant diseases. One used class of drugs known as anti-inflammatory drugs (NSAIDs) such, as aspirin are widely utilized for preventing and managing cardiovascular diseases due to their antithrombotic and anti-inflammatory properties. Recent research indicates that NSAIDs might also have effects against cancers expanding their potential applications in anti-inflammatory treatments. However the known side effects, like bleeding and ulceration limit the long term usage of NSAIDs for a significant portion of the population [118].

The researchers have provided details, about the range of peptides found in environments including their ability to combat inflammation. They have also discussed the inflammatory properties of newly discovered compounds derived from sponges, bacteria and microalgae [35].

The impact of peptides, on inflammation has predominantly been studied in mammalian cells. In this context researchers have explored the potential of peptides derived from various marine resources [119]. According to a study, three specific peptides (LREMLSTMCTARGA, AVGPAGPRG and VAPAWGPWPKG) found in the hydrolysate of a sea cucumber species called *A. Lecanora* displayed inflammatory

properties. The effectiveness of these peptides was measured at 76.3%, 66.6% and 69.9% respectively [120].

In a research, scientists examined how oyster peptides (OPs) can impact the system of the mucosa and intestinal micro flora in mice that have been made immunosuppressed, through treatment with chemotherapy called Cyclophosphamide (Cy). The mucosa immune system consists of lymphocytes, macrophages and plasma cells. Acts as the initial defense against potential harm from the environment. Cyclophosphamide has effects, on the mucosa [121].

Immunostimulating or immunomodulatory peptides consist of categories of peptides including anticancer peptides and anti-inflammatory peptides. Anticancer peptides can have effects, on cancer cells, such as promoting cell death inhibiting tumor growth and regulating the system. They typically have molecules and are primarily made up of neutral and aromatic amino acids (for example Arg- Arg-Trp-Gln-Trp-Arg). On the hand, anti-inflammatory peptides work by targeting the bodys defense mechanism against inflammation. They help regulate the secretion of inflammation inducing substances like cytokines or tumor necrosis factor alpha (TNF- α). Anti-inflammatory peptides are characterized by a content of both hydrophobic and polar acids (for instance Tyr-Leu) [122].

There are types of peptides that help reduce cholesterol levels in the body each, with its unique way of working. They have the potential to either interfere with enter hepatic circulation, causing a rise in the elimination of cholesterol and bile acids through feces, or to enhance the expression of lipoproteins (LDL), achieving the same outcomes. It is known that many of these peptides work by inhibiting an enzyme called 3-hydroxy-3-methylglutaryl CoA (HMG CoA) reductase, which ultimately leads to the release of cholesterol. These peptides primarily consist of acids such, as proline, valine and glycine (for example; Gly-Gly-Val) [46]. Nevertheless, in a study it was found that the reduction in cholesterol brought about by peptides is primarily linked to their primary or higher-order structure, rather than their specific amino acid composition. In fact, hydrophobic peptides have the ability to bind with cholesterol and bile acid [123].

The human sense of taste can perceive four primary tastes; sweet, sour, bitter and salty. Additionally, umami is recognized as the fifth taste by our sense of taste. While initially not considered a biological characteristic of peptides, scientific evidence has demonstrated that peptides derived from marine products and their derivatives can exhibit all five tastes [124]. This discovery holds significant potential for the growth of the food industry. Researchers conducted a study to examine the creation of protein hydrolysates from the heads and backbones of salmon, mackerel and herring. Afterward, a group of highly trained sensory evaluators assessed the sensory characteristics of these hydrolysates. Protein hydrolysates made from herring were found to have the strongest flavor, while those made from salmon were considered more enjoyable. This study revealed that the type of raw material used (heads or backbones) and the enzyme employed had minimal impact on sensory and nutritional characteristics. Instead, it was the choice of fish species that played a significant role in determining the taste profile of the protein hydrolysates [125].

To summarize, we have previously discussed the main biological effects triggered by peptides. However, researchers have also explored other bioactivities apart from the ones mentioned. These include antithrombotic, mineral binding, opioid, anti-stress and insomnia relief effects [126]. This emphasizes that the effects triggered by peptides may depend on various factors such as their structure, amino acid composition and the protein family they originate from.

IV. RESULTS & FUTURE RESPECTS

Marine bioactive peptides are functional components that can prevent and treat disease in maintaining health. These peptides have demonstrated various bioactivities, including antioxidant, antimicrobial, anti-inflammatory, and ACE inhibitory properties, making them valuable candidates for potential therapeutic applications. Their ability to modulate immune responses and regulate metabolic processes highlights their potential in combating conditions like cardiovascular diseases, diabetes, and obesity. The increasing demand for functional food increases the need for functional food components. Bioactive peptides its positive effects on health increase consumer awareness, research and production trends of bioactive peptides. While there is an increasing demand for in vitro research to enhance our understanding of marine bioactive peptide mechanisms and promote their commercial utilization, the specific components remain unidentified. It is important to identify marine peptides and to investigate the mechanism of the effects on health. We anticipate that bioactive peptides will soon be accessible for commercial use. In essence, the future of marine-derived bioactive peptides is characterized by growth in various industries, sustainable practices, and advancements in research and development. These peptides are poised to play a crucial role in addressing health-related challenges, promoting overall well-being, and finding applications in both creating novel functional products within the food industry and advancing pharmaceuticals.

REFERENCES

- [1] A.A. Zaky, J. Simal-Gandara, J.B. Eun, J.H. Shim, and A.M. Abd El-Aty, "Bioactivities, applications, safety, and health benefits of bioactive peptides from food and by-products: A review," *Frontiers in Nutrition*, vol. 8, pp. 815640, 2022.
- [2] J. Zhou, Q. Han, T. Koyama, and S. Ishizaki, "Preparation, Purification and Characterization of Antibacterial and ACE Inhibitory Peptides from Head Protein Hydrolysate of Kuruma Shrimp, *Marsupenaeus japonicas*," *Molecules*, vol. 28(2), pp. 894, 2023.
- [3] S.B. Mada, C.P. Ugwu, and M. M. Abarshi, "Health promoting effects of food-derived bioactive peptides: A review," *International Journal of Peptide Research and Therapeutics*, vol.26, pp. 831-848, 2020.
- [4] N.H. Ishak, and N.M. Sarbon, "A review of protein hydrolysates and bioactive peptides deriving from wastes generated by fish processing," *Food and Bioprocess Technology*, vol.11, pp. 2-16, 2018.
- [5] C. Jo, F.F. Khan, M.I. Khan, and J. Iqbal, "Marine bioactive peptides: Types, structures, and physiological functions," *Food Reviews International*, vol. 33(1), pp.44-61, 2017.
- [6] L. Vercruyse, J. Van Camp, and G. Smaghe, "ACE inhibitory peptides derived from enzymatic hydrolysates of animal muscle protein: A review" *Journal of Agricultural and Food Chemistry*, vol. 53(21), pp. 8106-8115, 2005.
- [7] S.K. Ulug, F. Jahandideh, and J. Wu, "Novel technologies for the production of bioactive peptides," *Trends in food science & technology*, vol. 108, pp. 27-39, 2021.
- [8] I.P.S. Fernando, T.U. Jayawardena, and J. Wu, "Marine proteins and peptides: Production, biological activities, and potential applications," *Food Innovation and Advances*, vol. 2(2), pp. 69-84, 2023.
- [9] O. Villamil, H. Vaquiro, and J.F. Solanilla, "Fish viscera protein hydrolysates: Production, potential

- applications and functional and bioactive properties." *Food chemistry*, vol. 224, pp. 160-171, 2017.
- [10] P. Sezer, I. Okur, M.H. Oztop, and H. Alpas, "Improving the physical properties of fish gelatin by high hydrostatic pressure (HHP) and ultrasonication (US)," *International Journal of Food Science & Technology*, vol. 55(4), pp. 1468-1476, 2020.
- [11] J. A. Cárcel, J.V. García-Pérez, J. Benedito, and A. Mulet, "Food process innovation through new technologies: Use of ultrasound," *Journal of Food Engineering*, vol. 110(2), pp. 200-207, 2012.
- [12] W. Qu, H. Ma, J. Jia, R. He, L. Luo, and Z. Pan, "Enzymolysis kinetics and activities of ACE inhibitory peptides from wheat germ protein prepared with SFP ultrasound-assisted processing," *Ultrasonics Sonochemistry*, vol. 19, pp. 1021-1026, 2012.
- [13] Q. Liang, X. Ren, H. Ma, S. Li, K. Xu, and A.O. Oladejo, "Effect of low-frequency ultrasonic-assisted enzymolysis on the physicochemical and antioxidant properties of corn protein hydrolysates," *Journal of Food Quality*, vol. 2017, pp. 1-10, 2017.
- [14] X. Zhu, Z. Chao, Z. Liang, and H. Cheng, "Amino acids production from fish proteins hydrolysis in subcritical water," *Chinese Journal of Chemical Engineering*, vol. 15, pp. 456-460, 2008.
- [15] A.K.M. Asaduzzaman, and B.S. Chun, "Recovery of functional materials with thermally stable antioxidative properties in squid muscle hydrolyzates by subcritical water," *Journal of Food Science & Technology*, vol. 52, pp. 793-802, 2013.
- [16] E. Nguyen, O Jones, Y.H.B. Kim, F. San Martin-Gonzalez, and A.M. Liceaga, "Impact of microwave-assisted enzymatic hydrolysis on functional and antioxidant properties of rainbow trout *Oncorhynchus mykiss* by-products," *Fisheries Science*, vol. 83, pp. 317-331, 2017.
- [17] S.U. Kadam, B.K. Tiwari, and C.P.O'Donnell, "Application of novel extraction technologies for bioactives from marine algae," *Journal of agricultural and food chemistry*, vol. 61(20), pp. 4667-4675, 2013.
- [18] H.X. Jin, H.P. Xu, Y. Li, Q.W. Zhang, and H. Xie, "Preparation and evaluation of peptides with potential antioxidant activity by microwave assisted enzymatic hydrolysis of collagen from sea cucumber" *Acaudina molpadioides* obtained from Zhejiang province in China," *Marine drugs*, vol. 17(3), pp. 169, 2019.
- [19] C. Grosso, P. Valentão, F. Ferreres, and P. B. Andrade, "Alternative and efficient extraction methods for marine-derived compounds," *Marine Drugs*, vol. 13(5), pp. 3182-3230, 2015.
- [20] J. M. Bélanger, and J.J. Paré, "Applications of microwave-assisted processes (MAP™) to environmental analysis," *Analytical and bioanalytical chemistry*, vol. 386, pp. 1049-1058, 2006.
- [21] M.I. Shaik, and M.S. Norizah, "A review on purification and characterization of anti-proliferative peptides derived from fish protein hydrolysate," *Food Reviews International*, vol. 38.7, pp. 1389-1409, 2022.
- [22] F. Mahmoodani, M. Ghassem, A.S. Babji, S.M. Yusop, and R. Khosrokhavar, "ACE inhibitory activity of pangasius catfish (*Pangasius sutchi*) skin and bone gelatin hydrolysate," *Journal of food science and technology*, vol. 51, pp. 1847-1856, 2014.
- [23] R. Rabail, M.R. Khan, H.M. Mehwish, M.S.R. Rajoka, J.M. Lorenzo, M. Kieliszek and R.M. Aadil, "An overview of chia seed (*Salvia hispanica* L.) bioactive peptides' derivation and utilization as an emerging nutraceutical food," *Frontiers in Bioscience-Landmark*, vol. 26(9), pp. 643-654, 2021.
- [24] J.L. Wu, S.Y. Ge, Z.X. Cai, H. Liu, Y.X. Liu, J.H. Wang, and Q.Q. Zhang, "Purification and characterization of a gelatinolytic matrix metalloproteinase from the skeletal muscle of grass carp (*Ctenopharyngodon idellus*)," *Food chemistry*, vol. 145, pp. 632-638, 2014.
- [25] X. Wang, H. Yu, R. Xing, and P. Li, "Characterization, preparation, and purification of marine bioactive peptides," *BioMed research international*, 2017.
- [26] P.R. Levison, "Large-scale ion-exchange column chromatography of proteins: comparison of different formats," *Journal of Chromatography B*, vol. 790(1-2), pp. 17-33, 2003.
- [27] T. Lafarga, F.G. Ación-Fernández, and M. Garcia-Vaquero, "Bioactive peptides and carbohydrates from seaweed for food applications: Natural occurrence, isolation, purification, and identification," *Algal research*, vol. 48, pp. 101909, 2020.
- [28] C. Tamvakopoulos, "Mass spectrometry for the quantification of bioactive peptides in biological fluids," *Mass spectrometry reviews*, vol. 26.3, pp. 389-402, 2007.
- [29] F.S. Youssef, M.L. Ashour, A.N.B. Singab, and M. Wink, "A comprehensive review of bioactive peptides from marine fungi and their biological significance," *Marine drugs*, vol. 17(10), pp. 559, 2019.
- [30] S.A. Cunha, and M.E. Pintado, "Bioactive peptides derived from marine sources: Biological and functional properties," *Trends in Food Science & Technology*, vol. 119, pp. 348-370, 2022.
- [31] K. Erdmann, B.W. Cheung, and H. Schröder, "The possible roles of food-derived bioactive peptides in

- reducing the risk of cardiovascular disease,” *The Journal of nutritional biochemistry*, vol. 19(10), pp. 643-654, 2008.
- [32] World Health Organization (WHO). Global Status Report on Non communicable Diseases 2022; <https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases>.
- [33] D.Y. Pujiastuti, M.N. Ghoyatul Amin, M.A. Alamsjah, and J.L. Hsu, “Marine organisms as potential sources of bioactive peptides that inhibit the activity of angiotensin I-converting enzyme: A review,” *Molecules*, vol. 24(14), pp. 2541, 2019.
- [34] D.B. Matchar, D.C. McCrory, L.A. Orlando, M.R. Patel, U.D. Patel, M.B. Patwardhan, and R.N. Gray, “Systematic review: comparative effectiveness of angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers for treating essential hypertension,” *Annals of internal medicine*, vol. 148(1), pp. 16-29, 2008.
- [35] S.K. Kim, D.H. Ngo, and T.S. Vo, “Marine fish-derived bioactive peptides as potential antihypertensive agents,” *Advances in food and nutrition research*, vol. 65, pp. 249-260, 2012.
- [36] D. Cao, X. Lv, X. Xu, H. Yu, X. Sun, and N. Xu, “Purification and identification of a novel ACE inhibitory peptide from marine alga *Gracilariopsis lemaneiformis* protein hydrolysate,” *European Food Research and Technology*, vol. 243, pp. 1829-1837, 2017.
- [37] P. Diane, M.A. Packer, and M. Hayes. "Identification of bioactive peptides from a *Laminaria digitata* protein hydrolysate using in silico and in vitro methods to identify angiotensin-1-converting enzyme (ACE-1) inhibitory peptides," *Marine Drugs* vol. 21.2, pp. 90, 2023.
- [38] A. Ahmad, S. Sukarno, B. Slamet, and A.B. Sitanggang, “Enzymatic hydrolysis of marine fish gelatin for producing ACE inhibitor peptides: meta-analysis,” *The Annals of the University Dunarea de Jos of Galati. Fascicle VI-Food Technology*, vol. 46(2), pp. 188-206. 2022.
- [39] M.A. Mune Mune, Y. Miyabe, T. Shimizu, W. Matsui, Y. Kumagai and H. Kishimura, “Characterisation of bioactive peptides from red alga *Gracilariopsis chorda*,” *Marine drugs*, vol. 21(1), pp. 49, 2023.
- [40] M. Bartolomei, J. Cropotova, C. Bollati, K. Kvangarsnes, L. d’Adduzio, J. Li, and C. Lammi, “Rainbow Trout (*Oncorhynchus mykiss*) as Source of Multifunctional Peptides with Antioxidant, ACE and DPP-IV Inhibitory Activities,” *Nutrients*, vol. 15(4), pp. 829, 2023.
- [41] Y. Wang, F. Sun, Z. Wang, X. Duan, Q. Li, Y. Pang, and M. Gou, “Peptidomics Analysis Reveals the Buccal Gland of Jawless Vertebrate Lamprey as a Source of Multiple Bioactive Peptides,” *Marine Drugs*, vol. 21(7), pp. 389, 2023.
- [42] T.R. Senadheera, A. Hossain, D. Dave, and F. Shahidi, “Antioxidant and ACE-Inhibitory Activity of Protein Hydrolysates Produced from Atlantic Sea Cucumber (*Cucumaria frondosa*),” *Molecules*, vol. 28(13), pp. 5263, 2023.
- [43] H.L. He, D. Liu, and C.B. Ma, “Review on the angiotensin-I-converting enzyme (ACE) inhibitor peptides from marine proteins,” *Applied Biochemistry and Biotechnology*, vol. 169, pp. 738-749, 2013.
- [44] D.W. Cushman, and H.S. Cheung, “Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung,” *Biochemical pharmacology*, vol. 20(7), pp. 1637-1648, 1971.
- [45] Q.C. Meng, E. Balcells, L. Dell’Italia, J. Durand, and S. Oparil, “Sensitive method for quantitation of angiotensin-converting enzyme (ACE) activity in tissue,” *Biochemical pharmacology*, vol. 50(9), pp. 1445-145, 1995.
- [46] J. Bechaux, P. Gatellier, J.F. Le Page, Y. Drillet, and V. Sante-Lhoutellier, “A comprehensive review of bioactive peptides obtained from animal byproducts and their applications,” *Food & function*, vol. 10(10), pp. 6244-6266, 2019.
- [47] T. Toopcham, S. Roytrakul, and J. Yongsawatdigul, "Characterization and identification of angiotensin I-converting enzyme (ACE) inhibitory peptides derived from tilapia using *Virgibacillus halodenitrificans* SK1-3-7 proteinases," *Journal of Functional Foods*, vol. 14, pp. 435-444, 2015.
- [48] C.M. Montone, A.L. Capriotti, C. Cavaliere, G. La Barbera, S. Piovesana, Zenezini, R. Chiozzi, and A. Laganà, “Peptidomic strategy for purification and identification of potential ACE-inhibitory and antioxidant peptides in *Tetrademus obliquus* microalgae,” *Analytical and Bioanalytical Chemistry*, vol. 410, pp. 3573-3586, 2018.
- [49] A. Henriques, J.A. Vázquez, J. Valcarcel, R. Mendes, N.M. Bandarra, and C. Pires, “Characterization of protein hydrolysates from fish discards and by-products from the north-west Spain fishing fleet as potential sources of bioactive peptides,” *Marine Drugs*, vol. 19(6), pp. 338, 2021.
- [50] P. Vázquez, J.E. Zapata, V.C. Chamorro, S.F.G. Fillería, and V.A. Tironi, “Antioxidant and angiotensin I-converting enzyme (ACE) inhibitory peptides of rainbow trout (*Oncorhynchus mykiss*) viscera hydrolysates subjected to simulated gastrointestinal digestion and intestinal absorption,” *Lwt*, vol. 154, pp. 112834, 2022.

- [51] C. Fitzgerald, R.E. Aluko, M. Hossain, D.K. Rai, and M. Hayes, "Potential of a renin inhibitory peptide from the red seaweed *Palmaria palmata* as a functional food ingredient following confirmation and characterization of a hypotensive effect in spontaneously hypertensive rats," *Journal of agricultural and food chemistry*, vol. 62(33), pp. 8352-8356, 2014.
- [52] R. Intarasirisawat, S. Benjakul, J. Wu, and W. Visessanguan, "Isolation of antioxidative and ACE inhibitory peptides from protein hydrolysate of skipjack (*Katsuwana pelamis*) roe," *Journal of Functional Foods*, vol. 5(4), pp. 1854-1862, 2013.
- [53] World Health Organization (WHO). Fact sheets/Detail/Diabetes, 2023; <https://www.who.int/news-room/fact-sheets/detail/diabetes>
- [54] C. Acquah, C.K. Dzuovor, S. Tosh, and D. Agyei, "Anti-diabetic effects of bioactive peptides: Recent advances and clinical implications," *Critical Reviews in Food Science and Nutrition*, vol. 62(8), pp. 2158-2171, 2022.
- [55] L. Chiara, and A. Ianora, "Marine organisms with anti-diabetes properties," *Marine drugs*, vol. 14.12, pp. 220, 2016.
- [56] A. Katsarou, S. Gudbjörnsdóttir, A. Rawshani, D. Dabelea, E. Bonifacio, B.J. Anderson, and Å. Lernmark, "Type 1 diabetes mellitus," *Nature reviews Disease primers*, vol. 3(1), pp. 1-17, 2017.
- [57] D. Devendra, E. Liu, and G.S. Eisenbarth, "Type 1 diabetes: Recent developments," *Bmj*, vol. 328(7442), pp. 750-754, 2004.
- [58] M.A. Atkinson, G.S. Eisenbarth, and A.W. Michels, "Type 1 diabetes," *The Lancet*, vol. 383(9911), pp. 69-82, 2014.
- [59] P. A. Harnedy-Rothwell, C.M. McLaughlin, M. B. O'Keeffe, A.V. Le Gouic, P.J. Allsopp, E.M., McSorley, and R.J. FitzGerald, "Identification and characterisation of peptides from a boarfish (*Capros aper*) protein hydrolysate displaying in vitro dipeptidyl peptidase-IV (DPP-IV) inhibitory and insulinotropic activity," *Food Research International*, vol. 131, pp.108989, 2020.
- [60] J. P. Bantle, J. Wylie-Rosett, A. L. Albright, C.M., Apovian, N.G. Clark, M.J Franz, and American Diabetes Association, "Nutrition recommendations and interventions for diabetes: a position statement of the American Diabetes Association," *Diabetes care*, vol. 31, pp. 61-78, 2008.
- [61] J. Yan, J. Zhao, R. Yang, and W. Zhao, "Bioactive peptides with antidiabetic properties: A review," *International Journal of Food Science & Technology*, vol. 54(6), pp. 1909-1919, 2019.
- [62] M.E. Oseguera-Toledo, E. González de Mejía, R. Reynoso-Camacho, A. Cardador-Martínez, and S.L. Amaya-Llano, "Proteins and bioactive peptides: Mechanisms of action on diabetes management," *Nutrafoods*, vol. 13, pp. 147-157, 2014.
- [63] F. Jahandideh, and J. Wu. "A review on mechanisms of action of bioactive peptides against glucose intolerance and insulin resistance," *Food Science and Human Wellness*, vol. 11.6, pp. 1441-1454, 2022.
- [64] E.Q. Xia, S.S. Zhu, M.J. He, F. Luo, C.Z. Fu, and T.B. Zou, "Marine peptides as potential agents for the management of type 2 diabetes mellitus—a prospect," *Marine drugs*, vol. 15(4), pp. 88, 2017.
- [65] D. Zhu, Z. Yuan, D. Wu, C., Wu, El-Seedi, H.R., and Du, M. "The dual-function of bioactive peptides derived from oyster (*Crassostrea gigas*) proteins," 2023.
- [66] H. Admassu, M.A. Gasmalla, R. Yang, and W. Zhao, "Identification of bioactive peptides with α -amylase inhibitory potential from enzymatic protein hydrolysates of red seaweed (*Porphyra* spp)," *Journal of Agricultural and Food Chemistry*, vol. 66(19), pp. 4872-4882, 2018.
- [67] H. Hong, Y. Zheng, S. Song, Y. Zhang, C. Zhang, J. Liu, and Y. Luo, "Identification and characterization of DPP-IV inhibitory peptides from silver carp swim bladder hydrolysates," *Food Bioscience*, vol. 38, pp. 100748, 2020.
- [68] R. Jin, X. TengShang, J. Wang, and N. Liu, "Identification of novel DPP-IV inhibitory peptides from Atlantic salmon (*Salmo salar*) skin," *Food Research International*, vol. 133, pp. 109161, 2020.
- [69] S. Theysgeur, B. Cudennec, B. Deracinois, C. Perrin, I. Guiller, A. Lepoudère, and R. Ravallec, "New bioactive peptides identified from a tilapia byproduct hydrolysate exerting effects on DPP-IV activity and intestinal hormones regulation after canine gastrointestinal simulated digestion. *Molecules*, 26(1), 136.
- [70] W. G. Anderson, M. F. Ali, I. E. Einarsdóttir, L. Schäffer, N., Hazon, and J. M. Conlon, "Purification, characterization, and biological activity of insulins from the spotted dogfish, *Scyliorhinus canicula*, and the hammerhead shark, *Sphyrna lewini*," *General and comparative endocrinology*, vol. 126(1), pp. 113-122, 2002.
- [71] Y.I. Kwon, E. Apostolidis, and K. Shetty, "Inhibitory potential of wine and tea against α -amylase and α -glucosidase for management of hyperglycemia linked to type 2 diabetes," *Journal of Food Biochemistry*, vol. 32(1), pp.15-31, 2008.
- [72] L. Striegel, B. Kang, S.J. Pilkenton, M. Rychlik, and E. Apostolidis, "Effect of black tea and black tea

- pomace polyphenols on α -glucosidase and α -amylase inhibition, relevant to type 2 diabetes prevention,” *Frontiers in nutrition*, vol.2, pp. 3, 2015.
- [73] P. Patil, S. Mandal, S.K. Tomar, and S. Anand, “Food protein-derived bioactive peptides in management of type 2 diabetes,” *European journal of nutrition*, vol. 54, pp. 863-880, 2015.
- [74] M. González-Montoya, B. Hernández-Ledesma, R. Mora-Escobedo, and C. Martínez-Villaluenga, “Bioactive peptides from germinated soybean with anti-diabetic potential by inhibition of dipeptidyl peptidase-IV, α -amylase, and α -glucosidase enzymes,” *International journal of molecular sciences*, vol. 19(10), pp. 2883, 2018.
- [75] R. Chelliah, S. Wei, E.B.M. Daliri, F. Elahi, S.J. Yeon, A. Tyagi, and D.H. Oh, “The role of bioactive peptides in diabetes and obesity,” *Foods*, vol. 10(9), pp. 2220, 2021.
- [76] M. Luis, and E. González De Mejía, "Optimization of enzymatic production of anti-diabetic peptides from black bean (*Phaseolus vulgaris* L.) proteins, their characterization and biological potential," *Food & function*, vol. 7.2, pp. 713-727, 2016.
- [77] V. Rubén, C. Martínez-Villaluenga, and B. Hernández-Ledesma, "Release of dipeptidyl peptidase IV, α -amylase and α -glucosidase inhibitory peptides from quinoa (*Chenopodium quinoa* Willd.) during in vitro simulated gastrointestinal digestion," *Journal of Functional Foods*, vol. 35, pp. 531-539, 2017.
- [78] Z. Yu, Y. Yin, W. Zhao, J. Liu, and F. Chen, “Anti-diabetic activity peptides from albumin against α -glucosidase and α -amylase,” *Food chemistry*, vol. 135(3), pp. 2078-2085, 2012.
- [79] C.L. Jao, C.C. Hung, Y.S. Tung, P.Y. Lin, M.C. Chen, and K.C. Hsu, “The development of bioactive peptides from dietary proteins as a dipeptidyl peptidase IV inhibitor for the management of type 2 diabetes,” *BioMedicine*, vol. 5, pp. 1-7, 2015.
- [80] P. Krushner, and M. Gorrell, “DPP-4 inhibitors in type 2 diabetes: importance of selective enzyme inhibition and implications for clinical use,” *J Fam Pract*, vol. 59(2), pp. 1, 2010.
- [81] J. Ritian, X. Teng, M. Liao, L. Zhang, Z. Wei, R. Meng, and N. Liu, “Release of dipeptidyl peptidase IV inhibitory peptides from salmon (*Salmo salar*) skin collagen based on digestion-intestinal absorption in vitro,” *International Journal of Food Science & Technology*, vol. 56(7), pp. 3507-3518, 2021.
- [82] R.A. Sarteshnizi, Sahari, M.A., Gavlighi, H.A., Regenstein, J.M., Nikoo, M., and Udenigwe, C.C, “Influence of fish protein hydrolysate-pistachio green hull extract interactions on antioxidant activity and inhibition of α -glucosidase, α -amylase, and DPP-IV enzymes,” *LWT*, vol. 142, pp. 111019, 2021.
- [83] S. Patra, S. Nithya, B.Srinithya, and S.M. Meenakshi, “Review of medicinal plants for anti-obesity activity,” *Translational Biomedicine*, vol. 6(3), 2015.
- [84] Who 2021 <https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight>
- [85] L. Rajan, D. Palaniswamy, and S.K. Mohankumar, “Targeting obesity with plant-derived pancreatic lipase inhibitors: A comprehensive review,” *Pharmacological research*, vol. 155, pp. 104681, 2020.
- [86] L. Liu, Y. Wang, C. Peng, and J. Wang, “Optimization of the preparation of fish protein anti-obesity hydrolysates using response surface methodology,” *International Journal of Molecular Sciences*, vol. 14(2), pp. 3124-3139, 2013.
- [87] Y.W. Wang, and P.J. Jones, “Conjugated linoleic acid and obesity control: efficacy and mechanisms,” *International journal of obesity*, vol. 28(8), pp. 941-955, 2004.
- [88] X. Fan, Y. Cui, R. Zhang, and X. Zhang, “Purification and identification of anti-obesity peptides derived from *Spirulina platensis*,” *Journal of Functional Foods*, vol. 47, pp. 350-360, 2018.
- [89] T.Y. Chia, C.Y. Gan, M.H. Shafie, P.G., Yap, A.M. Rodhi, A. Ahmad, and E.J. Johns, “A comprehensive review on the pancreatic lipase inhibitory peptides: A future anti-obesity strategy,” *Electronic Journal of General Medicine*, vol. 20(3), 2023.
- [90] B. S. Sivamaruthi, K.Alagarsamy, S. Thangaleela, M. Bharathi, P. Kesika, and C. Chaiyasut, “Composition, microbiota, mechanisms, and anti-obesity properties of rice bran,” *Foods*, vol. 12(6), pp. 1300, 2023.
- [91] N. Raksha, T. Halenova, T. Vovk, O. Kostyuk, T. Synelnyk, T. Andriichuk, and L. Ostapchenko, “Anti-obesity effect of collagen peptides obtained from *Diplulmaris antarctica*, a jellyfish of the Antarctic region,” *Croatian Medical Journal*, vol. 64(1), pp. 21, 2023.
- [92] E. Shannon, M. Conlon, and M. Hayes, “In vitro enzyme inhibitory effects of green and brown Australian seaweeds and potential impact on metabolic syndrome,” *Journal of Applied Phycology*, vol. 35(2), pp. 893-910, 2023.
- [93] M. R. Kim, J.W. Kim, J.B Park, Y.K. Hong, S.K. Ku, J.S. Choi, “Anti-obesity effects of yellow catfish protein hydrolysate on mice fed a 45% kcal high-fat diet,” *International Journal of Molecular Medicine*, vol. 40(3), pp. 784-800, 2017.
- [94] Y. Zhang, S. He, X. Rui, and B.K. Simpson, “Interactions of *C. frondosa*-derived inhibitory peptides

- against angiotensin I-converting enzyme (ACE), α -amylase and lipase,” *Food Chemistry*, vol. 367, pp. 130695, 2022.
- [95] Y. Li, and J. Yu, “Research progress in structure-activity relationship of bioactive peptides,” *Journal of medicinal food*, vol. 18(2), pp. 147-156, 2015.
- [96] M. Sohaib, F.M. Anjum, A. Sahar, M.S. Arshad, U.U., Rahman, A. Imran, and S. Hussain, “Antioxidant proteins and peptides to enhance the oxidative stability of meat and meat products: A comprehensive review,” *International Journal of Food Properties*, vol. 20(11), pp. 2581-2593, 2017.
- [97] J. Pokorný, “Natural antioxidants for food use,” *Trends in Food Science & Technology*, vol. 2, pp. 223-227, 1991.
- [98] K. Suetsuna, H. Ukeda, and H. Ochi, “Isolation and characterization of free radical scavenging activities peptides derived from casein,” *The Journal of nutritional biochemistry*, vol. 11(3), pp. 128-131, 2000.
- [99] Y.H. Zhu, R. Liu, H. Wu, and L.C. Wang, “Progress of structure-activity relationship of bioactive peptides,” *China Journal of Traditional Chinese Medicine and Pharmacy*, vol. 27, pp. 2625-2628, 2012.
- [100] Y.Z. Wang, Y.Q. Zhao, Y.M. Wang, W.H. Zhao, P. Wang, C.F. Chi, and B. Wang, “Antioxidant peptides from Antarctic Krill (*Euphausia superba*) hydrolysate: Preparation, identification and cytoprotection on H₂O₂-induced oxidative stress,” *Journal of Functional Foods*, vol. 86, pp. 104701, 2021.
- [101] X. Zhang, D. Cao, X. Sun, S. Sun, and N. Xu, “Preparation and identification of antioxidant peptides from protein hydrolysate of marine alga *Gracilariopsis lemaneiformis*,” *Journal of Applied Phycology*, vol. 31, pp. 2585-2596, 2019.
- [102] J.F.X. Silva, K. Ribeiro, J. F. Silva, T.B. Cahú, and R.S. Bezerra, “Utilization of tilapia processing waste for the production of fish protein hydrolysate,” *Animal feed science and technology*, vol. 196, pp. 96-106, 2014.
- [103] R.A. Nazeer, and K. Anila Kulandai, “Evaluation of antioxidant activity of muscle and skin protein hydrolysates from giant kingfish, *Caranx ignobilis* (Forsskål, 1775),” *International journal of food science & technology*, vol. 47(2), pp. 274-281, 2012.
- [104] C.F. Chi, B. Wang, Y.M. Wang, B. Zhang, and S.G. Deng, “Isolation and characterization of three antioxidant peptides from protein hydrolysate of bluefin leatherjacket (*Navodon septentrionalis*) heads,” *Journal of functional foods*, vol. 12, pp. 1-10, 2015.
- [105] N.S. Sampath Kumar, R.A. Nazeer, and R. Jaiganesh, “Purification and identification of antioxidant peptides from the skin protein hydrolysate of two marine fishes, horse mackerel (*Magalaspis cordyla*) and croaker (*Otolithes ruber*),” *Amino acids*, vol. 42, pp. 1641-1649, 2012.
- [106] A. Bougatef, N. Nedjar-Arroume, L. Manni, R. Ravallec, A. Barkia, D. Guillochon, and M. Nasri, “Purification and identification of novel antioxidant peptides from enzymatic hydrolysates of sardinelle (*Sardinella aurita*) by-products proteins,” *Food chemistry*, vol. 118(3), pp. 559-565, 2010.
- [107] A.E. Aiello, and E. Larson, “Antibacterial cleaning and hygiene products as an emerging risk factor for antibiotic resistance in the community,” *The Lancet infectious diseases*, vol. 3(8), pp. 501-506, 2003.
- [108] J. Cruz, C. Ortiz, F. Guzman, R. Fernández-Lafuente, and R. Torres, “Antimicrobial peptides: promising compounds against pathogenic microorganisms,” *Current medicinal chemistry*, vol. 21(20), pp. 2299-2321, 2014.
- [109] B. Bertrand, and C. Munoz-Garay, “Marine antimicrobial peptides: A promising source of new generation antibiotics and other bio-active molecules,” *International Journal of Peptide Research and Therapeutics*, vol. 25, pp. 1441-1450, 2019.
- [110] E. F. Haney, S. C. Mansour, and R.E. Hancock, “Antimicrobial peptides: an introduction,” *Antimicrobial peptides: Methods and protocols* pp. 3-22, 2017.
- [111] T. Sarkar, M. Chetia, and S. Chatterjee, “Antimicrobial peptides and proteins: From nature’s reservoir to the laboratory and beyond,” *Frontiers in Chemistry*, vol. 9, pp. 691532, 2021.
- [112] J.A. Tincu, and S.W. Taylor, “Antimicrobial peptides from marine invertebrates,” *Antimicrobial agents and chemotherapy*, vol. 48(10), pp. 3645-3654, 2004.
- [113] J.A. Masso-Silva, and G. Diamond, “Antimicrobial peptides from fish,” *Pharmaceuticals*, vol. 7(3), pp. 265-310, 2014.
- [114] S.R. Brunner, J.F. Varga, and B. Dixon, “Antimicrobial peptides of salmonid fish: from form to function,” *Biology*, vol. 9(8), pp. 233, 2020.
- [115] P. Supungul, S. Klinbunga, R. Pichyangkura, I. Hirono, T. Aoki and A. Tassanakajon, “Antimicrobial peptides discovered in the black tiger shrimp *Penaeus monodon* using the EST approach,” *Diseases of aquatic organisms*, vol. 61(1-2), pp. 123-135, 2004.
- [116] I. Lassoued, L. Mora, A. Barkia, M.C. Aristoy, M. Nasri, and F. Toldrá, “Bioactive peptides identified in thornback ray skin's gelatin hydrolysates by proteases from *Bacillus subtilis* and *Bacillus*

- amyloliquefaciens,” *Journal of Proteomics*, vol. 128, pp. 8-17, 2015.
- [117] M. M. Mueller, “Inflammation in epithelial skin tumours: Old stories and new ideas,” *European journal of cancer*, vol. 42(6), pp. 735-744, 2006.
- [118] S. Chakrabarti, F. Jahandideh, and J. Wu, “Food-derived bioactive peptides on inflammation and oxidative stress,” *BioMed research international*, vol. 2014, 2014.
- [119] R. Ghanbari, “Review on the bioactive peptides from marine sources: Indication for health effects,” *International Journal of Peptide Research and Therapeutics*, vol. 25, pp. 1187-1199, 2019.
- [120] R. Ghanbari, A. Ebrahimpour, M. Zarei, A. Ismail, Abdul-Hamid, A., and N. Saari, “Purification and characterization of nitric oxide inhibitory peptides from *Actinopyga lecanora* through enzymatic hydrolysis,” *Food Biotechnology*, vol. 30(4), pp. 263-277, 2016.
- [121] X.W. Xiang, H.Z. Zheng, R. Wang, H. Chen, J.X. Xiao, B. Zheng, and Y.T. Ding, “Ameliorative effects of peptides derived from oyster (*Crassostrea gigas*) on immunomodulatory function and gut microbiota structure in cyclophosphamide-treated mice,” *Marine Drugs*, vol. 19(8), pp. 456, 2021.
- [122] P. Minkiewicz, J. Dziuba, A. Iwaniak, M. Dziuba, and M. Darewicz, “BIOPEP database and other programs for processing bioactive peptide sequences,” *Journal of AOAC International*, vol. 91(4), pp. 965-980, 2008.
- [123] K. Iwami, K. Sakakibara, and F. Ibuki, “Involvement of post-digestion hydrophobic peptides in plasma cholesterol-lowering effect of dietary plant proteins,” *Agricultural and biological chemistry*, vol. 50(5), pp. 1217-1222, 1986.
- [124] A. Iwaniak, P. Minkiewicz, M. Darewicz, K. Sieniawski, and P. Starowicz, “BIOPEP database of sensory peptides and amino acids,” *Food Research International*, vol. 85, pp. 155-161, 2016.
- [125] T. Aspevik, S. Steinsholm, B. Vang, M. Carlehög, J.A. Arnesen, and K. Kousoulaki, “Nutritional and sensory properties of protein hydrolysates based on salmon (*Salmo salar*), mackerel (*Scomber scombrus*), and herring (*Clupea harengus*) heads and backbones,” *Frontiers in Nutrition*, vol. 8, pp. 695151, 2021.
- [126] M. Vijaykrishnaraj and P. Prabhasankar, “Marine protein hydrolysates: their present and future perspectives in food chemistry—A review,” *RSC advances*, vol. 5(44), pp. 34864-34877, 2015.