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Review

A Systemic Review on Microalgal Peptides: Bioprocess and Sustainable Applications

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Abstract: Nowadays, microalgal research is predominantly centered on an industrial scale. In general, multipotent bioactive peptides are the advantages over focal points over utilitarian nourishment as well as nutraceuticals. Microalgal peptides are now profoundly connected with biological properties rather than nutritive. Numerous techniques are employed to purify active peptides from algal protein using enzymatic hydrolysis; it is broadly used for numerous favorable circumstances. There is a chance to utilize microalgal peptides for human well-being as nutritive enhancements. This exhaustive survey details the utilization of microalgal peptides as antioxidant, anti-cancerous, anti-hypersensitive, anti-atherosclerotic, and nutritional functional foods. It is also exploring the novel technologies for the production of active peptides, for instance, the use of algal peptides as food for human health discovered restrictions, where peptides are sensitive to hydrolysis protease degradation. This review emphasizes the issue of active peptides in gastrointestinal transit, which has to be solved in the future, and prompt impacts.

Keywords: microalgae; value added products; peptides; bioactivity; enzyme hydrolysis

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1. Introduction

Generally, algae are renewable sources and oxygenic photosynthetic organisms, including an enormous and wide variety of species grown in aquatic environments. Algae belong to a broad polyphyletic group, ranging from the monarchy of Prokaryotic (Monera) and eukaryotic (Protista). In view of their pigments, shape, cell wall, cellular division, and structural arrangement, algae are comprehensively classified into "microalgae" and "macroalgae". Macroalgae (seaweeds) are multicellular macroscopic nonvascular plants which grow faster and also contain chlorophyll pigment, and their size ranges up to 60 m in length. Relying upon the chlorophyll pigmentation, macroalgae are divided into chlorophyceae, rhodophyceae, and phaeophyceae, and the unicellular forms are called microalgae [1,2]. **Unlike** macroalgae, microalgae are microscopic unicellular organisms which cannot be seen with the naked eye. Both terrestrial and aquatic environment microalgae are present [3]. Due to their rapid growth, microalgae have an essential need for sunlight, atmospheric CO_2, minerals to produce biomass, biofuels, food feed, and bioactive components. Microalgae are monetarily displayed as pharmaceuticals [4], nutraceuticals [5–7], cosmeceuticals, biofuels and pollution control for wastewater treatment, food additives, and aquaculture [8–10]. **Upon** comparin the microalgal biomass produced competes in both quantity and quality and is a rich source of food protein than the traditional food proteins (e.g., fish, egg, and soybeans), [11,12]. Approximately, there are 200,000 species of microalgae present in diverse groups. A significant number of these microalgal species have recently been used for multi-purposes, including phospholipids, fodder, alginic acids or alginates, recycled water treatment, biofuel and cosmeceuticals [6,13,14]. According to various investigation and advancements the enzyme protein hydrolysates from bioactive functional foods, are a good source of nutritional quality and are available at

low costs from marine microorganisms [15]. The proteins, and protein hydrolysates or peptides of some notable microalgae species are profoundly connected with biological activities than being highly nutritive. They exhibit anti-oxidant [16], anti-hypertensive [17], immune-modulatory [18], anti-cancer [19], hepato-protective [15], anti-atherosclerotic [20], anticoagulant [21], anti-UV radiation, anti-osteoporosis [22] and anti-microbial [23] activities. Annually, microalgae are produced in low quantity when compared to seaweed, which has 5000 tons of dry matter per year (7.5×10^6 tons). Recently, *Chlorella* sp. of unicellular green algae has enormously expanded the development state of the nutritive bioactive supplement. The most common and widely used industrial species is *C. vulgaris*, which contains a high protein and essential amino acids EAA with an average dry weight of 51–58% [24]. The beneficial supplements of *Chlorella* include Vitamin B, minerals such as calcium, potassium, iron, sodium, and magnesium, carotenoids such as beta-glucan, beta carotene, chlorophyll, and the growth factor of *Chlorella sp*. [14,25]. Recently, globally, 30% of microalgal biomass production is sold to the feed industries [26]. The principle importance of these novel bioactive peptides, which contain high-quality proteins, and their structural diversity are still undiscovered. The isolation of these microalgal bioactive peptides is a growing demand nowadays [27]. This review emphasizes use of algal peptides bioprocess and its applications as a food for human health and the issue of bioactive peptides in the passage of a gastrointestinal study, which has to be solved in the future.

2. Development of Microalgal Bioactive Peptides from Proteins

Microalgal bioactive peptides comprises 2–20 amino acids in the length of the chain, showing analog as a hormones were released from the parent compound, which containing beneficial properties.The chemical residues gained importance due to its human health benefits and biological activities. Biologically active peptides acquired from microalgae are less revealed as functional constituents [4,28,29]. There are three unique strategies used to provide microalgal bioactive peptides, such as the extraction of chemical solvent, enzymatic protein hydrolysis, and microbial fermentation. Despite the fact that there is a loss of expected chemical residues in the byproducts such as peptides the technique of enzymatic hydrolysis is the most preferable method in the biopharmaceutical and food product industries [30,31]. This enzymatic hydrolysis is extensively used for a further separation method, which involves membrane ultra-filtration with multiple pore sizes such as 3, 5, 10, and 30 KDa and chromatography techniques, viz., size exclusion and ion-exchange [32]. In general, there are two different techniques for processing bioactive peptides such as fractionation and purification. In particular, the ultra-filtration (molecular weight) and chromatographic techniques (purification) such as ion-exchange, affinity, and gel-permeation are used for the fractionation of microalgal peptides. This technique is mainly to study consider the structural features and mass determination of microalgal peptides using LC-MS and LC-MS/MS spectrometry. Investigations are made with protein hydrolysates [31,33] and the preparation of bioactive peptide-derived microalgae proteins with different approaches. Enzymatic hydrolysis is the most common and widely used to synthesize bioactive peptides from microalgal proteins for clinical applications, and it expels the harmful substances to give a good yield and high purity, better than the organic solvent extraction. A few studies reported that enzyme-assisted extraction method provides great yield and a high amount of bioactive components. The hydrolysates of individual peptides showing bioactivity can be identified from the original protein. Currently, crypteins are recently termed peptides of novel therapeutic and they show natural bioactivities [28,34].

3. Isolation of Bioactive Peptides from Proteins

The hydrolysis of proteins into free amino acids can be determined using the equation proposed elsewhere [35]. The search for functional proteins and bioactive peptides from microalgae has increased significantly [36]. However, the isolation of proteins using organic solvent extractions delivered targeted compounds with toxic residues. Therefore,

proteolytic enzyme-assisted extractions (PEAEs) were employed, resulting in high yield and enormous activities instead of organic solvent extractions. A few studies have reported ultrasound sonication, and the mechanical grinding method can be employed as well. PEAEs include pepsin, trypsin and α-chymotrypsin (gastrointestinal enzymes) [37–41]. In recent advancement of technology, electrophoresis combines conventional membrane filters to separate highly charged bioactive peptides, thereby making simpler by means of being quicker for separation through chromatography [42].

4. Application of Microalgal Bioactive Peptides

4.1. Antioxidant Microalgal Peptides

In our human system, the antioxidant plays a major role in reducing oxidative stress reactions. Oxidative stress causes major disease factors and mechanical imbalance among vasoconstrictors and vasodilators in hypertension progression [43]. Reactive oxygen species (ROS) is one of the factor to cause diseases, tumors, ageing, hypertension, diabetes, endothelial dysfunction, and neurological disorders [44,45]. Due to certain ecological conditions, pollution, smoke from chemicals, and an increased diet of fat and alcohol, these disorders can be highly increased. It is essential to look through these natural antioxidants as a shelter and elective hotspot for protection against ROS-induced dieases. Therefore, researchers are finding a new way to alternate dietary food supplements as an excellent resource with potent antioxidant ability and one such revelation state that microalgal seaweeds are a nutritious source with natural antioxidant stability among various marine flora and fauna.

The aromatic and hydrophobic amino acids and functional compounds exhibited antioxidant properties [29,46]. The algal peptide is found with abundance and is a source of antioxidant activity that possesses these amino acid properties represented in Table 1.

Table 1. Microalgae-derived anti-oxidant peptides.

Source of Microalgal Peptides		Enzymatic Treatment	Activity	Reference
Navicula sp.	*N. incerta*	Papain	Cytotoxic effect in HepG2/CYP2E1 cells	[47]
	N. incerta	Pepsin	Shows DPPH superoxide radical quenching activity	[41]
	N. incerta	Papain	Antioxidant in HepG2/CYP2E1 cells	[47]
Chlorella sp.	*C. pyrenoidosa*	Aqueous; Filtration and centrifugation	Cytotoxicity in skin fibroblasts	[48]
	C. vulgaris	Pepsin	Superoxide radical quenching growth inhibition and results in cell cycle arrest in human gastric cell lines	[6,46]
	C. ellipsiodea	Hydrolysis with pepsin	Free radical scavenging activity	[49]
	C. ellipsiodea	Aqueous extraction	It shows moderate free radical scavenging	[50]
Tetraselmis sp.	*Tetraselmis suecica*	Aqueous extraction	Free radical scavenging; H_2O_2 in a monkey kidney cell line	[49]
Spirulina sp.	*Spirulina platensis*	Thermolysin	DPPH radical scavenging activity, peptides exhibited increased antioxidant activity with an IC_{50}	[51]

The bioactive peptides isolated from microalgae such as *Navicula incerta* and *Chlorella* sp. were investigated for its antioxidant capacity mainly on the basis of free radical scavenging, molecules, amino acids, molecular weight, and hydrophobicity [52,53]. *N. incerta* was hydrolyzed using Papain hydrolysates brought about in two antioxidative peptides such as PGWNQWFL and VEVLPPAEL.

Interestingly, these two peptide sequences instigated ethanol to protect the cytotoxic effect in HepG2/CYP2E1 cells due to antioxidant molecules being released by *N. incerta* [47]. The by-product of *C. vulgaris* was reported to contain various free radicals which demonstrated resistance to the intestine; however, there was no cytotoxic effect in human lung fibroblasts [46]. From the peptic hydrolysis of *Chlorella ellipsiodea* a pentapeptide (Leu-Asn-

Gly-Asp-Val-Trp) was identified which impacted on the ability of free radical scavenging activity in kidney cells [49]. Another examination on peptide derived from *C. pyrenoidosa*, reported that antioxidant properties against skin fibroblasts, and also, skin cells treated with UV irradiation demonstrated total cytoprotection within 72 h [48].

Scavenging activity is carried out by *C. ellipsoidea*, which was found to show a unique impact on DPPH free radicals, and antioxidant peptide (LNGDVW) and enzyme-assisted extraction from *Palmaria palmate* (red algae) against hydrogen and H_2O_2 free radicals treated with proteases enzymes [54,55] detailed that *Ulva* sp. has mitogenic hexapeptide amino acids (Glu-Asp-Arg-Leu-Lys-Pro) denominating the purified peptides (SECMA 1) which are active on human foreskin fibroblasts. The synthesis of peptides (ELWKTF) from *Gracilariopsis lemaneiformis* using protein hydrolysates provides the high antioxidant activity, and the significance of the DPPH radical-scavenging activity of these peptides (ELWKTF) shows an EC_{50} value of 1.514 mg mL^{-1} [56]. The extraction and digestion of *Dunaliella salina* can obtain four peptides (i.e., Ile-Leu-Thr-Lys-Ala-Ala-Ile-Glu-Gly-Lys, Ile-Ile-Tyr-Phe-Gln-Gly-Lys, Asn-Asp-Pro-Ser-Thr-Val-Lys, and Thr-Val-Arg-Pro-Pro-Gln-Arg), which were identified to contain 500–1000 Da fraction. Therefore, in vitro studies showed that the *Dunaliella salina* is a highly potent and renewable source that can simulate gastrointestinal digestion [57].

4.2. Anticancer Microalgal Peptides:

The anticancer treatments predominantly adopted to battle malignant growths such as radiotherapy and chemotherapy have been proven to have side effects and develop resistance. In that concern, anticancer bioactive peptides have attracted researchers with their multifunctional potency, sensitivity and stability, and they have shown beneficial activities with the presence of carotenoids, flavonoids, phenolic acids and so on. However, some publications reported that the anticancer peptides from the enzymatic hydrolysis preparation of natural or synthetic peptides derived from food protein sources provide diverse bioactive components, which show anticancer activities [58,59]. A few studies have reported the cytotoxicity on human cancer cells using marine algae-derived peptides, as shown in Table 2.

Table 2. Anticancer peptides using a microalgal source.

Peptide Sequence	Source	Cells	In Vitro/ In Vivo	Reference
Polypeptide VEC	*C. vulgaris*	Gastric cancer Anti-proliferative gastric cancer AGS cells	In vitro	[46]
Polypeptide CPAP	*C. pyrenoidosa*	HepG-2 cells	In vitro	[60]
Polypeptide Y2	*S. platensis*	MCF-7 and HepG-2 cells	In vitro	[61]

Table 2, uncovers the capability of marine algae peptides to prevent and treat tumors, and their significancestudied in detail [62], revealed the strategy of activating apoptotic processes, showing the significance of marine algae-derived anti-cancer peptides. In general, the anticancer peptides from marine sources display various mechanisms to kill cancer cells, including the induction of apoptosis, the imbalance of tubulin-microtubule, and the inhibition of angiogenesis [60]. The two well-characterized pathways of apoptosis are primarily initiated through caspase activation in cells [63].

4.3. Antihypertensive Microalgal Peptides

Hypertension is a silent killer disease and is often identified as a cardiovascular impact, prompting strokes and respiratory failures. Worldwide, there are one billion individuals affected due to the cause of hypertension [64]. The systemic blood pressure increases above 140/90 Hg due to food and environmental factors, leading to complex ailments known as hypertension [65]. Therefore, the mechanism involving vasoconstriction and vasodilatation

induces ROS and balances the systemic blood pressure. Angiotensin-converting enzyme (ACE-1) is a hydrophobic, monomeric amino acid and metalloprotease enzyme which plays a vital role in regulating blood pressure [66]. ACE-1 removes carboxyterminal dipeptide using proteolytic cleavage for the transformation of ACE-I to ACE-II. Therefore, ACE inhibitory peptides reduce the arterial blood pressure and are widely used to control hypertension [52,67,68]. The conversion from ACE to ACE-I, which directly inhibits the renin enzyme, is due to the blockage of reactive oxygen species (ROS) [69]. The determination of ACE inhibitory peptides mainly depends on the structure and composition of the amino acid (Table 3).

Table 3. Microalgal-derived Angiotensin Converting Enzymes ACE inhibitory peptides.

Amino Acid Sequence	Source of Peptides	Treatment	IC_{50} (mM)	Reference
Ile-Val-Val-Glu Ala-Phe-Leu Phe-Ala-Leu Ala-Glu-Leu Val-Val-Pro-Pro-Ala	*C. vulgaris*	Hydrolysis with pepsin; chromatography	315.3 63.8 26.3 57.1 79.5	[70]
Val-Glu-Cys-Tyr-Gly-Pro Asn-Arg-Pro-Gln-Phe	*C. vulgaris*	Hydrolysis with pepsin	29.6	[19]
Val-Glu-Gly-Tyr	*C. ellipsiodea*	Hydrolysis with alcalase	128.4	[49]
Ile-Ala-Glu Phe-Ala-Leu Ala-Glu-Leu Ile-Ala-Pro-Gly Val-Ala-Phe	*S. platensis*	Hydrolysis with pepsin; chromatography	34.7 11.4 11.4 11.4 35.8	[70]
Gly-Met-Asn-Asn-Leu-Thr-Pro Leu-Glu-Gln	*Nannochloropsis oculata*	Hydrolysis with pepsin; chromatography	123 173	[28]

Renin is determined as a single specific enzyme and shows specificity on angiotensinogen. Renin catalyze and controls ROS [46,69] and is also identified as purified peptide sequences, which showed inhibitory activity of ACE-I and stability against gastrointestinal study. In another study, the two species of *C. vulgaris* and *S. platensis* treated with protein hydrolysate for a fraction of peptides sequences, Val-Ala-Phe, Ile-Ala-Pro-Gly, and Ile-Ala-Glu were acquired, and they showed the maximum inhibitory concentration of ACE-1. *Chlorella* sp. derived microalgal sources were similar to study the antihypertensive and antioxidant properties. A few microalgal species, *C. vulgaris*, *C. ellipsiodea, N. incerta*, and *S. platensis*, produce anti-hypertensive peptides. Especially, this pepsin hydrolysate *C. vulgaris* produced a particularly high yield of antihypertensive peptides in both *in vivo* and *in vitro* examination [49,70], and also, isolated *C. ellipsiodea* derived ACE inhibitory Val-Glu-Gly-Tyr peptides in arterial low blood pressure, which shows that the minimum inhibitory concentration of enzymes binds to the active site. Moreover, food-derived bioactive peptides have been widely investigated, and the antihypertensive and anti-oxidative properties are dissimilar.

4.4. Anti-Atherosclerosis Microalgal Peptides

Fatty materials are responsible for the formation of plaques, resulting in atherogenesis, which leads to hypertension and coronary illness as a result of arteries. *C. undeacapeptide* derived VECYGPNRPQF amino acids gradually suppressed the gene expression level of E-selectin, endothelin-1, monocyte attractant chemo protein, E-selectin, vascular cell adhere molecule, and intercellular adhere molecule [71]. The interleukin IL-6 promotes atherogenic cytokine activity that occurs in the underlying stage and the implication in the development of atherosclerosis. The formation of Monocyte Chemoattractant protein MCP-1 in atherosclerosis is indicated by attracting into the subendothelial cell layer on monocytes. Both the IL-6 and MCP-1 produced and increased endothelial cell activation [72]. Two

bioactive peptides of *Spirulina maxima* LDAVNR and MMLD from digestive enzymatic hydrolysis on the intestine were reported to inhibit adhesion molecules by the reduction in the growth regulation of Mitogen –Activated Protein Kinase MAPKs, including P and E-selectin. Additionally, these anti-inflammatory peptides reduce reactive oxygen species production, which releases the inhibition of histamine and IL-8 expression [73]. The origin of atherosclerotic vascular diseases such as MCP-1, ET-1 VCAM-1, and ICAM-1 promotes the stage in the process of inflammation on atherosclerotic lesions in endothelial cells effectively [74,75], and these are listed in Table 4.

Table 4. Microalgae-derived anti-atherosclerotic peptides.

Peptide Sequences	Source	In Vitro/In Vivo	Reference
VECYGPNRPQF	*Chlorella* sp.	In vitro (endothelial cells Svec4-10 and macrophage RAW 264.7 cells)	[71]
NIGK	*Palmaria palmata*	In vitro	[20]
LDAVNR, MMLDF	*S. maxima*	In vitro (EA hy926 cells and U937 cells)	[73]

The key factor responsible for the inflammatory reaction is liable for ROS in the Red Blood Cells RBC activating receptor factor acetyl-hydrolase [76], where the early growth factor is targeted by a few inflammatory genes such as cell molecule adhesion, chemokines, and cytokines, etc. [77]. Thus, a derived form of microalgal peptides could be correlated with target molecules such as a reduction in the cell molecule, activating the red blood cell factor, the inhibition of acetyl hydrolase, and the histamine factor treated with anti-inflammatory reactive oxygen species of atherosclerosis.

4.5. Immuno-Modulatory Microalgal Peptides

The genetic engineering of microalgal bioactive peptides is an option to produce antibodies, pharmaceutical agents, and other valuable products such as vaccines, hormones, blood-clotting factors, growth factors, and anticancer and immune regulators [78]. The immune system activates immune cells to fights against antigens to eradicate the foreign substances, pathogenic agents, and pollutants [79]. Immunomodulating bioactive peptides promote the functioning of immune cells as sustained by increased leucocytes and induced cytokines. Immuno peptides regulate lymphocyte proliferation and also increase the phagocytic activity of macrophages in the human system [80,81]. Similarly, it is reported that *C. vulgaris* derived protein hydrolysate reported that both are inherited, and its specific immune function increases the lymphoblast, which develops T-lymphocytes and replenishes the delayed action of hypersensitivity. From the perception, the immunomodulatory mechanism of microalgae-derived peptides or proteins represented (Table 5) is prevalently associated with the pathway of T-lymphocyte and B-lymphocyte cytokine regulation [18,35,81]. A nucleotide-peptide complex derived from *C. vulgaris* possesses a factor for cell growth, promotes tissue repair, and has the capability of recovering the body [82].

Table 5. Microalgae-derived Immuno-modulating peptides.

Peptide Name or Sequence	Source	In Vitro/In Vivo	Reference
Protein hydrolysates	*C. vulgaris*	In vivo in mice	[18]
Protein hydrolysates	*Ecklonia cava* (Macroalgae)	In vivo in mice	[81]
Protein hydrolysates	*Porphyra columbina* (Macroalgae)	In vivo in rats	[35]

4.6. Anti-UV Radiation and Antiosteoporosis Microalgal Peptides

Exposure to solar ultraviolet (UV) causes harmful effects on the epithelial layer of the skin and induces progeria. The mechanism, which includes the protein–protein interaction, increases metalloproteinase expression, the degradation of collagen, increased cysteine residues, and chemoattractant monocyte protein [83]. Cysteine residues are extracellularly abundant and are associated with, signaling the molecule which represses the synthesis of procollagen by reducing the receptor transform growth factor receptor II, where the up-regulation of the metallo-matrix protease enzyme by protein–protein interaction [84] reported that the aqueous extraction from *Chlorella* sp. has the ability to reduce the production of metalloproteinase1, gene expression, proteins, and the increasing mRNA expression of procollagen, followed by Solar ultraviolet exposure and preventing ultraviolet radiation which suppresses the gene expression of elastin protein. In biotechnological applications of *Chlorella*-derived bioactive peptides, a handful of studies showed that anti-ultraviolet irradiated to the epithelial layer on the fibroblasts' skin after solar ultraviolet irradiation reduced [22].

Nowadays, osteoporosis is a type of vitamin deficiency that causes widespread human skeletal disorders, and it is an important health issue around the globe [85]. There are numerous signal transduction pathways involved in bone-forming cells [86]. At this time, osteoblastic cells are activated and highly expressed metalloprotein kinase cascades are activated with different markers, osteocalcin collagen I and alkaline phosphatase [87]. The transcription factors for various target genes (Smad1, Smad5, and Smad8) become phosphorylated, which regulates and interacts with activated bone morphogenetic protein receptors and forms a complex with Smad4 [88,89], identifying the purified *Nannochloropsis oculata* osteoblast-differentiator peptide (MPDW) from the application of algal by-products. Hence, the increased phosphorylation of peptides are expressed on metalloprotein kinases and smads. The microalgae-derived peptides and anti-UV radiation on antiosteoporosis are shown in Table 6.

Table 6. Microalgae-derived anti-UV radiation and antiosteoporosis peptides.

Peptide Name or Sequence	Source	In Vitro/In Vivo	Reference
Chlorella derived peptide	*Chlorella* sp.	In vitro studies in skin fibroblast of 966SK	[22,90]
MPDW	*N. oculata*	In vitro studies in human osteosarcoma and murine mesenchymal stem cell	[89]

4.7. Anti-Microbial/Anti-Bacterial Microalgal Peptides

The antibacterial/antimicrobial activity comprises short-chain peptides (50–100) in the form of complex proteins, and its net charge 3^{+}, which kills microbial pathogens with fewer chances of developing resistance, has been exhibited in some of the recent studies. The Antimicrobial peptides AMPs play a vital role in protection against infection [91]. This microbial cellular process propagated with bacterial cytoplasmic membrane has an amphipathic nature, which binds with both polar and non-polar activate sites [92,93]. Some of the peptides such as aeruginosins, aeruginoguanidins, aeruginosamides, microginins, microviridins and kasumigamide produced by *Microcystis aeruginosa* are highly toxic due to their toxins such as hepatotoxin cyclic peptide and alkaloid neurotoxin [94].

In vivo and in vitro studies suggested that *Spirulina* sp. controlled antimicrobial/antibacterial effect. The phenolic extraction of *Spirulina* sp. inhibits *Fusarium graminearum* growth, and the production of mycotoxins was also studied elsewhere [95]. Interestingly, microalgae-derived esters also showed significant antimicrobial and antioxidant properties [96]. The *S. platensis* antibacterial peptides showed Minimum inhibitory concentrationMIC with *Escherichia coli* and *Staphylococcus aureus.* Therefore, *S. platensis* were investigatd and found a potentially promising antimicrobial agent. The overall signif-

icance and the mechanism involved in the microalgal bioactive peptides are listed in Table 7 [97–102].

Table 7. Significance of microalgal bioactive peptides.

S.No	Peptide Sequences	Source	Mode of Preparation	Mechanism and Applications	References
1.	VECYGPNRPQF	*C. vulgaris*	Pepsin hydrolysate	Peptides prevent cell death due to the cause of hydroxyl radicals and also protect DNA damage. No cytotoxicity effect in human lung fibroblast cell line (WI38). It promotes gastrointestinal digestion.	[46]
2.	Val-Glu-Cys-Tyr-Gly-Pro-Asn-Arg-Pro-Gln-Phe	*Acaudina molpadioidea*	Bromelain and alcalase	Peptide inhibiting the activity was increased with gastrointestinal proteases.	[97]
3.	VECYGPNRPQF	*C. vulgaris*	Pepsin hydrolysates	Inhibits the growth and also promotes cell cycle arrest in human gastric cell lines.	[19]
4.	Pro-Gly- Trp-Asn-Gln-Trp-Phe-Leu—Val-Glu-Val-Leu-Pro-Pro-Ala-Glu-Leu	*N. incerta*	Papain hydrolysates	Cytotoxic effects in HepG2/CYP2E1 cells.	[15]
5.	Gly-Met-Asn-Asn-Leu-Thr-Pro-Leu-Glu-Gln	*N. oculata*	Protein hydrolysate	Hydrolysate fractions on human umbilical vein endothelial cell line (HUVECs).	[28]
6.	Ile-Ala-Glu, Phe-Ala-Leu, Ala-Glu-Leu, Ile-Ala-Pro-Gly and Val-Ala-Phe	*S. platensis*	Enzymatic hydrolysis	Antioxidant activity, antihypertensive activity, antimicrobial activity, antidiabetics activity and anti-obesity activity.	[98]
7.	WPRGYFL, GPDRPKFLGPF, WYGPDRPKFL, SDWDRF	*Tetradesmus obliquus*	Protein hydrolysate	Identification of antioxidant and ACE inhibitory peptides.	[99]
8.	GIVAGDVTPI	*S. platensis*	Enzymatic hydrolysis	Exerts an antihypertensive effect.	[100]
9.	Asp-Ala-Glu	Porphyra columbina	Protein extract hydrolysate	Immunosuppressive effect on rat splenocytes and also enriched IL-10 production. Protein extract hydrolysate gives high immunosuppressive effect, antihypertensive effect and antioxidant capacity.	[35]
10.	PHA,PHP,PHS	*Arthospira maxima* OF15	Enzymatic hydrolysis	Antioxidant, anti-hyluronase, anticollagenase, and anti-inflammatory activity.	[101]
11.	FGMPLDR MELVLR	*U. intestinalis*	Protein hydrolysates	ACE inhibitors with IC50 values of 219.35 µM were obtained.	[102]
12.	ELWKTF	*Gracilariopsis lemaneiformis*	Enzymatic hydrolysis	Glu-Lys is reliable for more antioxidant peptides, ELWKTF established from G. lemaneiformis. This marine algae gives a natural source as a novel antioxidant peptide.	[56]

5. Isolation and Production of Microalgal Peptides Using Different Technology

5.1. Enzyme-Assisted Extraction Process (EAE)

Microalgal peptides are derived through the protein and enzymatic hydrolysis process. The microalgal peptide-derived enzymatic hydrolysis is extensively used to increase the nutritional and functional values of foods [103]. The cell wall of the microalgal peptides made up of chemically complex heterogeneous molecules contains Ca+ and K+, which have sulfated and branched polysaccharides [104]. This enzymatic hydrolysis is used to break down the larger complex polypeptide molecule into smaller fragment pieces with a different arrangement of amino acid residues. Generally, the microalgal peptides provide molecular size reduction, free amino-acid sequence, and their composition [28]. Enzyme assisted extraction is low-cost, environmentally friendly, non-toxic, yield, catalytic, and

bioactivity for the extraction process [105], revealing that the importance of the enzyme assisted extraction process is to maintain the optimum temperature and pH for enzymes for high yield. Compared to the other conventional extraction methods, this enzyme-assisted extraction has obtained higher antioxidant activity [106]. Eventually, this method is useful for the extraction of the bioactive compound and provides a better yield of peptides in our further process.

5.2. Subcritical Water Extraction Process (SWE)

Apart from enzyme assisted extraction, the novelty of this subcritical water extraction (SWE) or pressurized liquid extraction (PHE) is a simple step method that is mainly used in the food industry for the production of different bioactive peptides which promote human health function. SWE is an easy, eco-friendly, and high energy hydrolysis technique [107]. Subcritical water hydrolysis is an advanced technique which provides amino acids and protein from vegetables and animal matter [108]. At an industrial level, this subcritical water technology is mainly applicable, and the cell extraction efficiency is 30- to 167-fold increased significantly [109]. It works at the temperature of 50–200 °C, an pressure of 50–300 psi, and an time of 5–10 min, when a small amount of organic solvent used. According to the published report, this extraction of a bioactive peptide is a most promising technique. While applying high temperature and pressure through extraction, the water has been changed due to the physical and chemical properties [58]. Another study indicates that a higher level of amino acid was degraded due to the compression of hot water treatment at 240 °C, and the concentration of amino acid was higher at 220 °C [110].

5.3. Recent Technological Microalgal Peptide Productions

There is a significant challenge for the synthesis of the industrial scale-up production of microalgal bioactive peptides. Regarding this study, there is an opportunity to produce a larger amount of microalgal bioactive peptides using the biotechnological technique. However, in industrial scale-up, the chemical method is highly expensive and not appropriate for biomass production. The greater advantage of this enzymatic hydrolysis is that it is a common and traditional method for the synthesis of microalgal peptides which contain membrane enzyme reactor systems, and it allows a fast reaction, low cost, increased yielding capacity, and pure byproducts in industrial-scale production [31]. Other than enzymatic hydrolysis, proteolytic enzymes are commonly used for the production of microalgal bioactive peptides (Table 8) [111–120]. The microbe-derived enzymes are Alcalase, Neutrase, and Flavourzyme. This protein hydrolysis method is the most crucial factor to determine the peptides' molecular weight and biofunctional activities on microalgal-derived peptides [121]. Additionally, more anti-oxidant protein peptides from beans have been isolated using enzymatic hydrolysis, as recently reported [122]. In addition, peptide extraction and the purification of peptides were separated by nanofiltration, electrodialysis with an ultra-filtration membrane (EDUF). Electrically driven forces were differentiated between mass flux and mass with ultra-filtration membranes. This EDUF provides better results to obtain more peptides [123]. Nonetheless, chromatography is also used to purify bioactive peptides. However, there is a limitation to using the chromatographic techniques dealing small peptides [124–126]. Fluorescence lifetime imaging (FLIM) is one of the techniques to investigate the interactive and non-interactive fractions on the binding, confirmation, and composition of a biological compound in the live cell. Hence, this is an inflexible technique determining physical interaction and cellular processing in the bioactive compounds. The mechanism and the interactions have been incredibly expanded to the antimicrobial peptides with bacterial cells [109,127].

Table 8. Protein extraction method from microalgae.

Protein Extraction Methods	Types		Reference
Conventional protein extraction methods	Enzymatic hydrolysis		[58,112]
	Physical Process		[36,113]
	Chemical extraction		[105,114]
Current extraction methods	Ultrasound-Assisted Extraction		[115,116]
	Pulsed Electric Field		[117,118]
	Other methods	Microwave assisted extraction (MAE)	[111,119]
		Subcritical wave extraction (SWE)	[116,120]
		Supercritical fluid extraction (SFE)	[120]

Nevertheless, FLIM not only examined the interaction and molecular changes in detail. Currently, in silico and OMICS technology such as transcriptomics, metabolomics and proteomics are being studied. By applying 2D electrophoresis, this can be used to separate proteins based on the isoelectric point and molecular weight in proteomics studies [128]. EAE and SWE are the best methods to implement for the extraction of microalgal peptides. On the other hand, these lactic acid bacteria produced bioactive peptides from fermentation extraction. Simultaneously, this EDUF technology isolated bioactive microalgal peptides which were employed in a mixture of complex peptides. Under another method, FLIM and in silico (proteomics and transcriptomics) finally exhibit the mechanism and action of bioactive peptides.

Considering all the extraction methods above, the synthesis of microalgal bioactive peptides displays diverse bioactivities of functional food, pharmaceuticals, nutraceuticals, and cosmeceuticals, despite the fact that there is a need to change the preparation of microalgal peptides using innovative techniques for scale-up. Upcoming research in this field is very advanced facilitating the investigation of the products of microalgal peptides in the gastrointestinal transit, and to eventually interpret the elaborated significance of in vivo studies. In the future, the market for bioactive peptides could be industrially scaled-up.

6. Conclusions

Recently, studies have reported that algal-derived biologically active peptides play a major part in health and the environment. The enzymatic hydrolysis of microalgal-derived bioactive peptides is a chance to design new biofunctional foods, pharmaceuticals, and cosmeceuticals. This survey reports the utilization of microalgal peptides as antioxidant, anti-cancerous, anti-hypersensitive, anti-atherosclerotic, and nutritional functional food. It also explores novel technologies for the production of active peptides. For instance, the use of algal peptides as food for human health found limitations, such as the sensitivity of peptides to hydrolysis protease degradation. This review emphasized the issue of active peptides in gastrointestinal transit, which has to be unraveled in the future.

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