Critical Reviews in Food Science and Nutrition

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/bfsn20

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CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION
https://doi.org/10.1080/10408398.2021.1895065

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REVIEW

Recent advances in the application of microalgae and its derivatives for preservation, quality improvement, and shelf-life extension of seafood

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ABSTRACT
Seafood is a highly perishable food product due to microbiological, chemical, and enzymatic reactions, which are the principal causes of their rapid quality deterioration. Therefore, ever-increasing consumers' demand for high-quality seafood along with a negative perception of synthetic preservatives creates opportunities for natural preservatives such as microalgae extracts. They are potential alternatives to reduce microbial growth, increase oxidative stability, and protect the sensorial properties of seafood. Research has shown that the inclusion of microalgae extracts into the aquatic animal's diet could enhance their meat quality and increase production. This review focuses on the direct application of various microalgae extracts as seafood preservative, and their functional properties in seafood, such as antioxidant and antimicrobial activities. Besides, the potential nutritional application of microalgae extracts as an alternative in aqua-feed and their impact on seafood quality (indirect application) are also presented. The safety aspects and regulatory issues of products from microalgae are highlighted.

KEYWORDS
Seafood; microalgae extracts; aqua-feed; antimicrobials; antioxidants; regulatory issues

Introduction

Seafood, comprising a large diverse group of fishes, mollusks, crustaceans, and echinoderms, has always been popular because of their high nutritional value as well as delicacy and palatability (Huang et al. 2018). However, these products are prone to rapid spoilage due to their high moisture content, neutral pH, and huge variety of small molecular weight components (Hong, Regenstein, and Luo 2017). Failing to control enzymatic and microbiological reactions that begin in seafood immediately after their capture, leads to changes in nutritional properties and sensory attributes (Inanli et al. 2020). The initial loss of seafood quality is a function of chemical reactions and indigenous enzymes, whereas complete deterioration of seafood is most often attributed to metabolic activities of microbes (Olatunde and Benjakul 2018).

Generally, fishery products are high in polyunsaturated fatty acids (PUFA), which make them more susceptible to oxidation (Guedes, Sousa-Pinto, and Malcata 2015). Secondary oxidation products such as aldehydes, ketones, epoxides, oligomers, hydroxyls, and other compounds lead to the formation of toxic compounds and negative biological effects. Color change, loss of nutrition, and formation of undesirable odors and flavors in fishery products have also been attributed to lipid oxidation and rancidity (Tavakoli et al. 2018). The distance between harvesting areas and processing centers, processing methods, processing facilities, and storage conditions are other important factors that contribute to the complexity of fishery product degradation (Olatunde and Benjakul 2018). To minimize or control unfavorable reactions in fishery products, diverse methods, and non-thermal anti-microbial technologies have been developed and used (Huang et al. 2018). Many synthetic antioxidants have been used in seafood to prevent the chemical, microbiological and enzymatic changes and extend the shelf-life. Nevertheless, the accumulation of these chemical components in the product may negatively affect human health (Huang et al. 2018). Hence, in the past few years, consumers have been requesting that such synthetic preservatives should be replaced with bio-preservatives, which are often rich in bioactive compounds, and have fewer side effect with respect to human health. Moreover, natural preservatives have mostly shown similar or even higher antioxidant and antimicrobial effects compare to chemical preservatives (de Oliveira et al. 2018).

Among these natural preservatives microalgae extracts appear to be a viable alternative. Microalgae are highly diverse photosynthetic microorganisms, including photoautotrophic protists and prokaryotic cyanobacteria (blue-green algae). These microscopic organisms are found in both freshwater and marine environments (Dineshbabu et al. 2019). Recently, researchers have successfully shown that the

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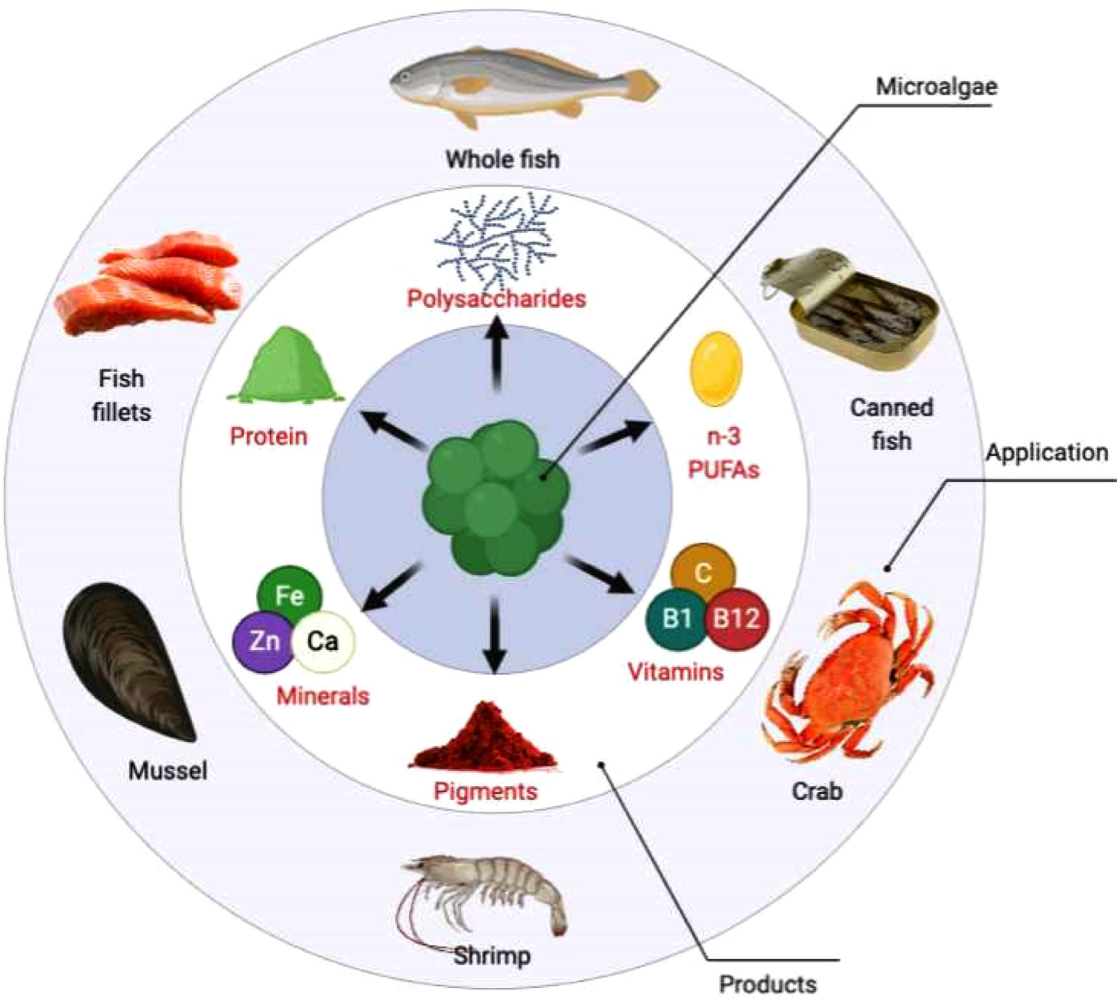

Figure 1. Schematic representation of different extracts from microalgae and their application for seafood production.

incorporation of microalgae extracts into seafood products can be an effective approach to reducing microbial growth, increasing oxidative stability and protecting sensorial properties and consequently prolonging their shelf life (Taghavi, Haghighat Khajavi, and Safari 2019). The application of these components into seafood could enhance their nutritional value by providing proteins, minerals, vitamins, lipids, carbohydrates, and fiber (Fields et al. 2020). Researchers also believe that the inclusion of microalgae into the diet of aquatic animals could improve their meat quality and possibly even their nutritional value (Li et al. 2014).

Although there have been several prior review articles on the use of microalgae extracts in functional foods (Bernaerts et al. 2019; Matos et al. 2017), the antioxidant and antimicrobial properties of microalgae extracts for application in seafood have not been reviewed. The knowledge of potential hazards associated with microalgae, especially those that accumulate and have toxic effects is also limited. Therefore, the focus of this review is to provide up-to-date information about the most recent published data regarding the application of microalgae and its derivatives for preservation, quality improvement, and shelf-life extension of seafood. The current state-of-the-art in the extraction, utilization and functional properties of microalgae extracts, such as antioxidant and antimicrobial activities, in seafood products are reviewed. The potential nutritional application of microalgae as an alternative in aqua-feed and their impact on seafood quality are also discussed. Ultimately, the safety aspects and regulatory issues of products from microalgae are highlighted. An overall concept of the coverage of this review is shown in Figure 1.

Seafood spoilage mechanisms

The term "spoilage" in the seafood literature has been used to describe any chemical, microbial and enzymatic changes that result in undesirable sensorial attributes (Olatunde and Benjakul 2018). The following section will focus on the three most common mechanisms occurring during the processing and storage of seafood products.

Microbial spoilage

Microorganisms are the primary causes of spoilage in many fishery products (Tavakoli et al. 2018). Seafoods contain significant amounts of free amino acids, a high content of water, and higher pH postmortem than most terrestrial animal products. These indigenous characteristics enhance the growth of microorganisms, especially those that survive well with a wide range of temperatures. They cause about 25–30% of the quality loss of seafood products (Hassoun and Çoban 2017). Wide numbers of bacteria (Gram-positive and Gram-negative), yeasts, and molds have been reported to be responsible for seafood spoilage. For example, Marshall (2014) reported that species within the families of *Enterobacteriaceae* and *Vibrionaceae* are the major contributing bacteria to seafood spoilage. Hassoun and Çoban (2017) have stated that psychrophilic bacteria such as *Shewanella sp.* and *Pseudomonas sp.* are the major group of microorganisms responsible for spoilage of chilled stored fish. Olatunde and Benjakul (2018) reported that aerobic or facultative anaerobic psychotropic Gram-negative bacteria, such as *Moraxella*, *Shewanella putrefaciens*, *Acinetobacter*, *Pseudomonas*, *Photobacterium*, *Aeromonas*, *Flavobacterium,* and *Vibrio* are the major group of organisms responsible for seafood deterioration. These microorganisms produce different metabolites such as aldehydes, alcohols, organic acids, biogenic amines, sulfides, histamine, and ketones, resulting in seafood degradation (Olatunde and Benjakul 2018).

Oxidation spoilage

Oxidation spoilage is another common spoilage route, especially for seafood containing high amounts of PUFA (Dehghani, Hosseini, and Regenstein 2018). Lipids can undergo various types of oxidation, including thermal oxidation, enzymatic oxidation, photo-oxidation, and auto-oxidation. However, auto-oxidation has been considered as the most common process causing oxidative spoilage. Auto-oxidation occurs through a free radical chain reaction, and expands during three phases: initiation, propagation, and termination (Hassoun and Çoban 2017). The initiation phase starts with a labile hydrogen atom being abstracted from a fatty acyl chain, and this may be catalyzed by irradiation, heat, metals, or ions to form a free-radical. The free-radicals react quickly with oxygen to form the peroxyl-radical, which can further react with other fatty acyl chains, thereby forming hydroperoxides and a new free radical through the propagation phase. Newly formed free-radical can then continue the chain reaction. The termination phase occurs when there is a build-up of free radicals that then interact to form non-radical products. The rate of oxidation depends on the degree of lipid unsaturation, oxygen availability, and the presence of metals, light, temperature and moisture (Olatunde and Benjakul 2018). Hydroperoxides are the primary products of oxidation, and are not stable. Secondary products of lipid oxidation are formed as a result of hydroperoxide decomposition. Aldehydes, ketones, hydrocarbons, volatile organic acids, alcohols, and epoxy compounds are among the secondary oxidation products formed (de Oliveira et al. 2018). These components can react with proteins, amino acids, and vitamins to lead to the development of a broad spectrum of unpleasant odor and flavor compounds, discoloration, and loss of protein functionality (Inanli et al. 2020).

Enzymatic deterioration

The autolysis of seafood also begins immediately after the completion of rigor mortis (Olatunde and Benjakul 2018). The activities of indigenous enzymes during autolysis leads to protein degradation (Freitas, Vaz-Pires, and Câmara 2020). The secondary changes in seafood properties are usually caused by proteases and lipases (Olatunde and Benjakul 2018). During the preliminary postmortem phase, textural changes of the sea cucumber *Stichopus japonicas* were attributed to proteolytic activities of cathepsins and cytosolic enzymes (the calpain system) (Xiong et al. 2020). After "belly burst" which occurs in many aquatic animals (e.g., whole fishes and sea cucumbers), a diverse range of enzymes such as phospholipase, cathepsins, trypsin, lipases, and pepsin are released from the digestive system, resulting in protein decomposition and textural changes (Olatunde and Benjakul 2018). These microbial, chemical, and enzymatic degradations in fishery products can be controlled or minimized with the use of optimal preservation treatments.

Trend in perspective of microalgae extracts as edible products and bio-preservatives

Microalgae have been consumed as a part of the human diet for hundreds of years. Asian countries such as China, Japan, and Korea have commonly used microalgae as a part of their normal diet (Rajvanshi et al. 2019). Around the 1950s, microalgae started to be considered a promising sources of protein for human consumption. The microalgae *Chlorella* was the first species that was commercially cultured at the beginning of the 1960s, followed by *Arthospira* in the 1970s. At that time, the production of protein was the main goal. Later, in the early 1980s, the production of pigments (mainly β-carotene and astaxanthin) from *Dunaliella* and *Haematococcus* became the main goal. These pigments have been used as human food additives and animal feed, but were previously mainly produced synthetically. Around the 1990s, the production of PUFA with a focus on eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) was started to produce value-added food products and aquaculture feed (Jacob-Lopes et al. 2019). Since the beginning of the twentieth century, rapid progress was made in food science and technology, especially with respect to synthetic preservatives, genetic modification, new food crops, artificial colorants, and synthetic flavorings (Hashemi Gahruie et al. 2020). These advancements have made the daily diet more palatable and attractive. Although, as a consequence of this progress, many health-related problems have arisen that negatively affected the quality of human life (Tavakoli et al. 2018). Hence, the need for replacement of many of these synthetic components along with the increasing population and the greater demand for food, have provided an opportunity for microalgae to become more popular for human consumption (Bernaerts et al. 2019). These aqua-marine products have been found to be a rich source of intermediate and high-value components such as proteins, carbohydrates, lipids, vitamins, and a diverse range of food colorants (Ejike et al. 2017) that are more valuable than

Table 1. Main bioactive compounds extracted from microalgae.

Specie	Bioactive compounds	References
Spirulina sp.	Phycocyanin, biomass, γ-linolenic acid (GLA), polysaccharides, vitamin E, *n*-3 fatty acids, flavonoids, vitamin B12 (cobalamin)	Vaz et al. 2016; Matos et al. 2017
Chlorella sp.	Biomass, ascorbic acid, sulfated polysaccharides, sterols, *n*-3 fatty acids, canthaxanthin, astaxanthin, peptide, oleic acid, zeaxanthin, violaxanthin, lutein, terpenoids, alkaloids, phytol, phenol	Othman et al. 2018; Taghavi et al. 2019; Vaz et al. 2016
Dunaliella salina	Carotenoids, powders, β-carotene, *cis*-β-carotene, oleic acid, linolenic acid, palmitic acid, diacylglycerols, sterols	Vaz et al. 2016; Dineshbabu et al. 2019
Haematococcus pluvialis	Astaxanthin, lutein, zeaxanthin, canthaxanthin, lutein, oleic acid, β-carotene	Su et al. 2020; Guedes et al. 2015
Chlamydomonas reinhardtii	β-Carotene, lutein	Bernaerts et al. 2019
Isochrysis galbana	Fatty acids, ascorbic acid	Vaz et al. 2016; Bernaerts et al. 2019
Porphyridium cruentum	Polysaccharides, phycocyanine, β-carotene, astaxanthin	Chacón-Lee and González-Mariño 2010; Rizwan et al. 2018
Neochloris sp.	Carotenoids	Herrero et al. 2015
Nostoc sp.	Phycocyanin, phenolic, terpenoids, alkaloids, phycobilins, vitamin B12	Rizwan et al. 2018; Vaz et al. 2016
Phaeodactylum tricornutum	β-Carotene, fucoxanthin, zeaxanthin	Bernaerts et al. 2019
Scenedesmus almeriensis	Lutein, zeaxanthin	Bernaerts et al. 2019
Parietochloris incise	Arachidonic acid	Rizwan et al. 2018
Hapalosiphonfontinalis	Vitamin B12	Rizwan et al. 2018
Tolypothrixtenuis	Vitamin B12	Rizwan et al. 2018
Cylindrospermum sp.	Vitamin B12	Rizwan et al. 2018
Eisenia bicyclis	Fucoxanthin	Herrero et al. 2015
Anabaena doliolum	Polyphenols	Herrero et al. 2015
Spongiochloris spongiosa	Polyphenols	Herrero et al. 2015
Porphyra tenera	Polyphenols	Herrero et al. 2015
Undaria pinnatifida	Polyphenols	Herrero et al. 2015
Sargassum muticum	Polyphenols	Herrero et al. 2015
Rhizoclonium hieroglyphicum	n-3 Fatty acids	Herrero et al. 2015

raising these algae for animal feeding and even direct use by humans. Numerous studies have shown that microalgae bioactive compounds have strong free-radical scavenging potential (Bernaerts et al. 2019). These characteristics made them a potential useful as antioxidant, antitumor, anti-inflammatory, anti-hypotensive, and anticancer substances (Dineshbabu et al. 2019). The main bioactive compounds extracted from different microalgae are shown in Table 1.

Recently, commercially cultivation of microalgae has become a sophisticated global industry, mainly focused on the cultivation of *Chlorella*, *Spirulina*, *Dunaliella*, *Nitzschia*, *Crypthecodinium*, *Nannochloris*, *Skeletonema*, *Schizochytrium*, and *Tetraselmis* (Ejike et al. 2017). China, Japan, the USA, Brazil, Myanmar, Spain, Israel, and Germany seem to be the major microalga producers (extracts and biomass) (Jacob-Lopes et al. 2019).

Commonly used microalgae in the seafood industry: macro- and micro-nutrients

Microalgae, although used by the seafood industry, are often also included in the aqua-marine industry along with fish, mollusk, shellfish, and other fishery products (van der Spiegel, Noordam, and Fels-Klerx 2013). Of the hundreds of microalgae species, only a few have been utilized for human consumption, most notably *Chlorella* and *Spirulina*, but also *Haematococcus*, *Dunaliella*, *Aphanizomenon*, *Schizochytrium*, *Scenedesmus*, *Odontella*, and *Porphyridium*. The microalgae *Arthrospira*, *Dunaliella*, *Chlorella*, *Haematococcus*, *Porphyridium cruentum*, *Crypthecodinium cohnii*, and *Schizochytrium* have been granted "Generally Recognized as Safe (GRAS)" status for human consumption by the US Food and Drug Administration (FDA) (Dineshbabu et al. 2019). As in human nutrition, products from several microalgae such as *Chlorella*, *Tetraselmis*, *Spirulina*, *Nannochloropsis*, *Nitzchia*, *Navicula*, *Chaetoceros*, *Scenedesmus*, *Haematococcus*, and *Crypthecodinium* have been used in aquatic animals' feed to improve the feed's sensorial properties, enhance the nutritional value, and/or extend the shelf life after capture by having their bioactive compounds distributed internally throughout the animal (van der Spiegel, Noordam, and Fels-Klerx 2013). These same bioactive compounds can be isolated and used as promising natural multifunctional substances in a broad range of seafood applications (Fields et al. 2020), which has made the application of microalgae important both from the seafood industry and from the consumers' health points of view. Some important species of microalgae used in these applications will be described below.

Spirulina sp. are a good source of carotenoids such as ß-carotene and astaxanthin (Rajvanshi et al. 2019). These compounds have been used in the fishery industry to improve skin pigmentation and muscle color. *Spirula* contains about 50-70% proteins by dry weight, depending on the strain (Ejike et al. 2017). Proteins from *Spirulina* contain all the essential amino acids in higher amounts than legumes which are the standard source of plant protein (Jung et al. 2019). *Spirulina* has also been reported as a good source of phycobiliproteins. These proteins have been shown to have free-radical scavenging characteristics, and this makes them

Table 2. Nutrition profile of different microalgae commonly used in the seafood industry.

Biomolecules (% dw)	*Spirulina*	*Chlorella*	*Dunaliella*	*Chlamydomonas*	*Scenedesmus*	*Haematococcus*	*Porphyridium*	*Isochrysis*	*Nannochloropsis*	*Schizochytrium*
Total crude protein	43-77	37-58	27-57	48	31-56	10-52	27-57	12-41	18-47	10-21
Total carbohydrate	8-22	8-27	4-41	17	6-28	34-37	12-57	13-48	7-40	12-24
Total lipid	4-14	5-28	6-22	21	8-21	15-40	5-14	17-36	7-48	43-74
DHA	0.30	2.60	0.36	ND	ND	ND	0.20	2.49	1.1(TFA)	7.60
EPA	0.25	0.40	0.60	ND	0.41	ND	3.13	0.22	3.47	0.08
C-Phycocyanin	9.27	ND	ND	ND	ND	ND	1.20	ND	ND	ND
C-Phycoerythrin	1.79	ND	ND	ND	ND	ND	4.22	ND	ND	ND
Carotenoids										
Β-Carotene	0.14	0.014	0.102	ND	0.070	0.05	ND	ND	0.048	0.00
Lutein/Fucoxanthin	ND	0.40	ND	ND	0.54	ND	ND	1.8(F)	ND	ND
Astaxanthin	ND	0.203	0.083	20.85*	0.150	3.07	ND	ND	0.640	1.25
Amino acids ($g\ 100\ g^{-1}$ protein)										
Leucine	9.26	14.2	13.41	14.0#	12.3	6.43	12.4	9.43	22.8	7.43
Valine	8.69	9.40	9.73	16.0#	10.9	5.11	5.32	6.00	20.1	5.43
Lysine	6.46	13.5	10.75	39.0#	11.1	4.10	11.7	6.14	22.8	4.52
Phenylalanine	6.53	8.46	9.71	31.0#	9.39	3.44	10.6	8.00	18.4	4.90
Methionine	4.43	6.17	5.52	16.0#	5.82	1.38	9.43	7.14	13.3	1.04
Tryptophan	2.45	3.55	2.95	80.0#	2.92	0.00	5.91	3.14	7.87	5.39
Threonine	6.94	7.61	8.55	41.7#	9.65	3.82	14.4	5.86	16.2	4.29
Histidine	2.65	4.47	3.65	8.3#	4.30	2.57	2.36	2.14	8.75	2.22
Vitamins ($mg\ kg^{-1}$ dw)										
Vitamin E	85.0	435	116	ND	ND	ND	ND	472	350	0.00
Vitamin B1	24.0	17.6	29.0	ND	ND	4.00	ND	14	70	0.02
Vitamin B6	13.7	7.70	2.20	ND	ND	3.00	ND	1.80	9.50	14
Vitamin B12	13.5	0.60	0.70	ND	ND	1.00	ND	0.60	0.85	0.54
Vitamin B3	145	82.5	79.3	ND	ND	60.0	ND	77.7	ND	140

Note: The values are average of data reported in 2 or more published articles, (Amaro et al. 2019; Bernaerts et al. 2019; Dineshbabu et al. 2019; Guedes et al. 2015; Herrero et al. 2015; Kent et al. 2015; Knutsen et al. 2019; Ljubic et al. 2020; Madeira et al. 2017; Soto-Sierra, Stoykova, and Nikolov 2018; Vaz et al. 2016; Ying et al. 2020)
*mg per chlorophyll *a*.
#g in 1000 g of dry weight; ND: no data available.

potential antioxidant, anticancer and antitumor agents (Rajvanshi et al. 2019). Recently, the World Health Organization (WHO) introduced *Spirulina* to NASA as a potentially better source of food nutrients for space men and women on planetary missions, as a small amount can provide a wide range of macro and micronutrients, and can presumably be successfully grown in space (Rajvanshi et al. 2019).

Haematococcus has been studied for use by the seafood industry as an "accumulated" form of natural astaxanthin (Sheikhzadeh et al. 2012). With optimum conditions, *Haematococcus pluvialis* can accumulate astaxanthin up to 5% of its dried weight (Xie et al. 2020). Astaxanthin (3,3′-dihydroxy-β,β-carotene-4,4′-dione) is a keto-carotenoid that contributes to the pinkish or reddish color of the muscle and skin of fish and crustaceans. Seafood coloring is the primary application of astaxanthin (Li et al. 2014).

Chlorella is a single-cell photoautotrophic green microalga. Studies have shown that *Chlorella* peptides have strong antioxidant activity in vitro and with cell-based assays. Similar to antioxidant capability, most of the peptides derived from *Chlorella vulgaris* and *Chlorella ellipsiodea*, have shown antihypertensive activity (Ejike et al. 2017). *Chlorella* sp. are high in β-1, 3-glucan, which act as a free-radical scavenger. Β-glucan has been reported to have strong immune-stimulant and antibacterial activity in various fish species (Dineshbabu et al. 2019).

Dunaliella is known for β-carotene accumulation (Jacob-Lopes et al. 2019). The average concentration of carotenoids in most of the microalgae has been reported to be between 0.1% and 2%, but *Dunaliella*, if grown using the optimal conditions of light and salinity, could yield up to 14% β-carotene (Levasseur, Perré, and Pozzobon 2020). This compound has been considered as the first-ever high-value product to be commercially produced from microalgae (Dineshbabu et al. 2019). β-Carotene is used as a pro-vitamin A and can contribute to vitamin A intake (Dineshbabu et al. 2019). Lutein, neoxanthin, zeaxanthin, violaxanthin, cryptoxanthin, and α-carotene are other important carotenoids that can be extracted from *Dunaliella* in sufficient quantities to be commercially interesting (Medeiros et al. 2021).

Nannochloropsis spp. contain a high level of PUFA (especially EPA); providing a source for aquaculture production (Knutsen et al. 2019). *Nannochloropsis* has also been reported as a potential source of antifungal compounds which can mitigate fungal contamination (Scaglioni, de Oliveira Garcia, and Badiale-Furlong 2019).

The nutritional profile of different microalgae commonly used in the seafood industry are shown in Table 2

Microalgae extracts and preparation methods

The biochemical composition of microalgae extracts depends on the species, collection time, and growing conditions (Vaz et al. 2016). Major bioactive compounds extracted from microalgae contained proteins, fatty acids (FA) (mainly omega-3), pigments, carotenoids, polysaccharides, and vitamins, which are highly valuable in seafood production to extend the shelf life and enhance the quality (Guedes, Sousa-Pinto, and Malcata 2015).

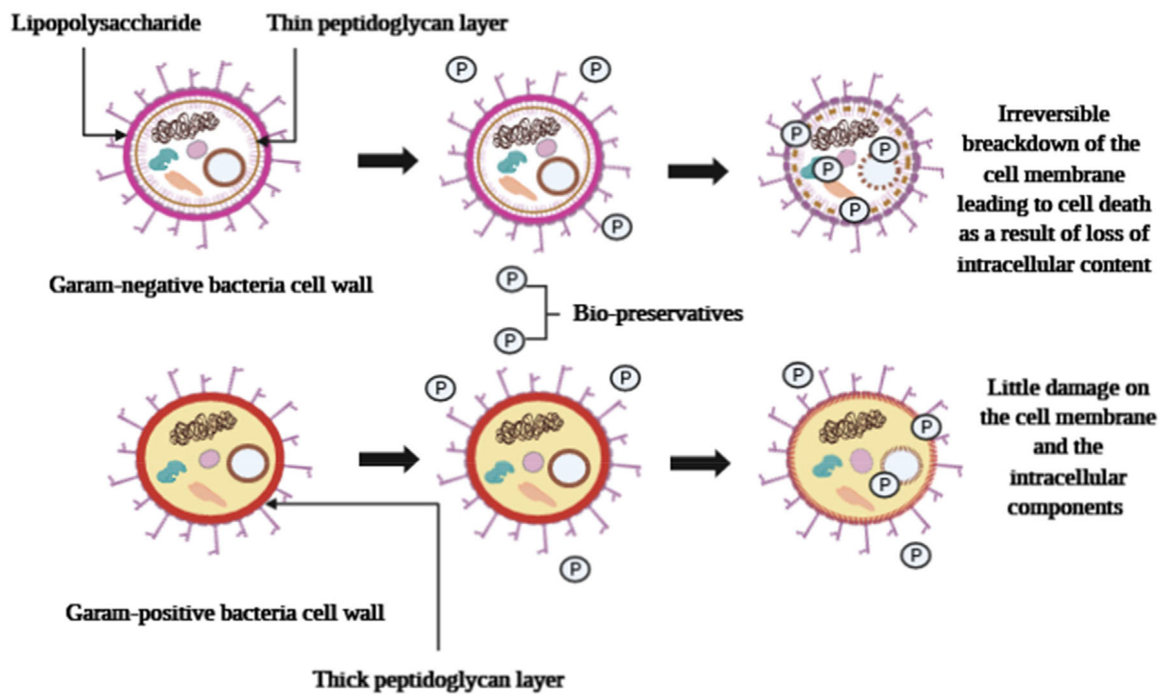

Figure 2. Response of Gram-positive and Gram-negative bacteria to preservatives according to Olatunde and Benjakul (2018).

Besides the physiological and biological functions of microalgae extracts, the sustainability of the processes used to extract and purify the bio-products is also important (Ursu et al. 2014). Recently, not only the efficiency of the extraction methods with the maximum bioactivity and optimum extraction yield have been taken into account, but also the development of environmental-friendly processes that would be preferred over conventional extraction methods (Rajvanshi et al. 2019; Soto-Sierra, Stoykova, and Nikolov 2018). These recent developments have tended to be in-line with the principals of "Green Chemistry Requirements (GCR)" related to extraction: (a) exploring and finding renewable resources; (b) utilization of alternative solvents (especially water); (c) using innovative and cost-efficient technologies; (d) reduction of waste, and production of co-products as an alternative; (e) fostering automation of the operations and minimizing the unit processes; and (f) the production of non-contaminated, non-denatured and biodegradable extracts (Herrero et al. 2015).

To comply with GCR principals, various extraction techniques have been reported. Generally, extraction of bioactive compounds has been done using two major steps, (1) pre-extraction or preparation of samples; and (2) the extraction process (Olatunde and Benjakul 2018). Drying (mainly freeze-drying), grinding, and solvent treatments have been the major processes in the pre-extraction stage of microalgae. The separation of soluble bioactive products from the selected solvents using a standard protocol is the second stage of the extraction process. Water, methanol, acetone, isopropanol, diethyl ether, ethanol, and hexane have been the most common solvents used for extraction (Mansour et al. 2019; Othman et al. 2018; Fayyad et al. 2019). Supercritical fluids, maceration, pressurized liquids, freeze-thawing, Soxhlet, ultrasound and/or microwave-assistance, and accelerating solvents have been the most common methods applied with the solvent extractions (Herrero et al. 2015; Yu et al. 2019; de Jesus et al. 2019).

Application of microalgae extracts for seafood preservation: Antioxidant and antimicrobial efficacy

In the seafood industry various preservatives have been used to extend the shelf-life of commercial products (Olatunde and Benjakul 2018). Among these components, microalgae provide a rich source of extracts and bio-preservative compounds that other raw materials may not offer (Madeira et al. 2017). Bioactive compounds in microalgae extracts can disrupt the cell membrane integrity of bacteria (Schuelter et al. 2019). By increasing the permeability of cell membranes, these active compounds may cause the extensive leaching of critical ions such as potassium, and other cytoplasmic contents. This process may finally lead to cell death. The disruption of bacteria cell membranes mainly caused by the insertion of the hydrophobic compounds of the extracts with the phospholipids of the bacterial cell wall. Gram-positive bacteria have a thicker cell wall compare to Gram-negative bacteria, meaning they are more resistant to inhibition by bio-preservatives (Olatunde and Benjakul 2018). Figure 2 shows a general summary of the potential mechanisms by which antimicrobial agents, such as microalgae extracts, inhibit bacterial growth (Olatunde and Benjakul 2018).

Microalgae polyphenols or extracts have been shown to significantly prevent lipid oxidation in seafood. For example, Taghavi, Haghighat Khajavi, and Safari (2019) investigated the role of alcoholic (ethanol, 96%) extracts of *Spirulina*

Table 3. Studies on potential applications of microalgae products for seafood production.

Microalgae	Concentration	Application	compounds	Reference
Chlorella minutissima, Isochrysis galbana, Picochlorum sp	0.5, 1, and 1.5% w/v	Canned fish burger	Powder	Ben Atitallah et al. 2019
Heterochlorella luteoviridis	2% (w/w)	Salmon packaging	Extract	Carissimi et al. 2018
Chlorella vulgaris, Spirulina platensis	0.05%, 0.1% w/v	Trout fillet	Extract	Taghavi et al. 2019
Haematococcus pluvialis	0.28, 0.56 and 1.12 g 100 g^{-1} diet	Yellow croaker	Biomass	Li et al. 2014
Arthrospira platensis	15, 22.5, 30, 45 g 100 g^{-1} diet	Mullet	Biomass	Rosas et al. 2019
Haematococcus pluvialis	0.2%, 0.4%, 0.6% of diet	Chinese mitten crab	Powder	Long et al. 2017
Haematococcus pluvialis	0.2%, 0.4%, 0.6% of diet	Chinese mitten crab	Powder	Su et al. 2020
Haematococcus pluvialis	100, 200 mg kg^{-1} dietary	Olive flounder	Extract	Pham et al. 2014
Haematococcus pluvialis	25, 50, 75, 100 ppm dietary	Orchid dottyback	Astaxanthin	Jiang et al. 2019
Spirulina platensis, Chlorella vulgaris	50, 75% of each into diet	African catfish	Powder	Raji et al. 2018
Nannochloropsis	4, 7, 10% of diet	Kuruma shrimp	Powder	Oswald et al. 2019
Tetraselmis chui, Chaetoceros muelleri, Isochrysis galbana	2% microalgae on a dry weight basis	Hard clam	Biomass	Chen et al. 2015
Schizochytriumsp	100, 150 g kg^{-1} diet	Atlantic salmon	Biomass	Kousoulaki et al. 2016
Nannochloropsis oceanica	7.5, 15% of diet	Spotted wolffish	Biomass	Knutsen et al. 2019

platensis and *Chlorella vulgaris* on the prevention of lipid oxidation and extending the shelf life of rainbow trout (*Oncorhynchus mykiss*) fillets at refrigerator temperature ($4 \pm 1\,^{\circ}C$). The results for peroxide value, free FA (FFA), and thiobarbituric acid have shown that samples treated with the microalgae extracts (0.1%) significantly controlled the increase in these indexes. The results of the sensory evaluation have also shown that in samples treated with algae extracts, there were fewer negative effects on texture, color and odor (Taghavi, Haghighat Khajavi, and Safari 2019). Polyphenols, phycobiliproteins, and vitamins are the most efficient water-soluble antioxidants in microalgae (Guedes, Sousa-Pinto, and Malcata 2015). Antioxidants compounds or systems retard lipid oxidation by inhibiting the formation of free radicals or interrupting their extension using the following multiple steps and mechanisms: (a) scavenging species that are induced by peroxidation; (b) chelating metal ions, so they cannot produce reactive species that induce oxidation reactions; (c) quenching O_2 and thereby inhibiting the formation or extension of peroxides; (d) breaking and interrupting the auto-oxidative chain reaction; and/or (e) diminishing the localized O_2 concentrations. The ability to interrupt free-radical chain reactions have been considered as the main criterion for ascertaining the efficiency of antioxidants (Olatunde and Benjakul 2018).

Microalgae biomass and powder have been utilized in seafood to enhance the quality and extend the shelf-life by reducing microbial growth and chemical reactions. A recent study by Ben Atitallah et al. (2019) has shown that addition of *Chlorella minutissima*, *Isochrysis galbana*, and *Picochlorum* sp. powder into canned fish burger prepared from common barbel (*Barbus barbus*) significantly improved the total sensory acceptability, texture analysis indexes (hardness, chewiness, gumminess, cohesiveness), nutritional value and functional properties (water and oil holding capacities) as compared to the controls. Analysis of the microbial contamination has also shown that no foodborne pathogens, mold, or yeast grew during two months of storage at $4\,^{\circ}C$. The incorporation of the microalgae also improved the antioxidant potential of the burgers.

For ease of implementation, microalgae extracts or biomass can be incorporated in edible films and packaging materials used for seafood preservation. Carissimi, Flôres, and Rech (2018) have developed biodegradable edible films for salmon packaging using cassava starch enriched with *Heterochlorella luteoviridis* and/or *Dunaliella tertiolecta* biomass. The films have been characterized by their physical, mechanical, optical, barrier, and antioxidant properties. The film contains 2% of *Heterochlorella luteoviridis* biomass showed the best physicochemical, mechanical, and antioxidant properties for salmon packaging. The final results have shown that the enrichment had significantly prevented lipid oxidation and moisture loss in samples over 6 days. Chentir et al. (2019) showed that incorporation of phycocyanin extracted from the microalgae *Arthrospira* sp into gelatin films significantly increased the DPPH radical scavenging capacity and limited the growth of Gram-positive (*Staphylococcus aureus*, *Listeria monocytogenes, Icrococcus luteus*) and Gram-negative (*Pseudomonas* sp., *Escherichia coli*, *Salmonella typhimurium*) bacteria. Balti et al. (2017) have developed bioactive films from spider crab (*Maja crispata*) chitosan incorporated with *Spirulina* extract. The results have shown that the incorporation led to considerable DPPH radical scavenging capacity and antibacterial properties of the film against *L. monocytogenes*, *P. Aeruginosa, E. coli*, *S. typhimurium, P. aeruginosa* and *S. aureus.* An overview of the literature reporting studies on the application of microalgae extracts in seafood products is shown in Table 3.

The effect of microalgae pigments on seafood quality

Along with chemical and microbial characteristics, sensorial properties are important determinants in consumers' attitudes to their choice of seafood (Tavakoli et al. 2018). Appropriate color is an integral attribute of sensory quality of seafood. The impression of pale color is often reflecting a poor quality while, natural and bright colors unconsciously are associated with high-quality seafood (Guedes, Sousa-Pinto, and Malcata 2015). To achieve a suitable color and the appropriate appearance desired by customers, optimized dietary levels of pigments for aquaculture species is necessary. From the point of view of consumers, the concept of "sustainable organic farming" has appeared to be favorable, including the use of natural forms of pigments as an alternative to synthetic forms (Guedes, Sousa-Pinto, and Malcata 2015).

Apart from the direct application of microalgae in seafood as bio-preservatives, research has shown that the incorporation of microalgae pigments into the diet of aquaculture species could improve their meat quality and nutritional value (Madeira et al. 2017; Rosas et al. 2019). Microalgae pigments have been classified into three groups: carotenoids, phycobiliproteins, and chlorophylls, responsible for yellow-orange, red-blue, and green color, respectively (Levasseur, Perré, and Pozzobon 2020). Carotenoids are the major groups of orange and yellow naturally occurring pigments that have been found to be abundant in microalgae (Guedes, Sousa-Pinto, and Malcata 2015). These richly colored compounds are known for their antioxidant potential as well as strong dyeing capability. Carotenoids are frequently utilized in seafood and aqua-feed production (Ejike et al. 2017). Carotenoids have been divided into two main groups; (1) carotenes, those that are oxygen-free and are composed of hydrogen and carbon (e.g., a-carotene, b-carotene, g-carotene, and lycopene); and (2) xanthophylls that are oxygenated derivatives of carotenes (e.g., astaxanthin, canthaxanthin, zeaxanthin and cryptoxanthin) (Vaz et al. 2016). B-Carotene is the most important pigment in seafood production. The natural form of this carotenoid in fishes has shown stronger colorant and antioxidant effects than the synthetic forms and it is also more easily absorbed (Guedes, Sousa-Pinto, and Malcata 2015).

Although the major known source of this pigment is the microalgae *Spirulina*, the most important species for industrial-scale production has been *Dunaliella salina*, with accumulation of up to 15% of dry weight (Vigani et al. 2015). Astaxanthin is another high-value carotenoids, used in the diet of aquaculture species to improve both nutritional value and quality of the farmed species (e.g., crab, shrimp, and salmon) (Pham et al. 2014). Microalgae *Haematococcus pluvialis* and *Chlorella zofingiensis* are the richest sources of astaxanthin (Levasseur, Perré, and Pozzobon 2020).

Phycobiliproteins are hydrophilic protein-pigment complexes, present in all cyanobacterial species such as *Phorphyridium*, *Spirulina*, and *Oscillatoria*. These protein-based pigments are classified as phycocyanins (blue pigments), allophycocyanins (pale-blue pigments), and phycoerythrins (red pigments) (Dineshbabu et al. 2019). Phycocyanin are a promising commercial ingredient in *Spirulina* frequently used for seafood (and other food products) wishing to have an antioxidant claim. Chlorophylls are another group of microalgal pigments with commercial value. These pigments have been used in seafood production as natural dyes and health-promoting substances (Guedes, Sousa-Pinto, and Malcata 2015; Levasseur, Perré, and Pozzobon 2020).

The pink color of salmon flesh has always been associated with the feeding of microalgae carotenoids. Lobster, crab, shrimp, and many fish species such as koi carp and seabream are other aquaculture species being that need colorants in their diet to improve their flesh color (Dineshbabu et al. 2019). Long et al. (2017) reported that dietary supplementation of green algae *Haematococcus pluvialis* powder (0.2, 0.4 and 0.6%) enhanced the coloration and protein content of Chinese mitten crab (*Eriocheir sinensis*). Su, Yu, and Liu (2020) did a study to compare the effects of dietary supplementation with *Haematococcus pluvialis* powder and synthetic astaxanthin on carotenoid composition, astaxanthin isomers, and the quality of Chinese mitten crabs. The results have shown that *H. pluvialis* appeared better able to increase the astaxanthin accumulation and potential nutritive quality of the crabs. Pham et al. (2014) have reported that dietary inclusion of *H. pluvialis* extract at a concentration of 100 mg kg^{-1} improved total skin pigmentation and the color of olive flounder (*Paralichthys olivaceus)*. Guedes, Sousa-Pinto, and Malcata (2015) stated that *Hematococcus pluvialis* has been shown to increase the reddish skin coloration of penaeid shrimp (*Litopenaeus vannamei*). Jiang et al. (2019) investigated the effects of different astaxanthin concentrations (25, 50, 75, and 100 ppm) as a dietary supplement, at different supplementation times (0, 14, 28, 42, 56, and 70 days), on the coloration of *Pseudochromis fridmani* using *Haematococcus pluvialis* as a natural and Carophyll Pink as a synthetic color. The authors concluded that natural astaxanthin from *Haematococcus pluvialis* had been more effective in giving the desired fish coloration.

For any given concentration tested in comparative studies between microalgae pigments (as a natural source) and synthetic colors in aquaculture production, it often took less time for synthetic pigments to reach a maximum yield. However, the color always appeared to be less favorable than the coloration provided by the microalgae pigments equivalents. Since the coloration yield by microalgae pigments equivalents had not yet reached a maximum level at the given concentration, the color of the fishery product could still be enhanced. These results encourage a shift away from synthetic colorants toward natural sources; while also improving seafood quality and consumer acceptability.

The effect of microalgae omega-3 FA on seafood quality

Microalgae are reported to have a lipid content between 2% and 23% of the dried weight. Omega 3 FA comprise 30–70% of the total lipids (Dineshbabu et al. 2019). Some microalgae species such as *Phaeodactylum tricornutum*, *Crytthecodinium cohnii*, and *Schizochytrium sp*. are capable of accumulating a higher yield of PUFA (Torres-Tiji, Fields, and Mayfield 2020). Omega-3 FA are the main group of PUFA. This category mainly includes EPA, DHA, α-linolenic acid, and γ-linolenic acid (Katiyar and Arora 2020). Various fish and other aquatic animal species were enriched with these FA using dietary supplementation either to enhance their nutritional quality or to fulfill their dietary requirements for essential FA (Chen, Tseng, and Huang 2015). The traditional source of omega-3 FA in aqua-feeds has been fish oil produced along with fish meal from marine fishes. However, fishes are rich sources of these nutrients as they consume microalgae as a part of their daily diet (Torres-Tiji, Fields, and Mayfield 2020). EPA, DHA, and α-linoleic acid are the omega-3 FA used in seafood production, and are abundantly found in some microalgae strains. For example, the DHA content of *Schizochytrium limacinum* can account for up to 25%, EPA content of *Phaeodactylum tricornutum* and *Nannochloropsis* sp. can account for up to 39% (Ahmmed et al. 2020), and linoleic acid content of *Nannochloropsis* can account for up to 3% of the total FA content (Guedes, Sousa-Pinto, and Malcata 2015).

Numerous studies have shown that the incorporation of microalgae omega-3 FA in the diet of aquatic animals is a way to enhance seafood quality and provide the healthy FA that humans need in their diet. For example, a recent study by Oswald et al. (2019) has shown that fish oil replacement with *Nannochloropsis* lipid in the diet of kuruma shrimp (*Marsupenaeus japonicas*) could increase the amount of omega-3 FA content of the edible shrimp flesh. Chen, Tseng, and Huang (2015) investigated the FA profiles and biomembrane lipid peroxidation of hard clams (*Meretrix lusoria*) fed the dietary microalgae *Isochrysis galbana*, *Chaetoceros muelleri*, and *Tetraselmis chui* for 8 weeks. Results have shown that FA (particularly EPA and DHA) in the polar lipid fractions of clams reflected those of the dietary microalgae species. Clams fed *Tetraselmis chui* and *Chaetoceros muelleri* had accumulated higher proportions of non-methylene interrupted (NMI) FA than those fed *Isochrysis galbana*. Lipids of the clams fed *I. galbana* have shown the highest proportion of DHA among the tested groups. Sarker et al. (2020) have reported that incorporation of DHA-rich *Schizochytrium sp.* into the diet of Nile tilapia (*Oreochromis niloticus*) could increase the DHA and total lipid content of the fish fillets. Kousoulaki et al. (2016) have shown that the pure spray dried microalgae *Schizochytrium sp.* as a source of long-chain omega-3 polyunsaturated FA in an *Atlantic salmon* diet decreased the amount of gaping in fish fillets.

New sources with high omega-3 FA content will be increasingly needed for aquaculture production. It is also important to achieve desirable levels of omega-3 FA that impart health advantages to consumers, especially in light of the growing attention to utilize plant-based oils as partial replacements for fish oil in the diet of farmed aquaculture species. The marine microalgae *Crypthecodinium cohnii* has been grown successfully using controlled conditions to produce a high-lipid product that is rich in omega-3 FA. The trade name of the product produced from *Crypthecodinium cohnii* is "Aqua Grow" (Hardy 2008).

The application of microalgae proteins to seafood production

Some species of microalgae are rich in protein that compete favorably, in terms of quality and quantity, with conventional food proteins including egg, fish, and soybean (Ejike et al. 2017). For example, most strains of *Spirulina*, a few strains of *Chlorella,* and *Nannochloropsis* have been reported to have between 40% and 65% crude protein content (Dineshbabu et al. 2019). Microalgae proteins contain well-balanced amino acid profiles, comparable to those of eggs and soybeans. However, these proteins may be associated with lower digestibility, efficiency ratio, and biological characteristics than egg and casein. The amount of synthesized proteins in microalgae depends on different factors such as species type, lighting (period and quality), growth phase and medium modification along with how they had been manipulated to affect the environmental stress (Ejike et al. 2017).

Microalgae protein products have been classified based on their protein content and the processing involved as whole-cell protein, concentrates, isolates, hydrolysates, and bioactive peptides (Soto-Sierra, Stoykova, and Nikolov 2018). Whole-cell protein is protected by a cellular structure that may not be bioavailable since the intracellular proteins are not readily accessible to digestive enzymes (Bernaerts et al. 2019). Microalgae whole-cell protein differs significantly among different species and may be from 40% to 50% of the total protein content (Soto-Sierra, Stoykova, and Nikolov 2018). Although the presence of cellulose in the microalgae cell wall makes it difficult to digest, mechanical or chemical treatments, and alternative heating have seemed to substantially modify their digestibility by the breakdown of proteins into smaller peptides (Rajvanshi et al. 2019). For the preparation of concentrated protein products (60–95% dry weight), such as protein concentrates, hydrolysates, isolates, and bioactive peptides; soluble proteins from microalgae cells should be released using either a single technique or a combination of mechanical (such as bead milling, ultrasonication, high-pressure homogenization, pulse electric field, and microwave) or non-mechanical (chemical and enzymatic) cell digestion techniques. Then, the extracted protein should be concentrated (Soto-Sierra, Stoykova, and Nikolov 2018).

Several studies have reported promising functionality properties for microalgae proteins. Microalgae proteins have shown beneficial surface-related properties such as emulsifying and foaming activity. For example, Ursu et al. (2014) investigated the emulsifying ability of proteins from *Chlorella vulgaris*. The authors concluded that the emulsifying capacity and/or stability of the microalgae protein remained comparable or even higher than some commercial proteins such as sodium caseinate. Similar results were obtained for the emulsifying properties of protein isolates from the green microalgae *Tetraselmis* sp. by Schwenzfeier et al. (2013). On the other hand, microalgae proteins can be favorable for direct application with seafood because of their hydrodynamic properties such as gelling or thickening. Garcia et al. (2018) studied the rheological properties of proteins extracted from *Tetraselmis suecica* and concluded that they had better gelation behavior compared to whey protein isolate. Similar gelling behavior was reported by Chronakis (2001) for protein isolate from *Arthrospira platensis*. During this study, an increase in viscosity above 50 °C was observed, which resulted in an elastic gel upon heating to 90 °C. The authors have reported that protein isolates from the microalgae *Arthrospira platensis* had favorable gelling ability at concentrations between 1.5% and 2.5%.

Microalgae proteins are also an important food source in aquaculture production. In aquaculture, producers commonly rely on formulated feeds to ensure optimal growth,

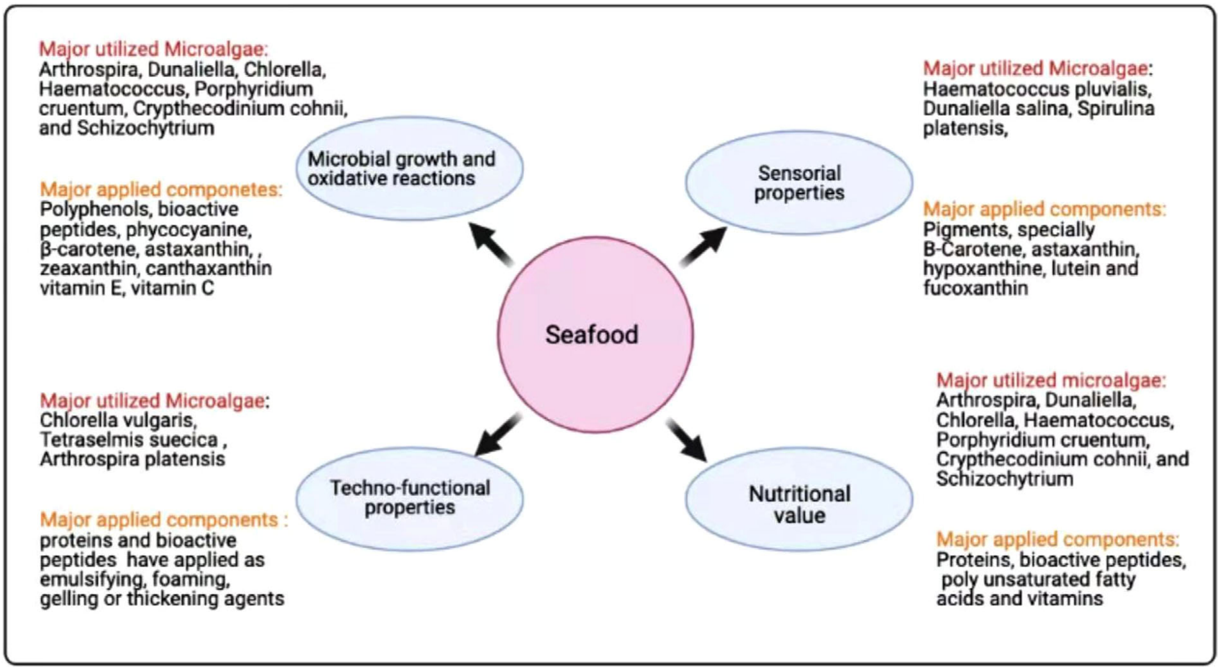

Figure 3. Application of the major microalgae species as well as their major bioactive compounds in different areas of seafood.

health, and quality of the farmed species (Rajvanshi et al. 2019). Fishmeal is usually supplied as the primary protein source in commercial aqua-feeds. However, the continuous growing consumption of seafood, the ever-increasing price of fishmeal, and the decline in fishmeal production have led to the search for suitable protein sources for aqua-feed production (Dineshbabu et al. 2019). Microalgae can be a suitable alternative to fishmeal due to their high protein content and the presence of all essential amino acids. Many physiological and toxicological studies on the application of microalgae protein in aqua-feed were done earlier to show their beneficial effects on seafood quality and production ratio. For example, Ju, Deng, and Dominy (2012) utilized a defatted microalgae *Haematococcus pluvialis* meal as a protein ingredient to partially replace fishmeal protein (12.5, 25, 37.5 or 50%) in diets of Pacific white shrimp (*Litopenaeus vannamei*). After 8 weeks of feeding, shrimp fed the four defatted microalgae-added diets had better color quality than the shrimp fed the control diet. In another study by Tibbetts, Yasumaru, and Lemos (2017), reliable digestion of diets supplemented with protein from the microalgae *Nannochloropsis granulata* for rainbow trout (*Oncorhynchus mykiss*) and Pacific white shrimp (*Litopenaeus vannamei*) have been shown to be effective (Tibbetts, Yasumaru, and Lemos 2017). Sarker et al. (2020) have reported that incorporation of protein-rich defatted biomass of *Nannochloropsis oculata* into the diet of Nile tilapia (*Oreochromis niloticus*) could increase the in-vitro protein hydrolysis and protein digestibility of the fish fillets. Microalgae proteins have become a valuable and cost-effective alternative for fishmeal in aqua-feeds, and their utilization in feed supplementation could ease the impending crisis for fishmeal-based aquaculture. It will be helpful to minimize the gap between seafood production and demand, by reducing the burden on aqua-feed production.

The application of microalgae vitamins and minerals to seafood production

Microalgae can accumulate high levels of vitamin A, B1, B2, B6, B12, C, E, K, niacin, nicotinate, biotin, and folic acid (Madeira et al. 2017). Vitamins are essential primary aqua-feed ingredients in the farming of all stages of several fish species, shrimps, bivalves, and mollusks; and microalgae provide them as part of their diet rather than as synthetic additives (Dineshbabu et al. 2019). These components are essential for the growth and development of aquatic animals as they are not able to synthesize them. Vitamins have an important antioxidant role in the scavenging of reactive oxygen species (ROS) (Levasseur, Perré, and Pozzobon 2020). The vitamin content of microalgae differs among the different species, and has been correlated with growth conditions and growth phase. The variation has been greatest for vitamin C, from 1 to 16 mg g^{-1} dry weight. Some species of *Chlorella* were reported to have higher amounts of vitamins than many cultivated land plants used for food. *Spirulina spp.* have also been reported to have over 10-fold more β-carotene (provitamin A) than any other foods, and more vitamin B12 compared to any fresh plant or aquatic animal feed source (Guedes, Sousa-Pinto, and Malcata 2015). High amounts of vitamin D3 present in the flesh of salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) originated from microalgae and zooplankton in their diet (Ljubic et al. 2020).

Microalgae also have a high and varied composition of minerals, among which the most abundant are calcium, potassium, phosphorous, magnesium, iron, sodium, sulfur, zinc, and copper (Dineshbabu et al. 2019). The mineral content of microalgae ranges from 2.2% to 4.8% of total dry weight (Guedes, Sousa-Pinto, and Malcata 2015). These atoms have shown a strong potential as natural sources to replace inorganic mineral salts commonly used for aqua-feed. It has also been reported that natural forms of many of the minerals are more bioavailable to the aquatic animals than synthetic forms, and can be manipulated or replaced by controlling bio-absorption (Guedes, Sousa-Pinto, and Malcata 2015). Guedes, Sousa-Pinto, and Malcata (2015) have also reported that the incorporation of mineral-rich microalgae into salmon diets could improve the texture and flavor of the fish.

The effect of microalgae carbohydrates on seafood quality

Microalgae are a good source of carbohydrates ranging from 10% to 25% of dry weight (Dineshbabu et al. 2019). The major microalgae used for polysaccharides extraction are *Tetraselmis sp.*, *Isochrysis sp.*, *Porphyridium purpureum, Porphyridium cruentum*, *Rhodella reticulate,* and *Chlorella sp.* (Levasseur, Perré, and Pozzobon 2020). Carbohydrates in microalgae are mainly composed of a mixture of amino sugars, neutral sugars, and uronic acids. They are very different from one species to another. Glucose, rhamnose, xylose, mannose, galactose, fucose, arabinose, ribose, glucosamine, galactosamine, glucuronic acid, and galacturonic acid have been reported as the most common monosaccharides of the microalgae carbohydrates (Dineshbabu et al. 2019; Levasseur, Perré, and Pozzobon 2020). Carbohydrate content can be manipulated through the growing period to reach up to 40% of dried weight in certain species. For example, the microalgae *Scenedesmus obliquus* may be able to accumulate carbohydrates between 10 and 47% of its dried mater depending on growing conditions. The same is true for *Chlorella vulgaris* which could synthesize carbohydrates between 9% and 41% of its dry matter content (Dineshbabu et al. 2019).

Microalgae polysaccharides have been classified in terms of physiological function into three groups: (a) energy reserve polysaccharides, (b) structural polysaccharides that participate in cell wall formation, and (c) polysaccharides involved in cell communication (Bernaerts et al. 2019). Structural polysaccharides from microalgae are of interest for use with seafood and other foods due to their relatively easy extraction and their rheological properties such as gelling or thickening capability (García, Vicente, and Galán 2017). The microalgae *Chlorella* sp. is a rich source of β-glucan, which is a polysaccharide composed of β-D-glucose. β-glucan has been reported to have significant antibacterial activity in fishes (Dineshbabu et al. 2019). Rajasekar et al. (2019) have reported that the supplementation of 2% sulfated polysaccharide from *Spirulina platensis* into a feed for the Zebra fish *Danio rerio* could enhance the reproductive performances and nutritional properties of the fish. The high carbohydrate content of microalgae could also enhance the growth rate of omnivorous fishes such as the common carp (Guedes, Sousa-Pinto, and Malcata 2015). An overview of the literature reporting studies on the application of the major microalgae species as well as their major bioactive compounds in different areas of seafood are shown in Figure 3.

Safety aspects and regulatory issues of products from microalgae

Several studies have reported on the safety issues hazards related to microalgae, which may in some cases contain toxins, allergens and heavy metals (Medeiros et al. 2021). It is important to know the safety of these materials if they are to be used for animal or human food. The toxin producing microalgae often belong to the *Cyanophyceae* class (Jacob-Lopes et al. 2019). No toxins have been detected in the microalgae *Chlorella* and *Spirulina*. However, microcystine toxins have been found in *Aphanizomenon flos-aquae*. Allergens have been found in airborne cyanobacteria such as *Nostoc muscorum* and *Phormidium fragile* (van der Spiegel, Noordam, and Fels-Klerx 2013). Another safety issue is the possibility of heavy metals accumulation in microalgae. Since microalgae are the primary level of the aquatic food chain, they can transfer accumulated heavy metals to levels of the trophic chain further up in marine environments (Medeiros et al. 2021). Between the microalgae varieties that reached commercial production, some already have been classified as GRAS (as previously mentioned) by the US FDA. Furthermore, the microalgae *Nannochloropsis sp.*, *Phaeodactylum tricornutum*, *Nitzschia sp.*, *Haematococcus pluvialis*, *Schizochytrium sp.*, and *Dunaliella sp.* have been classified as "no known toxins" by the FDA. In terms of microalgae-based products, DHA from *C. cohnii*, β-carotene from *Dunaliella salina*, astaxanthin esters from *Haematococcus pluvialis*, single-cell oils from *Schizochytrium sp.* and *Ulkenia sp.*, have already been approved as safe food ingredients by the FDA and the European Food Safety Authority (EFSA) (Jacob-Lopes et al. 2019).

Regulatory aspects and safety requirements of microalgae extracts vary among different countries. For instance, in 2017, the European Committee for Standardization (CEN) has established a technical committee (TC 454) of more than 30 specialists to develop standardization deliverables for “Algae and Algae Products”. The goal was to develop new European standards within five years that will increase consumer trust and improve the algae market overall. In Europe, three levels of regulations were considered for the application of microalgae and its extracts in food or feed: (a) general food safety regulations: this regulation states that microalgae cells or derivatives shall be subjected to the food safety regulations such as European Countries regulation EC No 852/2004 or European Parliament and the Council’s regulation on Food Safety EC No 178/2002, regarding the hygiene of foodstuffs; (b) novel food ingredients: this regulation updates the recent technological progress and clarifies the categories of food which constitute novel foods; (c) nutrition and health claims: this regulation covers the health claims on food and feed products based on the generally accepted scientific evidence. In the USA, microalgae has been included as an agricultural commodity in the 2018 Farm Bill. This confirms that the US Department of Agriculture (USDA) supporting microalgae as a reliable crop in a variety of ways; from scientific research and market programs to crop insurance. Asian countries, especially China and Japan, are interested in the microalgae-based industry because of the desire to open sustainable new markets in food and feed sections. The China government has regulated food production, food processing, food packaging, drug production, and trade regulation. In 2015, The Food Safety Law has amended and enforced by the National Medical Products Administration (NMPA). The New Food Safety Legislation takes into account the new raw materials and their function. Currently, a new guideline titled "Opinions on Deepening Reform and Strengthening Food Safety Work" has published in 2019. These guidelines have proposed serious supervision, strict standards and accountability (Novoveská et al. 2019).

Overall, the designation of the safety aspects of microalgae (or microalgae-based products) depends on the factors such as time and costs to perform safety tests. Considering the increasing number of scientific information generated by academy, startups, and multinational companies indicating the feasibility of microalgae cultivation to obtain biomass or high-added-value products, this scenario is likely to be changed in the soon future.

Conclusion and future remarks

Microalgae biomass and its derivatives (extracts and bioactive compounds) have increasingly attracted the interest of seafood manufacturers and consumers. These products have multiple functions, such as natural pigments, potential antimicrobial agents and antioxidants, health-related nutritional compounds, and ingredients with improved technological properties to be added to seafood. However, there are still many challenges faced in the production and utilization of microalgae biomass or its derivatives in food industries. These challenges are mainly related to the production cost, safety evaluation, and application of ideal concentrations. Hence, future studies are still necessary to determine the optimum concentration for the use of microalgae extracts on a large scale in the seafood industry and to develop effective strategies to prevent the occurrence of spoilage in the food product as well as to enhance the consumer's well-being. Furthermore, it is essential to conduct more clinical trials in humans to attest to the efficacy, safety, bioavailability, and beneficial effects of microalgae-based products.

Disclosure statement

The authors declare they have no conflicts of interest.

Abbreviations

FA	Fatty Acid
PUFA	Polyunsaturated fatty acid
EPA	Eicosapentaenoic Acid
DHA	Docosahexaenoic Acid
GRAS	Generally Recognized as Safe
FDA	Food and Drug Administration
WHO	World Health Organization
NASA	National Aeronautics and Space Administration
NMI	Non-methylene Interrupted
GCR	Green Chemistry Requirements
CEN	European Committee for Standardization
EC	European Countries
USDA	Department of Agriculture of the United States of America
NMPA	National Medical Products Administration

Funding

This work was supported by National Key R&D Program of China (2018YFD0901001). Thanks to the China Scholarship Council (CSC) for the Ph.D program.

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