This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. This Accepted Manuscript will be replaced by the edited, formatted and paginated article as soon as this is available.

You can find more information about Accepted Manuscripts in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.
Marine protein hydrolysates: their present and future perspectives in food chemistry – A review

Vijaykrishnaraj M and Prabhasankar P*

Flour Milling Baking and Confectionery Technology Department

CSIR-Central Food Technological Research Institute

Mysore – 570 020

* Corresponding author

Tel.: +91 821 2517730

Fax: +91 821 2517233

E-mail address: psankar@cftri.res.in
Abstract

Marine protein hydrolysates are usually prepared by enzymatic digestion of different proteases at controlled pH and temperature. Biologically potential peptides and essential amino acids were scientifically proved their important biological activities. The time has come to rethink about marine bio-diversity in utilization of protein hydrolysates, which can be playing in nutritional benefits and also plays a significant role in functional ingredients for food industries. This manuscript reviews overview of various marine based protein hydrolysates preparation, purification and bioavailability of bioactive peptides with recent technology tools. Fractionated peptides with biological activities for major health issues and claiming as functional ingredients for food processing.

Keywords: Enzymatic hydrolysis, bioactive peptides, marine functional ingredients, microencapsulation, Food proteomics.

Introduction

The surface of our planet is covered with 70% of saline water and rest of the part is filled with remaining resources. Earth structure was layered into different layers in that hydrosphere is entirely covered with marine water. Marine source plays a vital role in our ecosystem as well as the food web in the oceanic community. Marine biodiversity is one of the largest biodiversity on the earth, based on their adaptive mechanism huge variants of living organisms are abundant from micro to macro levels\(^1\). Recently the research has focused on marine Biomolecules, which is biological potential in healthcare, drug molecules and functional food ingredients. The Greek philosopher Aristotle was given the definition about marine “the ladder of life” that he described 500 species and several were from marine\(^2\). Spatially the marine environment divided into different zones and mainly pelagic and benthic zones. Pelagic zone covered from surface of the ocean layer to the photic zone. Benthos is the deeper area of the ocean layer and light cannot penetrate to in this zone. Overall these two different regions are habitat for various living organisms\(^3\).

Marine ecosystem is having vast abundance of living organisms, which can come from estuaries and wetland ecosystem. Life in the sea has been fascinating thousands of years. The study of those organisms and their importance in food science and nutrition are very scanty; this is the time to accept and to find the sources will be beneficial in all the way to human beings. The wondering microorganisms are responsible for the majority of an atmospheric oxygen fixation to the Earth. These tiny organisms can also responsible for the primary producer of the marine food web. These microscopic organisms habituated in seas, and ponds, lakes are helping to recycle the nutrition. According to the size, it is classified from micro to mega planktons. 20-200µm level has been categorized micro planktons, more than that in size is classified mega planktons of the sea. Overview of marine life and its impacts on earth have been depicted in Fig.1. Secondary metabolites of those organisms are playing vital role for physiological functions. Mega planktons were the highly influenced by the sea as well as to the humans\(^3\).

Zones of the oceans depend on the depth of the floor and sea. Surface layer of the sea is called epipelagic zone. It covers up to 200 m from the surface layer of the water. Most of the tiny living things will be abundant in this area because they need sunlight and energy to build them.
Mesopelagic and bathypelagic zones cover up to 1000 m from the pelagic zone, in this zone is huge abundant of floating organisms to swimming organisms habitat. The bio-actives present in the marine and other aquatic resources can rescue and render the health effects of the chronic diseases. Fish is one of the major marine foods consumed all over the world because of its nutrition benefits. Seafood processing discards and account approximately three-quarters of the total weight of catch it includes trimmings, fins, frames, heads, shells, skin and viscera. Large quantity of fishes were collected worldwide every year, approximately 50% of protein rich fish processed by-products discarded and used as animal feed and fish meal. The use of marine foods and its by-products as substrate leads to a novel approach for potential discovery of high-value bio-actives. Fish and fish by-product hydrolysates and active ingredients were the “Big-dream” of marine biotechnology industry; these products are in low quantity however their value has high, and also with tremendous potential of these innovative bio-molecules.

There is a high potential in marine bioprocessing industries to convert and utilize marine food products and their by-products as valuable functional ingredients. Seafood from both fisheries and aquaculture was supplied to world markets, providing approximately 2.9 million people with at least 15% of the protein of their average per animal protein intake. World aquaculture production of fish, crustaceans, mollusk, etc. has been increased yearly. According to FAO, Asia (580 millions) is the largest producer of aquaculture followed by Africa (1.4 million) and Europe (2.8 million). Marine organisms provide functional compounds like PUFA (Polyunsaturated fatty acids), protein and its bioactive peptides, minerals, vitamins and polysaccharides.

Human body undergoes physiological imbalances and an exposure to extrinsic toxic substance that disturbs normal functions provides various health problems. On other hand, processed food products or foods due to physical, chemical and biological characteristic leads to food spoilage/loss of nutrition. Proteins or peptides from food have been found, physiologically active or bioactive either directly from the food or by hydrolysis either in vitro or in vivo. Protein hydrolysates have exhibited potent biological activities like antihypertensive, antioxidant, antimicrobial, immunomodulatory and anticancer effects, etc. Nutrition point of view is comparing to other diet sources, and marine source provides favorable fatty acid.
composition DHA (Docosahexaenoic acid) & EPA (Eicosapentaenoic acid) have proven health benefits\textsuperscript{17}.

The Wondered aquatic organism had numerous bioactive compounds, which can protect themselves from predators and as well as leads to health benefits for Humans. Protein is among one of the major biological macromolecules which is physiologically involved in the metabolism and also in diet\textsuperscript{18}. Protein hydrolysis was carried out in intestine of mammalian immune system in the presence of a lot of proteolytic enzymes. Digested protein leads to absorb in the body and elucidates functionality. Marine protein hydrolysates prepared by enzymatic, simulated gastrointestinal digestion, solvent extraction and fermentation process. Hence, it can be suggested that marine-derived hydrolysates or bioactive peptides alternative source of synthetic ingredients\textsuperscript{10}. A significant research effort has been related to marine bioactive peptides and their biological potential activities. The relationship between food and health, bioactive peptides have shown to develop functional foods, defined as food with specific health benefits\textsuperscript{20}. Recently focused on improving the bioavailability and bioaccessibility of these marine protein hydrolysates was noticed by researchers and to validating functional ingredients for healthy foods. The objective of this review is to provide an overview in the chemistry of marine protein hydrolysates, their production, purification, characterization and perspectives in food chemistry.

**Methods for marine protein hydrolysates preparation**

The word peptide comes from the Greek word “πεπτίδια” which is translated as “small digestible”. Proteins are known as the various Physico-chemical process and sensory properties of foods and also act as a functional as well as health promotional ingredients\textsuperscript{4}. Preparation of protein hydrolysates from different marine sources and adopted methods showed in Table 1. Marine bioactive peptides have been prepared by enzymatic hydrolysis, solvent extraction and microbial fermentation from the protein present\textsuperscript{14}. Protein hydrolysis, cleavage of peptide bonds can be carried out enzymatically or by chemical processes. Chemical process including alkaline or acid hydrolysis tends to release and difficult to control, yield will be modified amino acids\textsuperscript{10}. In recent years, extraordinary research evidence has been showed food-derived bioactive
peptides and proteins have beneficial effects on human health. These food proteins are easily
digested and released the soluble peptides, which is greater resistant to gastric acid, heat, and
proteolytic enzymes. These peptides are 3-20 amino acids from the digested protein and although
some have been reported to be >20 amino acids\textsuperscript{21}. Essential proteins of vertebrates and
invertebrates muscle are myosin, actin, and collagen. Myosin present in thick filamentous and
action in thin filamentous responsible for contraction, regulatory proteins tropinin, and
tropomyosin also present\textsuperscript{7}. Marine protein hydrolysates have a broad range of ionic strength,
good solubility and tolerate steady heat without precipitating\textsuperscript{6}. Proteins of our foods can act as
health promoters in two ways, first acting indigestible substances in the digestive tract and trap,
expel toxins. Then it is lowering the re-absorption of cholesterol in large intestine\textsuperscript{22}.

Numerous methods have been utilized to release bioactive peptides from meat and marine
food protein, but enzymatic hydrolysis of whole protein is vast majority techniques. Several
researchers have succeeded to produce bioactive peptides from the milk protein followed by
\textit{lactobacilli} fermentation\textsuperscript{23-24}. However, \textit{lactobacilli} fermentation is less successful in meat and
marine food protein due to lower proteolytic activity. Indeed of our best of knowledge, no
microbial fermentation carried out to produce protein hydrolysates in muscle proteins\textsuperscript{25}.
Enzymatic hydrolysis is one of the best methods to prepare marine protein hydrolysates, and can
lead to producing short sequence peptides that can be obtained by \textit{in vitro} hydrolysis of protein
substrates using valid proteolytic enzymes. Proteolytic enzymes sources can be microbes, plants,
and animals in order to develop bioactive peptides\textsuperscript{26}. Usually, enzymatic reactions avoid side
reactions and do not reduce nutritional value of protein source. Native proteins are well-packed
structures with secondary and tertiary structures due to the amino acid linking sequence. These
interactions based on catalytic cleft of site of the proteins\textsuperscript{10}. However, enzymatic hydrolysis
method is preferred in the food and pharmaceutical industries because other methods lead to
release organic solvents and toxic substances in the hydrolysates. The hydrolysis reaction should
be carefully controlled in order to maintain and deliver the equal quality of the end products.
Physico-chemical conditions of the reaction media should be optimized for the activity of the
enzymes. The choice of proteolytic enzyme in hydrolysis is playing vital role because it provides
cleavage patterns of the peptide bonds\textsuperscript{27}. 
Degree of hydrolysis (DH), defined as a percentage of cleaved peptide bonds, is used to
describe hydrolysis of food proteins and serve as a monitoring parameter for the reaction.\textsuperscript{28} Quantification of the degree of hydrolysis is followed by different methods either
spectrophotometric or microkjeldahl for percentage of cleaved peptides. The rate of enzymatic
hydrolysis subsequent increase or decrease and enzymatic reaction steady-state phase was
measured and revealed by DH, which help to understand the researcher, to further purification
of bioactive peptides to render the potentiality. Many amino acids side chain, reactive functional
groups which can react with reagents by cross-linking, intra and intermolecular or covalent
coupling.\textsuperscript{29} Simulation of gastrointestinal digestion of protein by \textit{in vitro} is recent findings to
hydrolyse the complex protein into bioactive peptides. Simulated human gastrointestinal
digestion was carried out by pepsin (gastric digestion) at pH 2 (acidic condition) followed by
trypsin and α-chymotrypsin (duodenal absorption) pH 6.5 - pH 7 neutralization of the peptides.\textsuperscript{30} Newer technologies have been developed to improve the process of enzymatic hydrolysis such as
immobilization of enzymes. Immobilized enzymes more easily controlled conditions, preventing
the generation of secondary metabolites from autolysis of enzymes and also recovering & re-use
the enzymes.\textsuperscript{31}

\textbf{Purification and characterization of bioactive peptides}

Isolation and purification of bioactive peptides are crucial famous for exhibiting their \textit{in}
\textit{vitro} and \textit{in vivo} bioactivity. Traditional way of purification can perform a mixture of peptides
from the hydrolysates like different kinds of chromatography and membrane based separation
techniques.\textsuperscript{4} Purification of those peptides is mainly based on their ionic charges, size, and
hydrophobicity. Electrophoresis can separate the migration of charged particles according to the
size and molecular weight. SDS-PAGE (Sodium Dodecyl Sulphate-Polyacrylamide Gel
Electrophoresis) was a preliminary analysis of any protein molecules for confirmation of mode
of the protein molecule. Membrane ultrafiltration and size exclusion chromatography would be
the best choice to concentrate peptides leads to molecular weight ranges, and to obtained
fractions may contain the low-molecular-weight peptides.\textsuperscript{15} Membrane process based on the type
of cut-off membrane and filtration methods used to produce the bioactive. Novel membrane
technology known as Electro dialysis-ultrafiltration (EDUF) is useful to separate cationic, anionic, neutral peptides of defined molecular sizes. Refining peptides with biological interest of white fish hydrolyzed were achieved by ultrafiltration and nanofiltration. Combination of those filtrations improved purification and diafiltration mode of most active fractions from the hydrolysates. By Using two different cut-off three kDa and ten kDa membranes in blue mussel protein hydrolysates yields active low-molecular peptides. It has proven that good radical scavenging activity and inhibited auto-oxidation. Many researchers found that ultrafiltration through membranes with low-molecular cut-off used to obtain enriched ACE (Angiotensin Converting Enzyme) inhibitor peptides.

HPLC is one of the standard methods for peptides separation and easier because packed and commercially available reverse-phase columns are used to reduce the human error. HPLC usually ties up with quantitative/qualitative equipment such as mass spectrophotometer. Liquid chromatography followed with tandem mass spectroscopy is the standard method for characterization peptide sequences. Matrix Assisted Laser Desorption/Ionization and Time of flight (MALDI-TOF) is backbone analysis for generating peptide profiles of protein hydrolysates or semi-purified fractions. Combination of size exclusion, reverse phase-HPLC, and Q-TOF-MS purified peptides from flounder fish has shown stronger antioxidant activity. Particularly amino-acid residues in the sequences of Pro, Ala, Val, and Cys contributed antioxidant property was claimed due to those methods. Fractionation process results, often peptide yield depends upon the amino acid residues and interest of peptides. Furthermore, purification steps guided based on the bio-assays in order to produce function and structure studies.

Nowadays consumers in demand for health benefits foods beyond basic nutrition. The high complexity and various range of biological peptides abundance challenge the capabilities of analytical methodologies. In silico and in vitro approaches aimed to discover the bioactive peptides from the food matrix. Recent “omic” approaches consist cell biology, immunology, biochemistry, synthetic chemistry and combination library of mass spectrometry, to identify and formulate the bioactivity of peptides in the food sample. In the field of proteins and molecular biology, 2DGE (2-Dimensional Gel Electrophoresis) is playing a lead role. Measuring mass of the peptides obtained by enzymatic hydrolysis of proteins and identification of proteins separated...
by 2DGE after trypic gel digestion. Due to higher resolution and separating power of 2D gels, identification of proteins pattern can be done using simple and easy MS instrumentation\(^\text{40}\). The availability of genome sequences and throughput higher technology foods can be analyzed at various levels. Recently power of proteomic technology combined with another technology called nanotechnology. Food proteomics is one emerging field can act in multidisciplinary action of authentication, safety and response of individual diet molecules in nutritional aspects\(^\text{41}\).

Recently High-performance liquid chromatography (HPLC)-chromatin immunoprecipitation (ChiP)-tandem mass spectrometry (MS/MS) was applied to characterize the storage proteins\(^\text{42}\). Biomarker discovery is another era in food proteomics for major chronic diseases causing proteins invention. For accuracy and addressing questions of bioavailability and bioefficacy, both systemically (i.e., Blood) and locally (in the gut) must be quantified and qualified in the food matrix. Development of nano proteomics can offer significant advantages over proteomics, highly sensitive, selective, high dynamic range of protein analysis in low volume samples. Novel polypeptides can bind specifically to the selected inorganic nanomaterials were genetically engineered using phage-display technologies contributing new field molecular biomimetics. Replacing organic matrix for analysis of traditional MALDI-TOF-MS functionalized nanoparticle probes employed (matrix free direct laser desorption ionization (DLDI-MS)\(^\text{43}\).

Conventional proteomic techniques such as immunoassays and protein microarrays are reliant a biomarkers analysis. 2-DGE and mass spectrometry (Peptide Mass Fingerprinting) and coupled liquid chromatography label free proteome and biomarker analysis\(^\text{41}\). Quantitative structure-activity relationship (QSAR) method describes relationship between bioactivity and structure. QSAR modeling principle is activity or function of the particular chemical can be studied its molecular Physico-chemical descriptors, electronic attributes, hydrophobicity and steric properties. Discovery of bioactive peptides from food proteins greatly advanced to understand structure and activity relationships of peptides. Freely available bioinformatics tools peptide cutter (http://www.expasy.ch/tools/peptidecutter/) was able to do in silico digestion of protein. Server will be using the enzymes trypsin, thermolysin, pepsin, and chymotrypsin individually, or combinations can retrieve the bioactive peptides\(^\text{44}\).
Foodomics has been defined as, a new discipline that studies the food and nutrition domains through the application of advanced omics technologies in order to improve consumer’s well-being, health, and confidence\textsuperscript{45-46}. Foodomics covers the new functional foods development, health supplements and understanding of molecules through molecular tools. Approaches like genomic/transcriptomic/proteomic and metabolomic have used significantly to study of foods/ingredients for profiling of the molecules, biomarker investigation related to food quality and bioactivity of the molecules\textsuperscript{47}. The human health effects were followed by nutrigenomics and nutrigenetics approaches. Proteomes are different from individuals, type of cells and depending on the cell activity and state. Proteome is a challenging task, because of extensive concentration in most of the least abundant proteins. Sample preparation, it includes reducing proteome complexity via fractionation and depletion lead to low abundant proteins. Proteomic studies include “bottom-up”, “shot-gun” and “top-down” approaches. MS is the last step in analytical technique of proteomic, which helps to identify the peptides\textsuperscript{48}. Improved mass spectrometers with better sensitivity and high accuracy in mass and resolution, to identify and quantify the complex protein mixtures in a single experiment. Major mass analyzers utilized for the proteomic studies are, TOF (Time-of-flight), Q (quadrupole), FT-ICR (Fourier transform ion cyclotron resonance) and IT (ion-trap). Some of the mass analyzers are combined in one mass spectrometer, QqQ (Triple quadrupole), Q-IT, Q-TOF, TOF-TOF, IT-FTIMS, etc.

Metabolome is the mixture of endogenous or exogenous low molecular weight entities approximately <1000 Da, which are presenting in the biological system. Metabolites are downstream products of the operated biological system. Metabolic pattern analysis is critical and very much interesting to understand the nutrition point of view because variations in the metabolic pathways due to diet\textsuperscript{49}. Complex of Metabolome is diverse in nature, in the physical and chemical properties (Sugars, amino acids, amines, peptides, organic acids, nucleic acid or steroids). Sample preparation entirely depends on the compounds yet to be analyzed. Two analytical platforms are used in metabolomics, MS, and NMR-based system. These techniques either applicable in alone or fused with other techniques like (LC-NMR, GC-MS, LC-MS, and CE-MS). On the other hand, MS/MS or MS\textsuperscript{n} experiments can be analyzed for ions at high resolution with (Q-TOF, TOF-TOF or LTQ-Orbitrap) provides additional structural information and identification of the metabolites\textsuperscript{50-51}.
Biological potential of bioactive peptides from marine protein hydrolysates

Numerous bioactivities peptides from have been arrived dietary proteins by enzymatic hydrolysis. Specific peptides will have individual or multifunctional activities suitable for functional foods or pharmaceutical products\textsuperscript{52}. The particular bioactivity of the marine peptides for various molecular disease targets based on structural conformation like physico-chemical characteristics of amino acid residues, chain length, molecular charge and bulkiness of chain\textsuperscript{15,53}. Numerous bioactivities have been described from bioactive peptides or protein hydrolysates derived from enzymatic hydrolysis in Table 2. Aquatic species and by-products majorly investigated in food science and nutrition for claiming antioxidant peptides, immunomodulatory peptides, anticancer peptides, antimicrobial and anti-inflammatory, etc\textsuperscript{54}. In Asian countries like Japan, China, and Philippines, marine organisms have been part of their diet and also used in traditional medicine for curing major chronic diseases\textsuperscript{55}.

Anticancer potentiality of bioactive peptides and depsipeptides has been isolated from marine animals like tunicates, sponges, soft corals, sea hares, nudibranchs, bryozoans, sea slugs and other marine organisms\textsuperscript{56}. Approximately more than 10,000 species of sponges has been diversified in nature and most of them are of marine origin. Three of the genera (Haliclona, Petrosia, and Discodemia) reported anticancer and anti-inflammatory activities. In sponges, mostly research going on cyclodepsipeptides, which are secondary metabolites with unusual amino acids and non-amino acid moieties\textsuperscript{57}. Jaspamide is cyclic depsipeptide identified from genus Jaspis and Hemiastrella. Structure of the molecule is 15-carbon macrocyclic rings containing three amino acids (Fig.4A). Homophymin A, which is cyclic 4-aminomethyl-2, 3-dihydroxyhexaenoic acid (Fig.4D) possesses potent anticancer activity. Geodiamolide H (Fig.4B) isolated from a Brazilian sponge Geodia corticostylifera. It has proven anti-proliferative activity against breast cancer cells by affecting the cytoskeleton. Phakellistatins (Fig.4C) identified from the western Indian Ocean sponge Phalkellia carteri. It was investigated for leukemia and those cyclic depsipeptides inhibited the growth of leukemia cell. Isolated cyclodepsipeptides bioactivities are reported in vitro. Didemnin (Fig.4E) existed from Caribbean tunicate Triddidemnum solidum and the bioactive peptide has greater potential of anti-tumor activity and antiproliferative activity against human prostate cancer cell lines. Another bioactive
peptide from mollusc (*Conus magnus*) Ziconotide (Fig.4F) is a 25 amino acid peptide with three sulphur bonds proved analgesic activity. A 60 kDa protein from the purple ink of the hare *Bursatella leachii* named as Bursatellanin-P showed anti-HIV activity. Marine animals based cyclic depsipeptides, and bioactive peptides need to investigate with further detailed mechanism and human intervention studies.

Innovations in nutraceutical are growing enormously because of modern consumer’s awareness about their health. Hydrolyzing protein from marine sources is not only an innovation; it is claiming necessary nutritional availability, intervention against human diseases, promoting food industries to produce functional foods. Cardiovascular diseases are major health disorder to 30% of world’s population deaths and is estimated that in 2020 heart diseases and stroke will be a major source of death. Oxidative stress is a common factor for all these chronic diseases, at present there is increasing interest in the utilization of food derived biologically active peptides as nutritional supplements or nutraceutical. Generated peptides from seafood waste, Pacific cod skin effectively showed ACE inhibitor, antioxidant by *in vitro* gastrointestinal hydrolysis. These peptides are directly structural amino acid composition and higher hydrophobic amino acids. The protein rich salmon muscle analyzed for computer-aided approach and experimental approach to bringing out ACE-inhibitory peptides. Derived salmon fish peptides are often consumed in the diet. Hypertension is another problem worldwide and affects 15-20% of all adults. Salmon skin collagen peptides powder has low-molecular-weight peptides purified and shown to have *in vitro* bioactivities of ACE-inhibitor. Squid gelatin hydrolysates of fractionated HSSG-III, investigated for antihypertensive effects on oral renal hypertensive rats (RHR) in long-term oral administration. HSSG-III of squid gelatin hydrolysates, *in vitro* ACE-inhibitory activity IC\(_{50}\) value was 0.33mg/ml. Oral administration in rats decreased systolic blood pressure and diastolic blood pressure of RHR. It was intent effect of blood pressure reduction *in vivo*. Salted Herring brine protein hydrolysates, different peptide fractions by ultrafiltration revealed antioxidant properties and functional properties. Isolation of peptides from the hydrolysates by ultrafiltration removed salt content of the fractions. Fractions between 50 kDa and 10 kDa showed good antioxidant activity *in vitro*. Meanwhile, functional properties of isolated fractions exhibited lower than sodium caseinate and BSA (Bovine Serum Albumin). Pectoral fin of salmon by-products rich in proteins, enzymatically driven hydrolysates carried out
with antioxidant and anti-inflammatory effects in order to verify the possibility of application. Isolated highly active SPHF1 (Salmon Protein Hydrolysates Fraction1) (1000-2000 Da) was potentially inhibited intracellular ROS (Reactive Oxygen Species) generation. It also inhibited lipid peroxidation and increased the level of GSH (Glutathione) in Chang liver cells. SPHF1 also had proven anti-inflammatory effects by inhibiting Nitric Oxide and proinflammatory cytokine production. It includes TNF-α, IL-6 and IL-1β in LPS induced RAW264.7 macrophage cells in vitro.

Simulated gastrointestinal digested salmon protein hydrolysates by RP-HPLC fractions carried out for in vitro antioxidant properties. Peptides reduce and chelate the metal cations for production of harmful free radicals such as iron-catalyzed conversion of hydrogen peroxide to hydroxyl radical. Skate is the popular seafood in South Korea, due to unique taste and flavor. By-products of skate skin protein hydrolysates investigated first time for ACE-inhibitory activity. Tuna liver by-products procured when processing of Tuna canned products. Tuna liver protein hydrolysates prepared by commercially available enzymes and fractionated with different pore size of ultrafiltration membrane. Hydrolysates showed dual bioactivity in vitro AchE (acetylcholinesterase) inhibitory and antioxidant activities. Above 10 kDa fractions, exhibited high AchE inhibitor activity than low-molecular fractions. Macroalgae are one of the popular sea foods in many oriental countries. Biofunctional ingredients for cardioprotective, antidiabetic and antioxidant have been investigated in Red algae (Palmaria palmate). Aqueous protein hydrolysates generated by alcalase and Corolase PP in vitro studies proved higher inhibitory effects of Type-II diabetes, ACE inhibitory and antioxidant properties. Soluble extracts of edible parts of mussel (Mytilus edulis) anticoagulant peptide (MEAP) isolated and investigated. MEAP prolonged the normal clotting time to 321±2.1 s on APTT (Activated Partial Thromboplastin Time), and 81.3±0.8 s on TT (Thrombin Time) is dose-dependent manner. MEAP can prolong the time of clotting by inhibiting the activation of FX in intrinsic tenase complex and conversion of FII (Prothrombin) to FIIa (Thrombin) in the prothrombinase complex. Calcium deficiency in high spread ration due to insufficient intake and diminished solubility of calcium by constituents of food and anti-nutritional factors. Nile tilapia (Orechromis niloticus) is distributed worldwide, and dumping of processed tilapia scale by-products is also increasing. Calcium binding peptide (DGDDGEAGKIG, Mw 1033.0 Da) was purified from...
tilapia scale protein hydrolysates. Asp and Glu residues in the peptide contributed substantial calcium binding capacity, physical and biochemical properties of femurs in Ca-deficiency rats was significantly improved the Calcium bioavailability. Oyster is a high source of quality nutrition in North East China and rest of other parts of the World. Oyster (Crassostrea talienwhanensis) evaluated the yield of TCA-soluble fractions and hydrolyzed by subtilisin, and attempt also made to isolate two antioxidant peptides by Nano-ESI/MS/MS. Hydrolysates passed through 3 kDa membrane exhibited hydroxyl and radical scavenging activity. Purified two peptides PVMGD (Mw 518 Da) and QGHV (Mw 440 Da) do not have a significant homology of other antioxidative peptides. In Another study, Oyster (Crassostrea gigas) hydrolysates have been derived from protease (Bacillus sp.SM98011), and production was pilot to plant scales. Antitumor and immunomodulating effects of hydrolysates on S-180 bearing BALB/c Mice were investigated. The weight coefficient of thymus and the spleen, NK cells activity, Spleen lymphocyte proliferation of phagocytic rate of macrophage cells in S-180 bearing BALB/c Mice proved significant difference on orally administrated of hydrolysates. Sea cucumber is another benthic marine organism distributed in the majority of ocean and highest diversity of shallow tropical waters. It also used as food in Asian countries like Philippines, Malaysia, Japan, Korea, and China. Extensive research on sea cucumber extracts for multiple biological potential activities has been carried out. Simulated gastrointestinal digested peptides of sea cucumber (Isostichopus badionotus) analyzed for antioxidant, antiproliferative and ACE inhibitory. Fractioned > 3 kDa and < 3 kDa showed ACE inhibitory and cytotoxic effects against colorectal cancer cells. Released multifunctional peptides are capable of resisting gastrointestinal enzymes and found higher concentrations of amino acids (Gly, Arg, and Ala). It played a significant role in physiological effects and reduced serum cholesterol levels. Pollock is commercial fish and is having enough meat and backbone, after processing by-products utilized in animal feed. Immune functions play a significant role in modulating the immune system and counter attack the chronic diseases. Purified and identified peptides from Pollock frame protein hydrolysates carried out for splenocyte lymphocyte proliferation and amino acid sequencing. Three peptides with high lymphocyte proliferation activities were separated, and their amino acid sequences were NGMTY, NGLAP and WT respectively. The proliferation rates were above 30% in 20µg/ml peptides. Hydrolysates from shrimp waste for functional properties and product applications.
Use of enzymes, approximately 40-50% could be isolated from certain species of shrimp, possibly the binding of protein or carbohydrate complex in the shrimp shells. Fractions of <10 kDa and 10-30 kDa exhibited after 72 h significantly inhibited the growth of both colon cancer and liver cancer cells by 60%\textsuperscript{76}. Marine oligopeptide preparation from chum salmon (\textit{Oncorhynchus keta}) by enzymatically found that enhancement of innate and adaptive immunities through the production of cytokines in mice. Gamma radiation-induced immunosuppressed female mice fed by marine oligopeptide and it proved augmentation of the relative numbers of the radioresistant CD\textsubscript{4+} T-cells. It also showed enhancement of IL-12 level in splenocytes, reduction level of NF-\kappa B through induction of I\kappa B in spleen and apoptosis inhibition of splenocytes. Therefore, Marine oligopeptide can be supplementary therapy and protective effect in cancer\textsuperscript{77}. Baked products are the widely consumed foods in the world and suitable vehicle for delivering the bioactive ingredients\textsuperscript{78}. Antimicrobial peptides identification from marine origin is lower than terrestrial origin. Enzymatic hydrolysis of fish muscle leather jacket (\textit{Meuchenia sp.}) purified fractions 9 and 12 carried out for antimicrobial MIC (Minimum Inhibition Concentration) assay. Fraction 12 exhibited MIC against \textit{Bacillus cereus} and \textit{Staphylococcus aureus} pathogenic bacteria\textsuperscript{79}. Red seaweed (\textit{Palmaria palmate}) protein hydrolysates carried out for next level studies to claim functional foods or health supplements. The renin inhibition assay showed bioactive properties of hydrolysates were retained during the baking process. Furthermore, developed seaweed hydrolysates bread did not affect the sensory quality of the product\textsuperscript{80}.

Commercially marine-derived protein hydrolysates and peptides were approved as functional ingredients in Japan. It is labeled as FOSHU (Several Food for specific health use) products. Lapis Support\textsuperscript{TM} (Tokiwa Yakuhin Co.Ltd.) and Valtyron\textsuperscript{®} (Senmi Ekisu Co.Ltd.) are examples of two such products sold in Japan\textsuperscript{76}. Lapis Support\textsuperscript{TM} is available in beverage format and Valtyron\textsuperscript{®} is incorporated in 33 other products like soft drinks, jelly and dietary supplements. Production of Valtyron\textsuperscript{®} is hydrolysis of the sardine muscle with commercially available food grade alkaline protease from \textit{Bacillus licheniformis}. Another, FOSHU approved functional product ‘Peptide soup’ made up on katsuobushi (bonito) hydrolysate generated with thermolysin\textsuperscript{81-82}. The active peptide LKPNM in the product showed the significant reduction of systolic blood pressure in mildly hypertensive subjects. In addition to beverage (Soup and Tea)
bonito peptide has sold as powdered ingredient and also in tablet form called as ‘Peptide ACE 3000’ in Japan (Nippon Supplement Inc.). Apart from this, other marine-derived protein hydrolysates without approved health claims sold as food supplements in Europe and North America. The products are Stabilium® 200, Protizen®, AntiStress 24, Nutripeptin™ and Seacure®. Nutripeptin™ (Nutrimarine Life Science AS, Norway) is a product of cod protein hydrolysate sold as having postprandial blood glucose lowering activity. Seacure® (Proper Nutrition, US) is a product of Pacific whiting hydrolysate marketed as a supplement for gastrointestinal health improvement. Furthermore, Fortidium liqumen® (Biothalassol, France) is a product from white fish (*Molva molva*) autolysate is commercially available and having multifunctional effects like antioxidant, anti-stress and glycemic index reducing agents. Based on the evidence of potential health benefits of marine protein hydrolysates or peptides had a promising role in functional ingredients or Nutraceutical. List of commercially available marine protein-derived products is the examples of the utilization of the protein hydrolysates for alternative health supplements. Although a number of studies existed for proven biological effects are *in vitro* or animal models. Time has come to understand the molecules in human intervention trials to study the biological effects of more detailed mechanism. Ultimately regulatory approval from various standard agencies like FDA, EFSA and FOSHU are required to reach the market.

Recent approaches to bioactive peptides and functional delivery systems

Microencapsulation is the entrapment of tiny molecules, liquid droplets and gasses in coating. Microencapsulation can allow the protection of a broad range of materials of biological interest leads to applied biomedicine and biopharmaceuticals. Recently this technology utilized in the food industry applications for providing high-value products or Nutraceutical. Bioactive peptides added products can undergo processing, storage, and transport. To protect the bioactivity encapsulated form is the suitable delivery system. Marine protein hydrolysates and their bioactive peptides applications and recent approaches schematic representation showed in Fig.2. Encapsulation of proteins depended on the type of proteins and envisioned health effect serve the vehicle of bioactive peptide. Nanotechnology is another technology that can utilize, create and manipulate the materials in devices or systems in nanometer scale. Entrapment of
bioactive peptides with nanotechnology is a promising carrier for active functional ingredients to
the industry. Nanoemulsions, functional hydrogels, and nanoparticles deliver the bioactives to
target organs. To carry through the bioactivities and improves the stability in gut system, as
well as bioavailability, these technologies will be suitable and helpful for development of
functional foods, nutraceutical or health supplements.

**Nutritionally enriched marine based processed food products**

Marine animal foods are rich in protein content on an edible fresh weight basis than most
terrestrial meats. Marine animals such as fish, crustaceans and mollusks are the wide consuming
sea foods among others. Marine animal’s food proteins are highly digestible and have a
biological value of releasing essential amino acids (EAA), which is closely recommended to the
human diet. Since, this EAA is lack in plant and other terrestrial proteins consumed by humans.
Aquatic food products are a suitable way for the addition of plant-based diet consumed by
human. Humans were counter-attacked by free radicals from both, inside the body and
surrounding environment exclusively reactive oxygen species (ROS) during metabolic process.
Addition to cause oxidative stress that leads to attack macromolecules, DNA, Proteins,
Carbohydrates and Lipids cause health disorders. In another side, oxidation of foods is a major
problem to cause deterioration of food quality leading to rancidity and reducing shelf life of the
products. To retard this issues, many synthetic antioxidants made by pharmaceutical and food
industries. However, those synthetic antioxidants must be under strict regulation due to potential
health hazards. To overcome these issues, natural antioxidants from food based biological
substances addressed recently. A present and future direction of marine protein hydrolysates in
food science and nutrition are diagrammatically represented as Fig.3. Due to their safety mode,
nutritional and therapeutic purpose using level of interest increased significantly. Marine
organisms believed to be a potential source of biologically active peptides for the development of
pharmaceuticals, functional ingredients and human nutrition. Development of bioactive peptides
from the seafood protein depends on two factors, the primary sequence of the protein substrate
and specificity of the enzymes usage. Structure-activity relationship of those generated peptides
is not still fully established, but few have been identified with the influence of biological action.
For example, Angiotensin converting enzyme (ACE (EC 3.4.15.1)) inhibitory peptides, binding
action strongly consequence by the presence of amino acids like tyrosine, phenylalanine, tryptophan, proline, lysine, isoleucine, leucine, valine and Arginine. For lipid lowering and antioxidant activity of the peptides also totally depends on their configuration of the amino acids either hydrophobic or hydrophilic residues\textsuperscript{90}.

In addition, marine food processing by-products like standard muscles, viscera, skins, trimmings, and shellfish can be used efficiently to produce Nutraceutical and functional food ingredients with biofunctional activity\textsuperscript{91}. Marine species and processing by-products contain plenty of proteins were yet undiscovered novel sequences encrypted within their primary structures with potential biofunctional activity. However growing scientific evidence shows that many marine-derived including molluscs, crustaceans and processing waste by-products, protein hydrolysates and peptides can promote health and addition to rendering the chronic diseases\textsuperscript{83}. Recently, Seaweed (\textit{Palmaria palata}) protein hydrolysates added in the bakery food (Bread) and validated heart health beneficial to human kind. Those incorporated breads are not affected the organoleptic characteristics, and it also improved the overall product quality with beneficial effects\textsuperscript{80}.

Peptides, 2-6 amino acids length are compared to complex protein; proteins are the less absorbance across the gastrointestinal tract. Their limitations may be based on intrinsic factors like physico-chemical and biological properties. The reason is a poor permeation of the biological membranes because of molecular size, physical and chemical instability, degradation by intrinsic proteolytic enzymes and aggregation. Transcription factors and signaling molecules adsorption, immunogenicity is thought play role in the process. Therefore, marine-derived bioactive proteins incorporated foods play a critical role to assess the biological potential \textit{in-vivo}\textsuperscript{83}.

In early 1950, humans began to consume the microalgae in one of their diets, either in the form of the capsule, powder, tablet, and pastille. Most consuming marine-derived microalgae species are \textit{Spirulina}, \textit{Chlorella}, \textit{Dunaliella}, and \textit{Aphanizomenon}, etc. They have rich proteins and essential phytochemicals that can contribute more physiological effects to the humans. Microalgae can easily be incorporated into food products like pasta, biscuits, breads, candies, yogurt and soft drinks, etc. It is reported that \textit{Spirulina} incorporated foods consumption can lead
to stimulating gastrointestinal tract *Lactobacilli* sp. Moreover, microalgae were also acted as animal nutrition to stimulate the physiological functions. Animal feed price is double the amount of the human diet. Animal feed industries are looking functionally and lower cost of food supplements, which can give more potential to animal, as well as the animal form owners. Microalgae (*Schizochytrium sp*) were incorporated into ruminants feed, and it’s proved that enrich the products of polyunsaturated fatty acids in the milk fat whereas saturated fat was reduced. Another study in rabbits showed that incorporated of microalgae (*Spirulina platensis*) in their feed has been proved reduction of serum cholesterol levels and increased high-density lipoprotein cholesterol. Poultry feed is another growing research in the world, addition of microalgae (*Chlorella sp*) powder 10% showed increased linoleic acid and DHA in egg yolk and reduced docosatetraenoic acid. Aquaculture industries are also benefited by these tiny microalgae because phytoplankton communities only primary feed for macro level organisms. Powdered or pellet form of microalgae can be used feed or pigments for carp, salmon, and shrimp. Being a simple aquatic, photosynthetic organisms are promising sources of novel products and applications.

Meat oxidation in stored or processed products is a significant concern in the food industry. Meat oxidation leads to oxidize the meat and produce off-flavor, reduced shelf life, dark colors, and potentially toxic products chemical reaction. Due to these problems, food industry sector can not able to deliver a fresh product to the consumers once processed the meat or chopping. To handle this matter, inhibit the oxidation of meat can be controlled by antioxidant peptides. Recently, antioxidant peptide from Goby muscle protein hydrolysates (GPH) obtained by treatment with various fish crude alkaline protease and determination against lipid peroxidation in turkey meat sausage during 25 day’s storage period. Malondialdehyde (MDA) is widely studied marker of oxidation stress and lipid peroxidation index in food products. When MDA reacts with TBA (Thiobarbituric acid) gives TBA reactive substances detectable by spectrophotometer at 532 nm. The decrease of TBARS probably due to peptides interaction and inhibited the oxidation in turkey meat sausage up to 12 days.

One of the most relevant and significant food processing technologies is extrusion cooking, which has been used since 1930s for the production of breakfast cereals, ready to eat
snacks and other texture products. Edible seaweed as an ingredient aimed to develop food-based application in extrusion products to make attractive and reach non-seaweed eaters also. Two Indians seaweed (Sargassum marginatum and Undaria pinnatifida) based semolina extruded pasta products were developed and their biofunctional and nutritional qualities of the products analyzed. However as far now, very few research article existed incorporated food products and recently maize-based extruded products of seaweed (Porphyra columbina) and their carries through properties of bioactive compounds profiling was conducted. Maize (control) and maize: seaweed extruded products were digested with gastrointestinal enzymes, and their in vitro studies of bioactive peptides potential of ACE inhibitor, as well as antioxidation properties was performed. Another recent studies utilization of marine mussel (Perna canaliculus) as an ingredient for product quality, biofunctional evaluation was revealed in gluten-free pasta products. Gluten is backbone of the food industries, on the other hand, it can cause allergy to genetically suspected consumers. Gluten free diet is the only solution to handle this problem for the consumers. Marine based protein can play replacement of other protein sources and helps to develop a network of other molecules for developing gluten free products. These are the situations may help food technologists to understand and do research on marine sources utilization in the nutritional retention and enriched functional ingredients.

Conclusions and recommendations for future research

Marine protein hydrolysates production and their biological potential activity studies are existed and evolving in the direction of development of functional foods, Nutraceutical and functional food ingredients. Currently, very few studies on the development of protein hydrolysates or bioactive peptides enriched food products, or coated products were present. In the modern world due to fascinating of time and lower availability of terrestrial food products, we have to look it other sources, which are having a huge biodiversity and lesser utilization in consumption. Health related disorder is another trend nowadays, to combat and treat these disorders we have to utilize these natural sources and bring it to the population in need. Future research and studies should be in multidisciplinary; to produce functionally enriched food products, improved bioavailability & stability and finally retention of biological potential activity.
Acknowledgements

The author Vijaykrishnaraj M gratefully acknowledges the funding agency, (Department of Science & Technology, Govt. of India) for providing DST-INSPIRE-Fellowship to conduct this research and also thankful for CSIR-CFTRI to access the facilities.
<table>
<thead>
<tr>
<th>Protein source</th>
<th>Techniques used</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marine Fish by-products</td>
<td>Separation: MWCO – fractionation 1, 5 and 10 kDa, Ion exchange chromatography, RP-HPLC &amp; Q-TOF MS</td>
<td>Ahn&lt;sup&gt;68&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Identification: Ultrafiltration, RP-HPLC &amp; Analytical HPLC, Gel filtration, RP-HPLC &amp; Q-TOF with ESI, Gel filtration, RP-HPLC &amp; ESI-MS(MS/MS), FPLC, RP-HPLC &amp; Q-TOF MS</td>
<td>Girgih&lt;sup&gt;7&lt;/sup&gt;, Lee&lt;sup&gt;67&lt;/sup&gt;, Bougatef&lt;sup&gt;100&lt;/sup&gt;, Himaya&lt;sup&gt;30&lt;/sup&gt;</td>
</tr>
<tr>
<td>Marine Fish</td>
<td>Separation: Pico-Taq HPLC, Ultrafiltration MWCO – 1, 3 &amp; 10 kDa and HPLC, SDS-PAGE &amp; HPLC, Precipitation, sequential ultrafiltration &amp; FAST-AAA MS, Ultrafiltration &amp; Nanofiltration, FPLC &amp; DH</td>
<td>Samaranayaka&lt;sup&gt;101&lt;/sup&gt;, Salampessy&lt;sup&gt;79&lt;/sup&gt;, Taheri&lt;sup&gt;66&lt;/sup&gt;, Vandanjon&lt;sup&gt;33&lt;/sup&gt;, Slizyte&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td>Shrimp &amp; shrimp by-products</td>
<td>Separation: DH, Amino acid analyzer</td>
<td>Sila&lt;sup&gt;105&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Identification: Ion exchange, gel filtration, RP-HPLC &amp; ESI-MS (MS/MS)</td>
<td>Huang&lt;sup&gt;104&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mollusks – Oyster, mussel</td>
<td>Identification: MWCO – 1,3 &amp; 10 kDa, size exclusion, amino-acid analyzer, RP-HPLC, off gel fractionation &amp; MS/MS</td>
<td>Aleman&lt;sup&gt;105&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Separation</td>
<td></td>
</tr>
<tr>
<td>--------------------------</td>
<td>------------------</td>
<td>-------</td>
</tr>
<tr>
<td><strong>Echinoderms</strong></td>
<td>MWCO - 1,3 &amp; 10 kDa, SE-HPLC, SDS-PAGE, ESI-Q-TOF MS/MS, &amp; SPR</td>
<td>Wang $^{73}$</td>
</tr>
<tr>
<td></td>
<td>MWCO – 1,3 &amp; 10 kDa, Gel filtration, RP-HPLC &amp; ESI-Q-TOF MS/MS</td>
<td>Jung $^{70}$</td>
</tr>
<tr>
<td></td>
<td>MWCO 3kDa, RP-HPLC</td>
<td>Wang $^{34}$</td>
</tr>
<tr>
<td><strong>Cartilaginous Skeleton - Fish</strong></td>
<td>Separation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DH, gel filtration, GC-MS</td>
<td>Bougatef $^{93}$</td>
</tr>
<tr>
<td><strong>Seaweed</strong></td>
<td>SDS-PAGE, GPC-HPLC</td>
<td>Harnedy $^{69}$</td>
</tr>
</tbody>
</table>

Table 2
Marine protein hydrolysates and their biological potential for functional ingredients

<table>
<thead>
<tr>
<th>Marine sources</th>
<th>Mode of hydrolysates</th>
<th>Bioactivities</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuna frame protein</td>
<td>Cocktail enzymes</td>
<td>Antihypertensive effect</td>
<td>Lee&lt;sup&gt;99&lt;/sup&gt;</td>
</tr>
<tr>
<td>Oyster – Mollusc</td>
<td>Pepsin</td>
<td>ACE Inhibitory</td>
<td>Wang&lt;sup&gt;83&lt;/sup&gt;</td>
</tr>
<tr>
<td>Oyster - Mollusc</td>
<td>Substilisin</td>
<td>ACE Inhibitory</td>
<td>Wang&lt;sup&gt;72&lt;/sup&gt;</td>
</tr>
<tr>
<td>Blue mussel - mollusc</td>
<td>Cocktail enzymes</td>
<td>Antioxidant peptide</td>
<td>Wang&lt;sup&gt;34&lt;/sup&gt;</td>
</tr>
<tr>
<td>Common smooth-hound – Shark</td>
<td>Crude enzyme</td>
<td>Antioxidants</td>
<td>Bougatet&lt;sup&gt;100&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pacific hake - fish</td>
<td>Gastrointestinal digestion</td>
<td>Antioxidants &amp; ACE inhibitory effect</td>
<td>Samaranayaka&lt;sup&gt;101&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pacific oyster - mollusc</td>
<td>Crude enzyme</td>
<td>Antitumor &amp; immunostimulants</td>
<td>Chen&lt;sup&gt;106&lt;/sup&gt;</td>
</tr>
<tr>
<td>Shrimp waste</td>
<td>Alcalase</td>
<td>Antioxidants</td>
<td>Dey&lt;sup&gt;107&lt;/sup&gt;</td>
</tr>
<tr>
<td>White fish</td>
<td>Crude enzyme &amp; ultra filtration</td>
<td>-</td>
<td>Vandanjon&lt;sup&gt;33&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pacific whiting fish</td>
<td>Dried hydrolysate powder</td>
<td>Intestinal protective effect</td>
<td>Marchbank&lt;sup&gt;108&lt;/sup&gt;</td>
</tr>
<tr>
<td>Atlantic salmon skin</td>
<td>Alcalase &amp; Papain</td>
<td>ACE inhibitor peptide</td>
<td>Gu&lt;sup&gt;65&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cod backbone waste</td>
<td>Protamax</td>
<td>Antioxidant and radio immune assay</td>
<td>Slizyte&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td>Alaska Pollock frame</td>
<td>Trypsin</td>
<td>Immunomodulating peptides</td>
<td>Hou&lt;sup&gt;75&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fish waste from different fish muscle.</td>
<td>Pepsin, pancreatin and thermolysin from B. thermoproteolyticus</td>
<td>ACE inhibitory and radical scavenging effect</td>
<td>Nakajima&lt;sup&gt;109&lt;/sup&gt;</td>
</tr>
<tr>
<td>Leather jacket - fish</td>
<td>Papain, bromealin and flavourzyme</td>
<td>Antimicrobial effects</td>
<td>Salampessy&lt;sup&gt;79&lt;/sup&gt;</td>
</tr>
<tr>
<td>Squid gelatin</td>
<td>Cocktail enzymes (protamax,trypsin, neutrase,alcalase)</td>
<td>Antihypertensive, anticancer &amp; antioxidant effect</td>
<td>Aleman&lt;sup&gt;110&lt;/sup&gt;</td>
</tr>
<tr>
<td>Squid skin gelatin</td>
<td>Pepsin</td>
<td>ACE inhibitor &amp; antihypertensive</td>
<td>Lin&lt;sup&gt;35&lt;/sup&gt;</td>
</tr>
<tr>
<td>Squid skin collagen</td>
<td>Esperase, pepsin &amp; pancreatin</td>
<td>ACE inhibitor</td>
<td>Aleman&lt;sup&gt;105&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Salmon by product</strong></td>
<td><strong>Cocktail proteases</strong></td>
<td><strong>Antioxidants &amp; anti-inflammatory effect</strong></td>
<td><strong>Ahn</strong>&lt;sup&gt;9&lt;/sup&gt;</td>
</tr>
<tr>
<td>------------------------</td>
<td>------------------------</td>
<td>---------------------------------------------</td>
<td>------------------</td>
</tr>
<tr>
<td><strong>Chum salmon</strong></td>
<td><strong>Complex protease</strong></td>
<td><strong>Immuno modulatory effect</strong></td>
<td><strong>Yang</strong>&lt;sup&gt;111&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Chum salmon skin</strong></td>
<td><strong>Complex protease</strong></td>
<td><strong>Neuroprotective effect</strong></td>
<td><strong>Yang</strong>&lt;sup&gt;77&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Sardinella by-products</strong></td>
<td><strong>Crude protease</strong></td>
<td><strong>Antioxidant effect</strong></td>
<td><strong>Bougatet</strong>&lt;sup&gt;93&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Seaweed – <em>P. palmata</em></strong></td>
<td><strong>Alcalase &amp; Flavourzyme</strong></td>
<td><strong>Cardioprotective, anti-diabetic &amp; antioxidants</strong></td>
<td><strong>Harnedy</strong>&lt;sup&gt;69&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Seaweed – <em>P. columbina</em></strong></td>
<td><strong>Pepsin &amp; Pancreatin enzymes</strong></td>
<td><strong>ACE inhibitors &amp; Antioxidants</strong></td>
<td><strong>Cian</strong>&lt;sup&gt;97&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Salmon by-products</strong></td>
<td><strong>Cocktail enzymes</strong></td>
<td><strong>Antioxidant-octa peptide</strong></td>
<td><strong>Ahn</strong>&lt;sup&gt;112&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Salmon flesh</strong></td>
<td><strong>Pepsin, trypsin &amp; chymotrypsin</strong></td>
<td><strong>Antioxidants</strong></td>
<td><strong>Girigh</strong>&lt;sup&gt;7&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Surimi by-products</strong></td>
<td><strong>Protamax &amp; Alcalase</strong></td>
<td><strong>Functional properties</strong></td>
<td><strong>Liu</strong>&lt;sup&gt;113&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Shrimp by-products</strong></td>
<td><strong>Alcalase</strong></td>
<td><strong>Caroteno proteins - antioxidant</strong></td>
<td><strong>Sila</strong>&lt;sup&gt;103&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Pacific cod skin gelatin</strong></td>
<td><strong>Gastrointestinal enzyme</strong></td>
<td><strong>ACE inhibitor &amp; cellular oxidative stress</strong></td>
<td><strong>Himaya</strong>&lt;sup&gt;30&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Sea cucumber</strong></td>
<td><strong>Gastrointestinal enzyme</strong></td>
<td><strong>Multifunctional peptides</strong></td>
<td><strong>Vega</strong>&lt;sup&gt;74&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Sphyrrna lewini Muscle – shark</strong></td>
<td><strong>Ethanol soluble Proteins</strong></td>
<td><strong>Antioxidant peptide</strong></td>
<td><strong>Wang</strong>&lt;sup&gt;114&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Blue mussel</strong></td>
<td><strong>CCl₄ treatment &amp; ultrafiltration</strong></td>
<td><strong>Anticoagulant peptide</strong></td>
<td><strong>Jung</strong>&lt;sup&gt;70&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*ACE – Angiotensin-Converting-Enzyme*
Figure Captions

Fig. 1 Diagrammatic representation of an overview of marine life and its impacts.

Fig. 2 Schematic diagram of recent and application of marine protein hydrolysates in Food Science & Nutrition.

Fig. 3 Schematic representation of present and future perspectives of marine protein hydrolysates in Food Science & Nutrition.

Fig. 4. Chemical structures of marine bioactive peptides and depsipeptides from marine animal sources; sponges, tunicates, mollusks – A. Jaspamide; B - Geodiamolide H; C – Phakellistatin; D - Homophymine A; E – Didemin; F – Ziconotide.
References


Fig. 2.

- Applications in Food Science & Nutrition
  - Functional Ingredients
  - Health promoting foods
  - Antioxidants
  - Gluten Free diet
Fig. 3.

Marine organisms

Microbes, Achaeta and Plants

Animals and Mammals

Preparation of marine protein hydrolysates

Purification and isolation of peptides

Evaluation of bioactivities

Fortification into foods

Improving the Bioavailability & Stability

In-vitro & in vivo validation

“Omics” approaches for Bioactives recovery

Simulation of gastrointestinal digestion
Marine source

Bio-Diversity

Marine Biomolecules

Prevent the foods from spoilage

Control the major chronic diseases

Provide nutrition

Marine Protein