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Review

Microalgal Proteins and Bioactives for Food, Feed, and Other Applications

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Featured Application: This review paper details current production of microalgae globally and isolated microalgal proteins and peptides and their associated health benefits, as well as details concerning microalgal lipids, vitamins and minerals. The use of microalgae in feed is discussed, along with potential uses in other applications such as cosmetics and functional foods.

Abstract: Microalgae are a known source of proteins, prebiotics, lipids, small molecules, anti-oxidants and bioactives with health benefits that can be harnessed for the development of functional foods, feeds, cosmeceuticals and pharmaceuticals. This review collates information on the supply, processing costs, target markets and value of microalgae, as well as microalgal proteins, lipids, vitamins and minerals. It discusses the potential impact that microalgae could have on global food and feed supply and highlights gaps that exist with regards to the use of microalgal proteins and ingredients as foods and supplements.

Keywords: microalgae; protein; bioactive peptides; lipids; prebiotics; feeds

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1. Introduction

According to the United Nations, the world population will be 9.7 billion people by 2050, and to meet its needs, the amount of food produced must be doubled [1]. Nowadays, more than 3.5 million deaths are caused by maternal and child malnutrition annually. According to the World Health Organization (WHO), around 45% of child mortalities are caused by malnutrition, and over one billion people have inadequate protein intake [2]. For these reasons, there is a need to find new, nutrient-rich protein sources [3]. Microalgae are a diverse group of microorganisms known as phytoplankton and their classification is under constant revision due to new genetic evidence [4]. Nevertheless, the majority of microalgae are unicellular, photosynthetic microorganisms producing oxygen and assimilating carbon dioxide by obtaining macro- and micronutrients from aquatic environments. The term 'microalgae' applies to both eukaryotic microalgae and prokaryotic cyanobacteria [5] and they play an important role in the marine food chain as the primary source of omega-3 fatty acids [6]. Microalgae can survive in wastewater, ocean water and brine water; they have a high growth rate and productivity, they do not compete with agricultural land and they have high CO_2-fixing efficiency [7,8]. Although the number of microalgae species was estimated to be 200,000 (according to AlgaeBase, current number of known species is about 160,000, www.algaebase.org (accessed on 11 December 2021), only 30,000 species are studied currently. A few microalgae species are known to have been consumed since ancient times—these include *Arthrospira platensis* (Lake Chad) [9], *Arthrospira maxima* (Lake Texcoco) [10], *Nostoc commune* (China) [11], *Nostoc flagelliforme* (China) [12] and *Aphanothece*

sacrum (Japan) [13]. The first large-scale production of *Chlorella vulgaris* started at Massachusetts Institute of Technology in 1951 [14], followed by the production of *Arthrospira* sp. initiated in 1973 by Sosa-Texcoco Ltd. in Mexico [15].

Microalgae species currently cultivated in large volumes include *Arthrospira* spp. (world annual production 5000 tons of DW), *Chlorella* spp. (world annual production 2000 tons of DW), *Nannochloropsis* spp. and *Haematococcus pluvialis* [16,17]. Asia and Australia produce the largest volumes of microalgae for the food and feed sectors, and production by European companies is currently estimated at around 5% of the global market [16]. According to Araújo and colleagues, annual microalgae production in Europe is estimated at 182 tons of microalga dry mass produced by 167 companies and 142 tons of dry mass *Arthrospira* spp. produced by 222 companies. The largest producers of microalgae in Europe are Germany, France, Italy, Spain and Portugal. In the last decade, an increase of 150% in growth was observed for the number of new algae producing companies in existence [17]. However, several factors limit the potential of the European microalgae market, including insufficient domestic demand for microalgae-based products and difficulties in achieving the commercial authorization of microalgae production in the EU. The market value of microalgae biomass depends on the production system and production costs, place of origin, certifications (e.g., organic production) and step in the value chain (Business to Business (B2B) or Business to consumer (B2C) segment). The business to consumer (B2C) value for some microalgae such as *Chlorella* sp. and *Spirulina* spp. was estimated at 150 and 280 EUR/kg of DW, respectively, and for *Nannochloropsis* sp. (the most relevant species for feed) the B2C market value can go to 1000 EUR/kg microalgae DW [17].

Microalgae are often produced and used in feeds and foods due to their high lipid content; however, they are also a rich source of sustainable protein that may be suitable for human and animal consumption. In general, microalgae produce large amounts of protein when they are cultured under non-stress conditions [18], in contrast with the over-accumulation of lipids and carbohydrates induced by stress conditions such as high salinity or nitrogen starvation [19]. In some microalgae species, including *Arthrospira* sp., *Chlorella* sp., *Scenedesmus* sp. or *Synechococcus* sp., the total protein content may exceed 50% [20]. The production of microalgae proteins requires the development of feasible and robust extraction techniques. To improve the efficiency of protein extraction, cell disruption using physical, chemical or enzymatic methods is frequently used prior to the protein extraction process. Mechanical and non-mechanical methods can be applied, e.g., the use of high pressure, bead milling, lytic enzymes, microwaves or chemical solvents [20]. The aim of this review is to summarize the current state-of-the-art of microalgae use, the methods of protein isolation from microalgae biomass and legislative regulations in Europe and the United States for the use of microalgae biomass in food.

1.1. Microalgae for Food and Functional Food Applications

Nowadays, most microalgae biomass is produced, and components extracted from microalgae, including omega-3 fatty acids, phycocyanins, carotenoids, peptides, enzymes and vitamins, are used in food supplements, food additives or for their health benefits in nutraceuticals or functional foods. The biomass compositions of commercially important microalgae species are summarized in Table 1 [3,21]. The components that contribute to the potential health benefits of microalgae include proteins and peptides, lipids and fatty acid methyl esters (FAMEs) and small molecules with antioxidant, anti-inflammatory and a myriad of other reported bioactivities [8].

Table 1. Compositional analysis of commercially available microalgae.

Species	Proteins [% DW]	Lipids [% DW]	Carbohydrates [% DW]
Arthrospira platensis	53–70	6–20	12–24
Chlorella vulgaris	49–55	3–36	7–42
Dunaliella salina	57	32	6
Haematococcus pluvialis	48	15	27
Nannochloropsis oceanica	29	19–24	32–39
Nannochloropsis sp.	29–32	15–18	9–36
Schizochytrium sp.	12	32	38–71

1.1.1. Proteins and Peptides

Microalgae are a rich source of proteins, which can make up to 70% of the biomass dry weight for some species. Well-known, protein-rich microalgae species include *Arthrospira*, *Chlorella*, *Aphanizomenon* and *Nostoc* [22]. Generally, microalgae proteins have a balanced total amino acid (TAA) profile and contain all of the essential amino acids (EAA). According to the FAO and WHO, amino-acid profiles of proteins extracted from *Arthrospira* correspond to those recommended for human consumption [23]. The factors which have to be considered in order to evaluate the suitability of microalgae proteins for human consumption include TAA and EAA content and protein digestibility, bioaccessibility and bioavailability. The cellulosic wall of most microalgae species may interfere with nutrient utilization if consumed. To assess protein quality, several methods are recommended, including the Protein Digestibility Corrected Amino Acid Score (PDCAAS) and the Digestible Indispensable Amino Acid Score (DIAAS) methods. Table 2 lists the PDCAAS values, protein content and cell wall composition for several microalgae consumed as foods, feeds or functional foods today. Quality protein sources such as egg, whey and soy have reported PDCAAS values in the range of 0.9–1.0 [24]. Unfortunately, no information about the DIAAS values of microalgae biomass or microalgae protein products for human foods is currently available [25]. However, high in vivo DIAAS values ranging from 1.0 to 3.6 were recently reported for dry intact-cell meal produced from *Pavlova* sp. biomass used to feed juvenile Atlantic salmon [26].

Table 2. Reported protein content, PDCAAS values and cell wall composition for well-known microalgae species.

Species	Protein [% DW]	PDCAAS	Cell Wall Composition
Arthrospira platensis	53–70 [21]	0.84 [27]	Peptidoglycan + outer membrane [28]
Chlorella sorokiniana	50 [29]	0.81 [29]	Glucosamin, rhamnose [30]
Chlorella vulgaris	54 [29]	0.77 [29]	Cellulose [31]
Dunaliella salina	57 [21]	n/d	No cell wall, glycocalyx-type cell covering [32]
Haematococcus pluvialis	48 [21]	n/d	Cellulose, mannan [33]
Isochrysis galbana	29 [34]	n/d	No cell wall [35]
Nannochloropsis gaditana	20–45 [36]	n/d	Cellulose (inner wall) + outer hydrophobic algaenan layer [37]
Nannochloropsis oculata	35 [34]	n/d	Cellulose [38]
Pavlova lutheri	29 [34]	n/d	Cellulose, hemicellulose [38]
Scenedesmus obliquus	50–56 [21]	n/d	-
Schizochytrium sp.	12 [39]	n/d	Galactose [40]

Table 2. *Cont.*

Species	Protein [% DW]	PDCAAS	Cell Wall Composition
Tetraselmis suecica	31 [34]	n/d	Polysaccharides (high content of 3-deoxy-d-manno-oct-2-ulosonic acid, galacturonic acid, galactose) [41]

n/d = not determined.

Meat and whey proteins are known to have PDCAAS values closer to 1 and are considered complete protein sources because of their amino acid content and digestibility (as measured using PDCAAS) values. The PDCAAS values found to date for selected microalgae (Table 2) are lower than 1, and this may result from the anti-nutritional factors present in microalgae, including the constituents of the algal cell walls, which may bind to available protein in microalgae when algae are consumed, preventing their complete digestion. Proteins also contribute to the rheological and stability properties of microalgae during manufacture and storage. In terms of health, these proteins are also a source of bioactive peptides with a wide range of different health effects when consumed [42]. Many microalgae species also produce commercially attractive enzymes with a wide range of potential uses, for example, enzymes with antioxidant activity including superoxide dismutase, catalase and peroxidase activities [43].

Peptides are short sequences of amino acids between two and thirty in length, with mass values less than 10-kDa [44]. They provide a health benefit to the consumer that goes above and beyond basic, human nutrition. Microalgae peptides can be generated using enzymes or are native and encoded from the algae genome. Both peptide types are associated with a wide range of hormone-like, biological activities [45]. The use of bioactive peptides in pharmacology was first described in 1950, when peptides of dairy origin were shown to enhance bone calcification in rachitic infants [46]. Table 3 lists the species of microalgae from which peptides with significant antimicrobial, antioxidant, anti-inflammatory, anti-hypertensive, and anti-atherosclerotic properties have been derived to date using enzymatic hydrolysis methods.

Table 3. Reported microalgae-derived peptides generated using enzymes found to have biological activity.

Species	Enzyme Used for Hydrolysis	Peptide	Effect	References
Arthrospira maxima	Trypsin, chymotrypsin, and pepsin	LDAVNR MMLDF	Anti-inflammatory	[47]
Arthrospira platensis	Thermolysin	FSESSAPEQHY	Antioxidant	[48]
Arthrospira platensis	Trypsin	n/d	Antitumor	[49]
Arthrospira platensis	Pepsin	IAE FAL AEL IAPG VAF	ACE-1 inhibitory	[50]
Chlorella ellipsoidea	Pepsin	LNGDVW	Antioxidant	[51]
Chlorella pyrenoidosa	Papain	n/d	Antitumor	[52]

Table 3. *Cont.*

Species	Enzyme Used for Hydrolysis	Peptide	Effect	References
Chlorella pyrenoidosa	Trypsin, pepsin	FLKPLGSGK QIYTMGK LFVAEAIYK QHAGTKAK	ACE-inhibitory DPP-IV inhibitory	[53]
Chlorella pyrenoidosa	Pepsin, flavourzyme, alcalase, and papain	VECYGPNRPQF	Ant-inflammatory Anti-atherosclerotic	[54]
Chlorella sorokiniana	Pepsin, mixture of proteases	n/d	DPP-IV inhibitory ACE-1 inhibitory Antioxidant	[55]
Chlorella vulgaris	Pepsin	VECYGPNRPQF	Protective effect on DNA Antioxidant	[56]
Chlorella vulgaris	Pepsin	IVVE AFL FAL AEL VVPPA	ACE-1 inhibitory	[50]
Isochrysis zhanjiangensis	Chymotrypsin	NDAEYGICGF	Antioxidant	[57]
Nannochloropsis oculata	Alcalase	LVTVM	ACE-inhibitory	[58]
Nannochloropsis oculata	Pepsin	GMNNLTP LEQ	ACE-inhibitory	[59]
Navicula incerta	Papain	n/d	Cytoprotective effect Antioxidant	[60]
Navicula incerta	Alcalase neutrase, pepsin, papain, trypsin, pronase-E, α-chymotrypsin	n/d	Antioxidant	[61]
Tetradesmus obliquus	Alcalase	WPRGYL GPDRPKFLGPF WYGPDRPKFL SDWDRF	Antioxidant ACE-1 inhibitory	[62]

n/d = peptide sequences not characterised.

1.1.2. Lipids

Microalgae are an excellent source of the polyunsaturated fatty acids (PUFAs) omega-3 and omega-6, as well as sterols [63]. Humans and mammals lack the delta-12 and delta-15 desaturase enzymes, which have the ability to convert oleic acid into linoleic and α-linoleic acids. Because of this, it is essential to include PUFAs in sufficient amounts (males and females 0.25 g of EPA and DHA daily) in the human diet [64]. Microalgae species such as *Nannochloropsis gaditana*, *Nannochloropsis oculata*, *Pavlova lutheri*, *Phaeodactylum tricornutum* and *Tetradesmus pseudonana* are an excellent source of eicosapentaenoic acid (EPA), while others such as *Schizochytrium* sp., *Isochrysis* sp. and *Pavlova lutheri* are rich in docosahexaenoic acid (DHA). Arachidonic acid is found in *Parietochloris incisa*; gamma linoleic acid is found in *Arthrospira* sp. as well as stearidonic acid [8]. Enhanced PUFA levels can be

achieved by applying various types of abiotic stress to microalgae during cultivation. The methods used to increase PUFA levels in microalgae include nutrient depletion, commonly known as nitrogen starvation, or adjusting salinity, pH and temperature conditions [65,66]. The percentage content of PUFAs found within the fatty acids and dry biomass of selected microalgae is summarized in Table 4.

Table 4. Content of PUFAs found in common microalgaespecies.

Species	Omega-3 [% of FA]	Omega-3 [% of DW]	References
Isochrysis galbana	EPA 25	EPA 5.3	[67]
Nannochloropsis oculata	EPA 20	EPA 8.3	[68]
Pavlova lutheri	EPA 12	EPA 2.3	[69]
Phaeodactylum tricornutum	EPA 20	EPA 7.7	[70]
Cryptheconidium cohnii	DHA 44	DHA 5.8	[71]
Schizochytrium sp.	DHA 43	DHA 11	[72]
Species	**Omega-6 [% of FA]**	**Omega-6 [% of DW]**	**References**
Arthrospira platensis	GLA 20–23	-	[73]
Porphyridium purpureum	ARA 24	AEA 0.8	[74]

Arachidonic acid (ARA); gamma-linolenic acid (GLA); anandamide (AEA).

Microalgae lipids also have potential for use in biofuel production [65]. In addition, sterols can find applications in pharmaceuticals due to their ability to lower blood cholesterol [75]. The main microalgae producers of sterols are *Isochrysis galbana*, *Tetraselmis suecica*, *Phaeodactylum tricornutum* and *Pavlova lutheri* [63].

1.1.3. Carbohydrates

Carbohydrates make up approximately 20% of microalgae biomass and usually accumulate in the form of starch or other polysaccharides, including β-glucans, sulfated polysaccharides and exopolysaccharides [8]. Nowadays, fermentable polysaccharides including starch, which is the main storage polysaccharide in microalgae, or cellulose, which is the main polysaccharide constituent in the microalgae cell wall, are widely explored for use in bioethanol and biofuels production [8]. Microalgae species known for their high carbohydrate contents and evaluated as feasible for biofuel production include the species *Porphyridium cruentum*, which has a carbohydrate content of between 40–57%, and *Spirogyra* sp., which has a carbohydrate content of 33–64% [76]. *Chlorella* sp. have carbohydrate contents of 50% of the dry mass of the alga [77]. Moreover, some polysaccharides and oligosaccharides from *Arthrospira* sp., *Nostoc* sp. and *Chlorella* sp. were looked at previously for their prebiotic effects [8]. The types of polysaccharide found within the biomass of selected microalgae and their potential applications are summarized in Table 5.

Table 5. Reported prebiotic potential of microalgae derived polysaccharides and oligosaccharides.

Species	Carbohydrate	Application	References
Arthrospira platensis	Sulfated polysaccharides—exopolysaccharides/glycogen	Antibacterial and antioxidant activity	[78]
Arthrospira platensis *Dunaliella salina* *Porphyridium* sp.	Polysaccharides	Plant bio-stimulants	[79]
Arthrospira platensis	Extracellular polysaccharides—exopolysaccharides/glycogen	Prebiotic/stimulate growth of *Lactobacilli*	[80]

Table 5. *Cont.*

Species	Carbohydrate	Application	References
Chlorella sp.	β-1,3-glucan	Immuno-stimulator, antioxidant, reduce blood lipid levels, thickener in the food industry	[81]
Phaeodactylum tricornutum	Mannose	Alternative to antibiotics, prebiotic effect	[8]
Porphyridium sp.	Sulfated polysaccharides	Thickening/lubrication agent	[82]

1.1.4. Pigments

The presence of pigments such as chlorophylls, phycobilins and carotenoids in microalgae is essential for light harvesting and stress mitigation. The key pigments produced in relation to light harvesting in microalgae include the chlorophylls, absorbing light mainly from the blue and red spectrum of light [83]. As a response to environmental stress, such as oversaturation of light intensity, high salinity or nitrogen limitations, photo-protective carotenoid pigments such as β-carotene, astaxanthin, zeaxanthin, lutein, canthaxanthin, fucoxanthin and lycopene are overproduced [84]. The most important commercial producers of carotenoids are the species *Dunaliella salina*, which produces β-carotene, and *Haematococcus pluvialis*, which produces astaxanthin. *Dunaliella salina* can accumulate up to 14% β-carotene of its DW, and *Haematococcus pluvialis* can accumulate up to 5% astaxanthin of its DW [85]. Carotenoids find application as food colorants, additives for aquaculture feed and, most recently, in cosmetics and pharmaceuticals for their anti-ageing, anti-inflammatory and anticancer properties [86]. Phycobilins as phycocyanin that are produced by *Arthrospira* sp. and phycoerythrin produced by *Porphyridium* sp. and *Rhodella* sp. are another important group of antenna pigments. At present, phycobilins are mainly used as natural food colorants, antioxidants and fluorescent agents [8].

1.1.5. Vitamins

Some species of microalgae contain high levels of different water and lipid-soluble vitamins, including vitamins A, B-complex, C, D2, D3, E and K [8]. *Nannochloropsis oceanica* is an excellent source of vitamin D [87], *Tetraselmis suecica* and *Dunaliella tertiolecta* contain high amount of vitamin E [88] and some microalgae including *Chlorella* sp. and *Dunaliella salina* accumulate vitamin C in considerable amounts. *Chlorella* sp. was also mentioned as a source of vitamin B12 [89]. Some studies show that the active form of vitamin B12 is normally not presented in microalgae because it is synthetized from pseudocobalamin; however, they can accumulate B12 from the aquatic environments where they are cultivated [90]. Vitamins found within the biomass of selected microalgae in high, significant amounts are summarized in Table 6.

Table 6. Reported vitamin levels in microalgae species.

Species	Vitamin	Vitamin Recommended Daily Allowance (RDA)	Vitamin [mg/100 g DW]	References
Arthrospira sp.	A	800 µg	0.34	[91]
Chlorella sp.	A		30.77	[91]
Arthrospira sp.	B3	18 mg	12.8	[91]
Chlorella sp.	B3		23.8	[91]
Arthrospira sp.	B9	200 µg	0.094	[91]
Chlorella sp.	B9		0.094	[91]

Table 6. *Cont.*

Species	Vitamin	Vitamin Recommended Daily Allowance (RDA)	Vitamin [mg/100 g DW]	References
Arthrospira sp.	C	60 mg	10.1	[91]
Chlorella sp.	C		10.4	[91]
Dunaliella salina	C		2500	[92]
Nannochloropsis oceanica	D3	5 µg	0.1	[87]
Tetraselmis suecica	E	10 µg	108.0	[88]
Anabaena cylindrica	K1	120 µg	20.0	[93]

1.2. Microalgae for Feed Applications

The use of microalgae biomass in animal feed dates to the 1950s and it is considered an effective way to include valuable nutrients and vitamins, EAAs, PUFAs, polysaccharides, minerals and pigments into feed to increase its nutritional value [3]. Currently, about 30% of total microalgae biomass produced globally is used as feed, and approximately half of this consists of *Arthrospira* sp. biomass [94]. The incorporation of microalgae into feed can benefit the animal's physiology by improving their immunity and disease resistance, as well as through stimulation of probiotic bacteria in the gut/rumen. Other benefits described include reproductive performance, improvements of feed conversion ratios and improvement in the meat quality of pigs, rabbits, poultry and ruminants. However, the findings of different studies are highly influenced by the microalgae biomass composition and the amount included in the diets of animals [3]. Interestingly, Madeira and colleagues claim that the efficiency of microalgae biomass incorporation into the diet of mono-gastric animals is improved by the simultaneous addition of carbohydrate-active enzymes as feed additives [3]. In fish aquaculture, microalgae are used to feed larvae, and the main species used include *Nannochloropsis oceanica*, *Chlorella vulgaris*, *Isochrysis galbana*, *Pavlova* sp., *Phaeodactylum tricornutum*, *Tetraselmis suecica*, *Skeletonema* sp., *Thalassiosira* sp. and *Haematococcus pluvialis* [16]. Astaxanthin extracted from *Haematococcus pluvialis* is widely used in salmon aquaculture as it gives salmon its typical "pink" color desired by the consumer [95]. Effects of the inclusion of microalgae biomass into the feed of different animals are summarized in Table 7.

Table 7. The effects of microalgae inclusion in the feed diet of different animals (ruminants, fish and mono-gastric).

Species	Animal, Duration of Experiment	Content of Microalga in Diet	Findings	References
Arthrospira platensis	Lambs 6 weeks	10–20%	Increase of weight (10%)	[96]
Chlorella sp.	Broiler chicks 4 weeks	1%	Increase of average daily gain (ADG)	[97]
Haematococcus pluvialis	Rainbow trout 30 days	0.3%	Decreased serum glucose, Triglycerides (TAG) and cholesterol levels	[98]
Isochrysis galbana	*Silver fish* 80 days	4.5–5%	Increased fish growth performance Increased content of omega-3 fatty acids	[99]

Table 7. *Cont.*

Species	Animal, Duration of Experiment	Content of Microalga in Diet	Findings	References
Nannochloropsis oceanica	Rabbits 5 weeks	4.5%	Increase of abundance of proteins related to amino acid catabolism and synthesis Results suggested that more tender meat may result from algae feeding	[100]
Porphyridium sp.	Chickens 10 days	5–10%	Decreased feed intake (10%) Decreased serum cholesterol level (28%)	[101]
Schizochytrium sp.	Dairy cows 6 weeks	4%	Decreased feed intake	[102]

1.3. Microalgae for Pharmaceutical Applications

Red biotechnology defines the use of biotechnology in the medical and pharmaceutical industries and health preservation [103]. There is demand for further screening of different microalgae species and strains and the development of new potential pharmaceutic agents derived from microalgae biomass [104]. Antioxidants hold potential for development as health-promoting ingredients and for maintenance of food quality and safety. Well-known antioxidants include carotenoids and peptides derived from microalgae. Carotenoids prevent cell damage by quenching cellular reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide or hydroxyl radicals, which result in increased oxidative stress and subsequent lipid peroxidation and protein oxidation. Oxidative stress results from an imbalance of homeostasis between oxidant and antioxidant species in the cell with excessive production of ROS and free radicals [105]. Carotenoids can protect human cells from inflammatory and metabolic disorders, early ageing, cardiovascular diseases, arthritis and cancer by quenching ROS and free radicals [86]. Several studies indicate that an increase in the intake of astaxanthin, for example, helps to prevent the development of type 2 diabetes mellitus (T2DM), reduces systolic blood pressure and protects the consumer from diseases associated with metabolic syndromes as well as atherosclerosis, neurodegenerative and cardiovascular diseases [106]. Moreover, a sufficient intake of β-carotene can decrease the damaging effect of free radicals associated with different types of cancer and plays a role in restoring the activity of antioxidant hepatic enzymes, which protect hepatic cells from xenobiotics, for example [86,107].

Microalgae sterols are lipids that make up the cell membrane and influence its fluidity and permeability. They can lower blood cholesterol significantly and are reported to reduce total cholesterol by 10% and LDL cholesterol by up to 15%. Species known to produce phytosterols in high amounts are *Isochrysis galbana* and *Pavlova lutheri*. Moreover, significant anti-cancer and anti-inflammatory effects of microalgae sterols were previously described. In a study by Ramos-Romero and colleagues, lipid extracts from *Nannochloropsis* sp. reduced plasma and liver cholesterol in rats significantly. In contrast, a lipid extract derived from *Nannochloropsis gaditana* was found to reduce blood glucose and LDL cholesterol, while the concentration of blood insulin and HDL cholesterol increased [75].

As mentioned earlier, PUFAs are an important group of bioactive molecules with significant, positive effects on human health. Eicosapentaenoic acid (EPA) helps with the regulation of blood pressure, regulation of the immune system response, protection against cancer and atherosclerosis and treatment of anxiety and depression. Docosahexaenoic acid (DHA) showed significant anticancer activity previously and has positive impacts on the functionality of the nervous system and human fetus development. Gamma linoleic acid is successfully used for the treatment of autoimmune diseases, allergies and obesity [8].

Another interesting group of microalgae bioactive molecules with the potential for use in pharmaceutical applications includes therapeutic proteins. The advantages of

recombinant therapeutic proteins over antibodies or proteins include simple synthesis, high specificity and selectivity and low accumulation in tissues. On the other hand, they have a short half-life due to their low stability and are expensive to produce [104,108–110]. In genetic engineering, therapeutic proteins are made in various host organisms such as bacteria, yeast, plant and mammalian cells and, more recently, in microalgae. However, all of these host organisms have some drawbacks. In the case of bacteria they do not make the same post-translation modifications of proteins as higher eukaryotes, so they are not appropriate for the production of eukaryotic proteins. Plant cells have different glycosylation patterns, and mammalian tissues are costly and instable. Microalgae cells may be effective hosts for the expression of recombinant therapeutic proteins [111–113].

1.4. Microalgae in Cosmetics and Cosmeceuticals

Cosmetics may be defined as any substance or mixture placed in contact with the skin or outer sparts of the human body such as the epidermis, hair, nails, lips, external genital organs, teeth and mucous membranes of the oral cavity that can clean them, perfume them, change their appearance, protect them and keep them in good condition or reduce body odors [113]. Cosmeceuticals are cosmetic products with biologically active ingredients aimed at having medical or drug-like benefits [114]. Microalgae and bioactive components extracted from microalgae are used in cosmetics as antioxidants, free-radical collectors, stress protectors, immune system boosters, odor maskers, make-up pigments, sunscreen protectors and anti-ageing agents. The different effects of active ingredients extracted from microalgae include blemish prevention, damaged skin reparation, seborrhea improvement, inflammation process inhibition, acceleration of the healing process and skin moisture maintenance [115,116]. Examples of some microalgae species that are in cosmetics and have potential for use in cosmeceuticals are summarized in Table 8.

Table 8. Microalgae with potential use in cosmetics or cosmeceuticals.

Species	Observed Effects	References
Arthrospira maxima	Skin protection Skin regeneration	[116]
Arthrospira platensis	Wrinkle formation prevention Early skin aging prevention	[116]
Chlorella vulgaris	Support collagen repair mechanism	[117]
Haematococcus pluvialis	Sunscreen protection	[117]
Nannochloropsis gaditana	Decreased oxidative stress in human dermal fibroblasts Skin protection Skin hydration	[118]
Nannochloropsis sp.	Tanning cosmetics	[117]

Microalgae are found in several products for personal skin care. For example, the company Soliance uses whole *Arthrospira* sp., and the peptide sequence LVMH, derived from *Chlorella* sp., is used in personal skin care products. Furthermore, the company Solazyme uses alguronic acid in its anti-aging skin products. Soliance also uses the alga *Skeletonema costatum* in hydrating skin products and uses *Dysmorphococcus* globosus in products muted to have anti-inflammatory effects [16]. Due to their unique cellular composition and content of PUFAs, including DHA, EPA, vitamins and folic acid, microalgae are also of great interest in the field of thalassotherapy. Thalassotherapy is a modern procedure working with seaweed and marine elements including microalgae, mud, sand or plankton for therapeutic and preventive health care purposes [119].

2. Isolation of Proteins and Functional Peptides from Microalgae

Although the incorporation of whole microalgae biomass into food and feed is well established, for the production of microalgae protein isolates and their subsequent successful incorporation into food products, the development of robust and feasible processes is required [20]. After the protein extraction, the solubility is increased and undesired color is removed, which leads to easier integration into the food product [120]. Firstly, to improve the efficiency of any extraction process, it is necessary to disrupt the cells and release the intracellular content to buffers of solvents. The composition of the microalgae cell wall is specific to each species, so the selection of a suitable disruption technique must also take into account, among other things, the cell wall composition [20]. The use of conventional extraction techniques generally results in low yields caused by protein degradation due to extreme temperatures and pH conditions used during the processes. Therefore, researchers are currently mainly focused on the development of novel, non-thermal extraction methods that employ enzymes and "green technologies" to increase extraction efficiencies and lower negative impacts on the environment [22]. During most of the extraction procedures, proteins are co-extracted with sugars, polyphenols and other compounds. For this reason, subsequent isolation and purification procedures are necessary. For the scheme of the whole process of protein extraction and the production of bioactive peptides, see Figure 1.

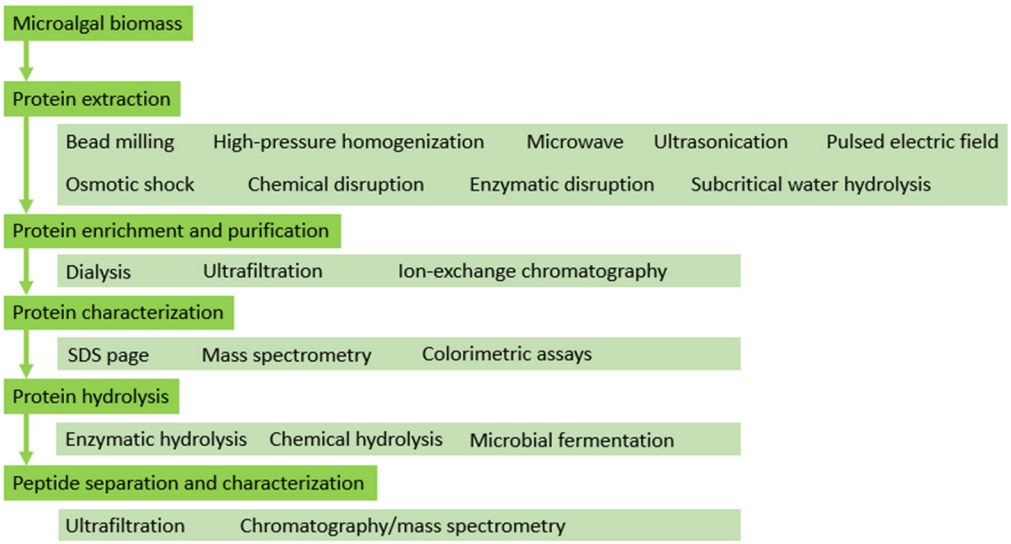

Figure 1. Production of bioactive peptides.

2.1. Protein Extraction

In general, microalgae cell disruption techniques can be divided into mechanical and non-mechanical techniques. The main advantages and disadvantages of selected techniques suitable for microalgae protein extraction are listed in Table 9. Mechanical methods are suitable for cell disruption; where the bioactive in question is not heat sensitive, a fast process method is required (Table 9). For heat sensitive actives such as proteins and peptides, enzymatic methods are preferred. In addition, enzymatic methods are considered to be more environmentally friendly. Enzymatic methods and the physical methods are both scalable. The yields of proteins obtained following the use of different disruption methods are shown in Table 10. Ultrasound and high-pressure homogenization combined with enzymatic treatment have resulted in protein yields of between 74–90% when applied to microalgae previously.

Table 9. Mechanical and non-mechanical techniques of microalgae cell disruption.

Mechanical Techniques of Cell Disruption		
Technique	**Advantages**	**Disadvantages**
Bead mills	Low dependence on cell wall composition [20] High efficiency [117] High biomass loading [118] Easy scale-up [118] Short processing time [119]	High energy consumption [118] Difficult/energy consuming control of temperature [118,119] Low selectivity [20]
High-pressure homogenization	Low dependence on cell wall composition [20] High efficiency [119] Easy scale-up [117] Simple [117] Applicable to highly concentrated microalgae pastes [119]	Difficult/energy consuming control of temperature [120] Low selectivity [120] High energy consumption [20]
Microwave	High efficiency [118,119] Short processing time [119] Easy scale-up [118]	Intensive heat production [118] Formation of free radicals [118]
Osmotic shock	Simple [119] Low energy consumption [20] Easy scale-up [119]	Low efficiency [117] High cost of salt [117,119]
Pulsed electric field	Easy scale-up [118] Mild conditions [118] Selective extraction of water-soluble compounds [119]	Medium has to be non-conductive [117,118]
Subcritical water hydrolysis	Possible to scale-up [121]	High capital cost [121]
Ultrasonication	Simple [20]	Intensive heat production [118] Low efficiency [118] Low selectivity Formation of free radicals [118] High energy consumption [117,119] Difficult scale-up [117]
Non-Mechanical Techniques of Cell Disruption		
Technique	**Advantages**	**Disadvantages**
Chemical disruption	Low energy consumption [117]	High dependence on cell wall composition [118] Risk of protein degradation [119] Contamination by solvents [118]
Enzymatic disruption	Low energy consumption, biological specificity, mild operational conditions, low capital investments [118] Suitable for thermo-sensitive compounds [117] High efficiency [119] Environmentally friendly [119]	High cost of enzymes [117,118] Long processing time [118] Low production capacity [118] Product inhibition [118] Difficult scale-up [119]

Table 10. Extraction methods used for extraction of proteins from selected microalgae species.

Species	Extraction Method, Conditions	Results	References
Arthrospira platensis	Aqueous two-phase system (16% sodium citrate, 18% PEG 1500 kDa)	Protein recovery 75%	[121]
Arthrospira platensis	Manothermosonication (probe 20 kHz, solvent sodium buffer)	Protein recovery 50%	[122]
Chlamydomonas sp.	Solvent extraction (tested solvents: water, methanol, ethanol, 1-propanol)	Highest yields using water	[123]
Chlorella sorokiniana	Aqueous two-phase system (30% K_3PO_4, 20% methanol and 3% NaCl)	Yield 84.2%	[124]
Chlorella vulgaris	Ultrasonic-assisted three phase partitioning(salt saturation 50%, slurry to t-butanol 1:2, sonication power 100%, irradiation time 10 min, frequency 35 kHz, duty cycle 80%, biomass loading 0.75 wt%)	Separation efficiency 74.6%Yield 56.6%	[125]
Chlorella vulgaris	Bead milling (DYNO-Mill Type MULTI LAB, 1 mm ZrO_2 beads, time < 1 min)	Yield 42%	[126]
Chlorella vulgaris	High pressure and high pH (pressure 2.7 kbar, two passes, pH 12)	Yield 98%	[127]
Chlorella vulgaris	Subcritical water extraction (277 °C, 5% of microalgae biomass loading, time 5 min)	Yield 31.2%	[128]
Haematococcus pluvialisNannochloropsis oculataChlorella vulgarisArthrospira platensisPorphyridium cruentum	High pressure homogenization (pressure 2.7 kbar, two passes)	Yield 41.0%Yield 52.3%Yield 52.8%Yield 78.0%Yield 90.0%	[129]
Haematococcus pluvialis	High pressure homogenization (pressure 2.7 kbar)	Yield 73%	[130]
Nannochloropsis sp.	High pressure homogenization (pressure 1.5 kbar, three passes)	Yield 91%	[120]
Tetraselmis sp.	Bead milling (DYNO-Mill Type MULTI LAB, ceramic beads 0.4–0.6 mm)	Yield 79%	[131]
Tetraselmis suecica	Bead milling (DYNO-Mill Type MULTI LAB, Y_2O_3 stabilized ZrO_2 beads 0.4 mm)	Yield 22.5%	[132]

Extraction techniques used for protein extractions of selected microalgae species together with gained protein recovery/yield are listed in Table 10.

2.2. Protein Purification

As mentioned above, proteins are co-extracted with other compounds, including polysaccharides, polyphenols and minerals, so, depending on the end application, they

may need to be enriched further using dialysis, ionic-exchange chromatography or other techniques based on molecular sizes or charges [20,44].

Dialysis is a separation method based on the selective passive diffusion of particles of different sizes through a semipermeable membrane. Dialysis is commonly used to remove minerals, salts, contaminants, reducing agents or preservatives [44].

Ultrafiltration is another type of membrane separation technique that can be applied in protein purification. In contrast to dialysis, the driving force of ultrafiltration is not passive diffusion, but the application of an external pressure. After application of the pressure, smaller molecules and molecules of solvents pass through the membrane and larger molecules are trapped by the membrane [44].

Ionic-exchange chromatography is a commonly used method for the separation of charged molecules as proteins, peptides and amino-acids. During the process, charged molecules dissolved in mobile-phase solvent interact with charger groups of the stationary phase [44]. Proteins can also be separated from other compounds using molecular weight cut off filtration. However, proteins less than the membrane size are only recovered along with salts, which can pose problems for later applications in food or feeds, for example. Proteins can also be salted out using ammonium sulphate precipitation, and this method is the most commonly described in the literature to date. Purification of protein extracts is usually achieved using charcoal filtration or TiO_2 clean-up methods, especially if proteins are to be characterised for their peptide content using mass spectrometry.

2.3. Protein Hydrolysis

Apart from proteins, bioactive peptides are one of the most commercially attractive microalgae products, with a wide range of potential uses in pharmacy, cosmetics and the production of food and feed. Because peptides remain inactive in the primary structure of proteins, they have to be released in gastrointestinal tract during food processing to become biologically active. The commonly used methods for the production of biologically active peptides are chemical or enzymatic hydrolysis and microbial fermentation [133].

Chemical hydrolysis of proteins is performed at higher temperatures (over 40 °C) and an extreme pH. The advantages of this method are low costs, simplicity and a short processing time, but on the other hand, the process lacks sensitivity and specificity, and some amino-acids can be destroyed [134].

Enzymatic hydrolysis is usually carried out in a reactor with a controlled pH and temperature by adding a protease or protease mixture (containing trypsin, pepsin, papain and α-chymotrypsin) to the protein concentrate. Compared to chemical hydrolysis, enzymatic hydrolysis is performed in lower temperatures, and the process has higher specificity, higher yields and a higher purity of the product, so it is a preferred hydrolysis technique in food and the pharmaceutical industry. However, peptidases are expensive, and it is difficult to adjust the desired pH and temperature during the whole process.

Microbial fermentation was evaluated as an eco-friendly method suitable for protein hydrolysis on a large scale. Other advantages include the elimination of hyper-allergic and anti-nutritional factors. For the purpose of protein hydrolysis by fermentation, lactic acid bacteria such as *Lactobacillus brevis*, *Bacillus subtilis*, *Enterococcus gallinarum* or *Pediococcus acidilactii* are frequently used [133]. As discussed by Sharma and colleagues recently [135], "fermented foods comprise very complex ecosystems consisting of enzymes from raw ingredients that interact with the fermenting microorganisms' metabolic activities. Fermenting microorganisms provide a unique approach towards food stability via physical and biochemical changes in fermented foods. These fermented foods can benefit consumers compared to simple foods in terms of antioxidants, production of peptides, organoleptic and probiotic properties, and antimicrobial activity".

2.4. Separation, Purification and Identification of Bioactive Peptides

Once peptides are released from the parent protein, the amino acid composition, hydrophobicity and molecular weight determine their bioactivity. After hydrolysis, the

peptides have to be separated from the mixture, usually with the use of membrane ultrafiltration, gel chromatography or liquid chromatography. For subsequent purification of peptides, several approaches are known, for example reverse-phase high performance liquid chromatography, purifying the peptides on the basis of their hydrophobic properties. To identify the peptides, several techniques can be used, e.g., liquid chromatography-tandem mass spectrometry with a quadrupole time-of-flight tandem mass spectrometer Q-TOF equipped with electrospray ionization (LC-MS/MS), ultralight performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) or a matrix-assisted laser desorption/ionization-time of flight spectrometer (MALDI-TOF-MS/MS) [133].

3. Application of Microalgae as Food, Functional Foods and Feed

Microalgae biomass is commonly used as a food supplement in the form of powder, tablets, capsules, flakes or pastes, but recently, it was incorporated into some food products including noodles, bread, pasta [136], ice-creams [137], cookies and biscuits [138], chocolate [139], gelled deserts [140], yoghurts [141] and cheeses [142]. The number of drinks and snacks containing microalgae biomass has doubled in western countries in the past few years. However, there are limitations concerning their incorporation into food products, including their bright green color, "fishy" aroma and the fact that they can stain surfaces due to their lipid content. Other drawbacks are legal and legislative issues, discussed in Section 4. From the techno-functional point of view, the most important properties of proteins are solubility, emulsification, nutritional quality and digestibility. Solubility is largely dependent on amino-acid composition and sequence, as well as conditions such as pH and the ionic strength of the solvent. The solubility typically increases when the pH is further away from the isoelectric point. In general, microalgae proteins have high solubility in a high pH and minimal solubility at a pH below 4. Due to its amphiphilic character, protein has a great emulsifying ability. Proteins are widely recognized as the major component influencing the rheological properties of food products and stability during storage [42]. Due to their high content of surface active proteins, various microalgae species were proved to have a great ability to stabilize proteins and foams and exhibited comparable stabilization properties in commonly used synthetic surfactants or animal-based proteins. In the future, microalgae proteins have the potential to replace surfactant and animal proteins in the food industry [143]. The techno-functional properties of selected microalgae species and their protein fractions are listed in Table 11, together with the effect of their incorporation into food. As outlined in Table 11, the emulsifying properties of selected proteins from different *Chlorella* sp. were excellent and comparable to egg protein in many instances. Microalgae proteins exhibit comparable to superior interfacial stabilization compared with animal- or plant-based proteins [143]. Their emulsions and foams exhibit minor pH-dependency due to a characteristically low isoelectric point and an extraordinary resistance to increased ionic strength.

Table 11. Techno-functional properties of selected microalgae species and effect of their incorporation into food.

Species	Fraction/Product	Effects	References
Arthrospira platensis Nannochloropsis gaditana Tetraselmis impellucida Scenedesmus dimorphus	Soluble protein isolate	High solubility at low ionic strength and pH < 6.5	[144]
Arthrospira platensis	Soluble protein isolate	High oil and water absorption capacity, high emulsifying capacity, high foam stability All properties strongly influenced by pH	[145]

Table 11. *Cont.*

Species	Fraction/Product	Effects	References
Arthrospira platensis	Biomass	Boost of fermentation performance of lactic acid bacteria (LAB)22 a	[146]
Arthrospira platensis	Biomass incorporated into bread (crostini)	Increased protein and phenolic content Increased antioxidant capacity Decreased protein digestibility	[147]
Chlorella protothecoides	Water soluble extract of lyophilized biomass	Emulsion stable for at least 7 days, resistant to high salt concentration (to 500 mM NaCl) at pH 2–9	[148]
Chlorella vulgaris	Protein extract	Emulsifying capacity and stability comparable or higher that to commercial emulsifiers	[127]
Chlorella vulgaris	Biomass incorporated into mayonnaise (replacement of eggs by *Chlorella* and acid casein curd)	Improved nutritional value and stability Better rheological properties Positive effect on sensory characteristics	[149]
Haematococcus pluvialis	Biomass incorporated into cookies	Increased phenolic content and antioxidant capacity Reduction in the rate of glucose released during digestion	[150]
Nannochloropsis sp. *Tetraselmis* sp.	Biomass incorporated into wheat tortillas	Increased phenolic content and antioxidant capacity No difference in physical parameters Sensory acceptable	[151]
Tetraselmis sp.	Soluble protein isolate	High emulsion stability at pH 5–7 at low ionic strength	[152]

4. Legislation Governing The Use of Microalgae

- The consumption history of an alga affects its regulatory status. Entry of a species or extracts from that species into the market is regulated by the Novel Food Regulation. This applies to species having not been used as food to a significant degree in any of the EU member countries before 15 May 1997. These algae need to undergo the authorization procedure in order to ensure their safety for human consumption (Regulation (EC) No 258/97).
- In the New Novel Food Regulation (EC) 2015/2283, an additional notification system is provided for species that have a demonstrated history of safe use for at least 25 years in a country outside of the EU. The notification system may provide an easier route to the EU market for some microalgae species that have not been used in Europe but are consumed elsewhere.
- The EU through Regulation (EU) 2017/2470 maintains an online list—the novel food catalogue—that contains the Union's list of all authorized novel foods. This legislation applies to microalgae intended to be used as food. This catalogue contains both European and imported algae, and to the current date there were 22 algae listed. The list is accessible at https://ec.europa.eu/food/safety/novel-food/novel-food-catalogue_en (accessed on 21 December 2021) and includes six microalgae, including *Arthrospira platensis*, *Chlorella luteoviridis*, *Chlorella pyrenoidosa*, *Chlorella vulgaris*, *Chlamydomonas reinhardtii and Spirulina* sp. when the list was accessed on 1 November 2021.

- In the US, the FDA regulates both US laws applicable to microalgae-based food products, which are the Federal Food, Drug and Cosmetic Act, regulating all food and food additives, and the Dietary Supplement Health and Education Act, regulating dietary ingredients and supplements. The FDA Center for Food Safety and Applied Nutrition governs all food ingredients and is responsible for their safety [16].
- The European Union and United States have largely different attitudes and regulations that apply to microalgae-based products. One of the main differences is the criterion for novel food definition and consequently the authorization process [16].

Some microalgae species were designated as generally recognized as safe (GRAS) by the FDA. Microalgae relevant for food or feed applications and their safety aspects are listed in Table 12.

Table 12. Safety aspects of selected microalgae species [16,152].

Safety Aspect	Species	Application
GRAS	*Arthrospira platensis*	Biomass
	Chlorella protothecoides	Biomass, oil
	Crypteconidium cohnii	DHA-rich oil
	Dunaliella bardawil	Biomass
	Haematococcus pluvialis	Astaxanthin
No toxins known	*Synechococcus* sp.	
	Tetraselmis sp.	
	Chlamydomonas reinhardtii	
	Haematococcus pluvialis	
	Chlororoccum sp.	
	Scenedesmus	
	Desmodesmus sp.	
	Parietochlors incisa	
	Navicula sp.	
	Nitzschia dissipata	
	Phaeodactylum tricornutum	
	Thalassiosira pseudonana	
	Odonrella aurita	
	Skeletonema sp.	
	Monodus subterraneus	
	Nannochloropsis sp.	
	Isochrysis sp.	
	Pavlova sp.	

4.1. European Regulation on Marketing of Microalgae for Food

In Europe, three main regulations apply to the marketing of microalgae and its components: (i) Regulation on Food Safety, (ii) Regulation on Novel Foods and Novel Food Ingredients, (iii) Regulation on Nutrition and Health Claims made on Food [16].

4.1.1. Regulation on Food Safety

The European Community Regulation on Food Safety (EC 178/2002) was published in the Official Journal of the European Communities (1.2.2002 EN L 31/1) and provides information regarding approaches to the development of any food legislation. It only works as a general framework for areas that are not covered by harmonized rules and gives definitions, principles and obligations covering all stages of food and feed production, processing and distribution. The regulation established EFSA, the European Food Safety Authority. Food safety regulations are applied to all food products introduced to the market, including products using microalgae biomass or its components.

The Food Safety regulation concerns food that is proved by a prolonged period of consumption, however, in case of new food products without a history of safe use on the market, these products are not introduced to the European market without meeting the conditions set out in the Regulation on Novel Foods and Novel Food Ingredients [16].

4.1.2. Regulation on Novel Foods and Novel Food Ingredients

Novel Foods and Novel Food Ingredients regulation is applied to foods and food ingredients that were not consumed on a significant level in Europe before May 1997. This concept includes, for example, microalgae oils rich in omega-3 fatty acids, which have been introduced to the market recently and thus fall under this regulation, despite the consumption of omega-3 fatty acids having a long history. Another example of a product regulated as novel food is a blue colorant extracted from *Arthrospira*, despite *Arthrospira* itself not being considered as a novel food and being consumed for several centuries. The risk assessment process leading to the commercialization of novel food product is usually time-consuming and expensive [16]. *Arthrospira* and *Chlorella* are not included in the novel food list, because they are not considered as novel and have a designation GRAS (generally recognized as safe) [143].

The main principle of this regulation is to ensure food safety for consumers, so that a product is not dangerous or nutritionally disadvantageous and is labelled properly. When companies intend to introduce a novel food or novel food ingredient to the market, firstly they must present the scientific information and a safety assessment report to a national authority for authorization. The process of authorization involves conditions of use, designation of novel food or food ingredient, specification and labelling requirements. After, the Commission asks the Standing Committee on Food Chain and Animal Health for its opinion, and, if the novel food or food ingredient is likely to have an effect on public health, it also asks the EFSA Scientific Committee for Food.

When the applicant considers its food or food ingredient as 'substantially equivalent' to a similar product which is already marketed in EU, the process can be simplified to a procedure called 'notification' [16].

4.1.3. Regulation on Nutrition and Health Claims Made on Foods

This regulation was introduced in 2006 and states that nutrition and health claims regarding food and feed products have to be based on generally accepted scientific evidence. These scientific assessments are only authorized in the EU by EFSA, which provides scientific opinions on health claims via the Panel on Dietetic Products for Nutrition and Allergies (NDA) [16].

4.2. United States Regulation on Marketing of Microalgae for Food

Any substance intentionally added to food is recognized as a food additive and thus, unless it is already GRAS, has to be subjected to premarket review and approval by the FDA. The Dietary Supplement Health and Education Act provides a framework for dietary supplement regulations, including current good manufacturing procedures, mechanisms for pre-market safety notifications of food ingredients and claims used in product labelling. When companies aim to market new dietary ingredients, the manufacturers and distributors have to notify the FDA about these ingredients. Additionally, they have to provide information on the basis that this new dietary ingredient can be reasonably expected to be safe when used as recommended. For types of food products other than dietary supplements or ingredients, it is not mandatory to ask for GRAS status; however, it is highly recommended to satisfy government safety assessment requests [16].

5. Challenges and Bottlenecks

Several barriers need to be addressed to ensure the successful incorporation of bioactive components extracted from microalgae biomass including proteins into food, feed, pharmaceuticals and other products. The main bottlenecks are (i) high production costs of microalgae biomass and its components, (ii) lack of knowledge about the impact of consumption of microalgae biomass and the digestibility and safety of microalgae and (iii) insufficient research into the development of new food products. One of the main bottlenecks regarding the development of microalgae protein as a food ingredient is the high cost of microalgae biomass production, recently estimated to be 3.4 EUR/kg for DW microal-

gal production in Spain [153] or 5.1 EUR/kg of DW for *Tetraselmis suecica* production in Italy [154]. Nowadays, selective separation of microalgae products is at an early stage, and most commercial facilities focus on one product, which is either dried biomass or extracted and purified specific high-value components such as omega-3 fatty acid (DHA, EPA) or pigments such as astaxanthin. Selective separation of different products using a biorefinery approach aims at optimal exploitation of various biomass components and their allocation to different markets. The separation of functional proteins requires mild conditions, and the costs are still too high currently to be economically viable. For example, where cell disruption using the bead-milling process is implemented, there is a huge amount of energy (1 kWh/kg) dissipated in the liquid fraction as heat, which corresponds to additional costs of approximately 0.15 EUR/kg. However, it was estimated that a 90% reduction of energy consumption for cell disruption can be achieved by the use of novel techniques such as pulse electric field (PEF) and ultrasound [153]. However, these technologies are not widely available currently and initial set-up costs are high.

6. Conclusions and Future Directions

The safety of microalgae biomass for consumption is another challenge. Van der Spiegel and colleagues warned that food safety of novel protein sources such as microalgae, seaweed or insects needs to be addressed. Potential hazards associated with their consumption include poisoning due to heavy metals, mycotoxins, pesticide residues and pathogens. Other problems are the presence of anti-nutritional factors, allergens and the modification of substances during processing that may increase allergenicity, for example. In the future, research should focus on the safety of novel proteins from microalgae in food products and on the degradation and accumulation of bioactives and contaminants during processing [155,156]. Other issues include the digestibility of microalgae proteins, which has not been adequately explored to date. Although many studies have evaluated the digestibility of microalgae, which is reported as 94% for *Arthrospira platensis* in some studies, other studies found that microalgae proteins have lower digestibility compared to standard protein sources such as egg, soya and pea protein [157], and that digestibility values for *Arthrospira platensis* were significantly lower at 78% [158]. The importance of the correct selection of methods to determine digestibility and bioavailability and standardization of these methods should not be underestimated. Furthermore, addition to the EU list of approved algae for use as a novel food is required beyond what is currently approved. At present, there is a limited number of microalgae species approved, and apart from omega-3-PUFA-rich oil extracted from certain heterotrophic microalgae, only *Spirulina* and *Chlorella* sp. exist on the market today, and they are used primarily for their food colorant potential rather than as a source of nutrients.

The future for microalgae use looks promising despite the aforementioned bottlenecks and challenges. They are a noted source of PUFAs and proteins, and plans to improve processing methods to make microalgae protein more acceptable to consumers should be pursued. These processing methods include cell disruption methods that can actually enhance the uptake of key nutrients including amino acids by the consumer, as well as methods to refine key ingredients from microalgae to generate acceptable powder formats with less sensory challenges compared to whole microalgae. Methods that can be applied to microalgae proteins include molecular weight cut off (MWCO) filtration and diafiltration, which are used in the processing of proteins from the dairy and pea protein industries for example. According to Enzing, Europe has some important advances to make in this field, and this topic is of high priority in terms of R&D funding policies. Some bottlenecks in the European microalgae industry are obvious, including suboptimal climatic conditions (low levels of sun hours and intensity, low temperature, high level of rainfalls), high labor costs, a lack of venture and seed capital for start-up companies, low entrepreneurial activity among researchers and engineers, low R&D investments by large companies, high cost of land and low domestic demand for microalgae-based products [16]. The European Commission's Green Deal targets numerous areas where microalgae production

and processing can play an important role. For example, the goal of becoming climate neutral by 2050, protecting biodiversity, developing a circular economy and contributing to the "farm to fork" strategy for sustainable food system development [112] could be a key driver of microalgae development for food, pharma and cosmetics in Europe and beyond.

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