Discover Food

Review

Bioactive compounds from micro-algae and its application in foods: a review

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Abstract

The marine ecosystem is a vast source of diversified biota that includes more than half of the world's biota including marine microorganisms; algae, invertebrates, and fish. Most of these organisms constitute complex biomolecules, allowing, them to thrive under extreme environmental conditions. These biomolecules are proteins, lipids, polysaccharides, phenolics, peptides, etc. perform various biological functions depending on the source. In addition to the basic functions**,** they also exhibit diverse functional properties such as antimicrobial, antioxidant, anti-inflammatory, anticoagulant, antidiabetic and antihypertensive properties etc., which make them potential candidates for application in the food and pharmaceutical industry. The marine microalgae and cyanobacteria are a very rich source of these functional molecules with several biological applications related with health benefits and food applications. The viewpoint details the potential and bioactive compound profile of marine micro algae, extraction and characterization of bioactive compounds from marine micro algae and its application in the food industry.

Keywords Bioactive compounds · Functional foods · Functional properties · Marine micro algae · Nutraceuticals

1 Introduction

Microalgae, a diverse assemblage of photosynthetic microorganisms, are captivating researchers with their ability to bridge the gap between environmental sustainability and nutraceutical potential. Not only do they efficiently capture carbon dioxide and light, converting them into biomass rich in primary metabolites (lipids, carbohydrates, proteins, and pigments), but they also hold great promise for producing high-value bioactive compounds [1]. With more than 7000 identified species [2], boasting rapid growth rates and the ability to thrive in diverse environments (freshwater, brackish water, seawater), microalgae offer a versatile platform for sustainable production of bioactive compounds [3, 4]. Their impressive adaptability comes from their ability to form highly resistant spores, ensuring survival in harsh conditions [5].

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The allure of microalgae extends beyond their primary metabolites. They show remarkable versatility in generating a wide range of valuable compounds, including lipids for biofuels, polyunsaturated fatty acids (PUFAs) for nutritional supplements, and carotenoids for food and pharmaceutical applications [6]. This impressive repertoire of chemicals, which includes peptides, carotenoids, steroids, fatty acids, polysaccharides, and halogenated compounds, exhibits a broad spectrum of biological activities, particularly antibacterial and anticancer properties [7]. Their modes of action range from targeting specific cellular pathways to inhibiting cell division and inducing apoptosis [1], effectively contributing to the prevention and treatment of various diseases, including cancer. For example, the potent antioxidant astaxanthin, abundantly found in certain microalgae species, emerges as a "super antioxidant" with a plethora of health benefits [2, 8, 9].

Microalgae-based bioactive compounds offer a win–win scenario for both environmental sustainability and economic viability. Their potential for co-production of high value added products presents an attractive strategy to offset the high production costs associated with microalgae cultivation and processing [10]. Furthermore, compared to land plants traditionally relied on for natural products, microalgae boast an estimated tenfold greater diversity in their bioactive compounds [11]. This extraordinary richness underscores their immense potential as a source of novel and valuable bioactive molecules (Table 1). Microalgae also possess inherent advantages that facilitate the extraction and utilization of their bioactive compounds. Unlike terrestrial plants, they lack intricate lignocellulosic cell walls and root systems, making them easily degradable and simplifying the extraction process [12]. This attribute differentiates them from land plants and enhances their suitability for bioactive compound extraction. In addition, microalgae production is largely independent of climatic fluctuations, leading to higher and more consistent yields compared to land-based crops [13]. This year-round reliability underscores their suitability for sustainable and consistent production of bioactive compounds for diverse applications, spanning the nutritional and pharmaceutical sectors.

The unique combination of diverse bioactive compounds, ease of processing, and consistent year-round production position microalgae as an untapped and valuable resource for the development of multifaceted food applications. As research delves into their bioactive potential, unlocking these tiny powerhouses promises to revolutionize not only the food industry but also various health and industrial sectors.

2 Microalgae as sources of bioactive compounds

Microalgae encompass various taxonomic groups, including diatoms, dinoflagellates, green algae, red algae, and blue-green algae. They exhibit diverse morphological characteristics, and their taxonomic identification is based on morphological and genetic features [14]. The physiological and ecological characteristics of microalgae vary widely depending on their taxonomic group and habitat. For example, they can be found in freshwater and marine environments, and their growth and metabolic activities are influenced by factors such as light, temperature, and nutrient availability [14].

2.1 Advantages and challenges of microalgae cultivation and harvesting for food applications

Microalgae boast immense potential as a sustainable source of bioactive compounds for food applications. However, successful commercialization is dependent on efficient cultivation and harvesting practices. The table (Table 2) combines the advantages and challenges discussed above, highlighting their relationship across key aspects.

3 Microalgal bioactive compounds for functional food formulation

The extraction, purification, and modification of bioactive microalgal compounds are crucial steps to exploit their potential for application in the food industry. Solvent extraction, enzymatic hydrolysis, membrane filtration, and chemical modification are among the key methods and technologies used in this process (Table 3). Each technique has its advantages and limitations, and careful optimization and selection are necessary to maximize the yield, bioactivity, and safety of the extracted compounds.

Table 1 Bioactive Compounds from marine, brackish, and freshwater microalgae and their applications in food

Environment	Microalgae	Bioactive compounds	Functional properties	Food applications	References
Marine	*Isochrysis galbana* (Coccolithophyceae)	DHA (omega-3 PUFA)	Anti-inflammatory, brain health, vision support	Infant formula, functional yoghurt, bread enrichment	[75]
	Alexandrium tamarense (Dinophyceae)	Saxitoxins, polyketide toxins, fatty acids	Toxin, nutrient source, omega-3 source	Toxin detection kits, functional foods, omega-3 supplements	[76]
	Haematococcus lacustris (as *Haematococcus pluvialis*) (Chlorophyceae)	Astaxanthin	Antioxidant, anti-cancer, skin health	Seafood, dietary supplements, pasta fortification	[77]
	Conticribra weissflogii (as *Thalassiosira weissflogii*) (Mediophyceae)	Silica, long-chain polyunsaturated fatty acids	Diatomaceous earth, omega-3 source	Food fortification, nutritional supplements, bio-based materials	[78]
	Dunaliella salina (Chlorophyceae)	β-carotene	Antioxidant, immune system support, vision health	Margarine, juices, yogurt fortification	[79]
	Phaeodactylum tricornutum (Bacillariophyceae)	EPA, fucoxanthin, polyunsaturated fatty acids	Omega-3 source, antioxidant, anti-inflammatory	Nutritional supplements, functional foods, skin care	[80]
	Dunaliella tertiolecta (Chlorophyceae)	Beta-carotene, glycolipids, dunaliellin	Provitamin A, membrane stability, antioxidant	Food coloring, nutraceuticals, antioxidant supplements	[81]
	Arthrospira platensis (as *Spirulina platensis*) (Cyanophyceae)	Phycocyanin	Antioxidant, anti-inflammatory, immune system support	Noodles, smoothies, protein bars	[82]
	Phaeocystis pouchetii (Coccolithophyceae)	Polysaccharides, long-chain polyunsaturated fatty acids	Prebiotic, omega-3 source, diatomaceous earth	Prebiotic supplements, omega-3 supplements, food fortification	[83]
	Nannochloropsis oceanica (Eustigmatophyceae)	EPA, DHA, astaxanthin, lutein	Antioxidant, eye health	Fortified foods, omega-3 supplements, colorant in seafood	[84]
	Chaetoceros calcitrans (Mediophyceae)	Omega-3 fatty acids, carotenoids, silica	Omega-3 source, antioxidant, diatomaceous earth	Omega-3 supplements, functional foods, food fortification	[85]
	Schizochytrium limacinum (Labyrinthulomyceteae)	Docosahexaenoic acid (DHA), astaxanthin, betaine, enzymes	Omega-3 fortification, antioxidant potential, nutritionalenhancement, sensory improvement	Infant formula and baby food, functional beverages, bakery products, confectionery and snacks, dietary supplements	[86–88]
	Lyngbya majuscula (Cyanophyceae)	Majusculamide C, aeruginosin 290A, cylindrospermopsin (CYN), majusculamide A	Potent antitumor and antiproliferative activities, immunomodulatory activity	Aquaculture feed, dietary supplement, functional food ingredient	[89]

Table 1 (continued)

Environment	Microalgae	Bioactive compounds	Functional properties	Food applications	References
Brackish	*Nannochloropsis oculata* (Eustigmatophyceae)	EPA (omega-3 PUFA)	Anti-inflammatory, cardiovascular health, cognitive function	Aquaculture feed, nutraceuticals, enriched eggs	[55]
	Nannochloropsis limnetica (Eustigmatophyceae)	EPA, DHA, astaxanthin, lutein, phycocyanin	Omega-3 fatty acids, antioxidant, eye health	Fortified foods, omega-3 supplements, colorant in seafood	[90]
	Tetraselmis chui (Chlorodendrophyceae)	Docosahexaenoic acid (DHA)	Brain health, vision development, cognitive function	Infant formula, dairy products, functional beverages	[26]
	Porphyridium purpureum (as *Porphyridium cruentum*) (Porphyridiophyceae)	Omega-3 fatty acids, phycobiliproteins	Cardiovascular health, antioxidant	Omega-3 supplements, food colorant, nutraceuticals	[91]
	Chaetoceros muelleri (Mediophyceae)	Omega-3 fatty acids, fucoxanthin, silica	Joint health, antioxidant, diatomaceous earth	Omega-3 supplements, functional foods, food fortification	[92]
	Tetraselmis suecica (Chlorodendrophyceae)	Omega-3 fatty acids, fucoxanthin, polysaccharides	Lipid-lowering, antioxidant, prebiotic	Functional foods, nutritional supplements, prebiotic additives	[26]
	Dunaliella salina (Chlorophyceae)	Glycerol	Humectant, sweetener, anti-freezing agent	Baked goods, confectionery, ice cream	[93]
	Prymnesium parvum (Coccolithophyceae)	Prymnesin, fatty acids, polysaccharides	Toxin, nutrient source, immune-modulating	Toxin detection kits, functional foods, nutraceuticals	[94]
	Skeletonema costatum (Mediophyceae)	Long-chain polyunsaturated fatty acids, silica	Omega-3 source, diatomaceous earth	Omega-3 supplements, functional foods, food fortification	[95]
	Thalassiosira pseudonana (Mediophyceae)	Silica, long-chain polyunsaturated fatty acids	Diatomaceous earth, omega-3 source	Food fortification, nutritional supplements, bio-based materials	[78]

Table 1 (continued)

Environment	Microalgae	Bioactive compounds	Functional properties	Food applications	References
Freshwater	*Chlorella vulgaris* (Trebouxiophyceae)	Chlorophyll	Detoxification, antioxidant, immune system support	Dietary supplements, juices, leafy green alternatives	[96]
	Haematococcus lacustris (as *Haematococcus pluvialis*) (Chlorophyceae)	Astaxanthin	Antioxidant, anti-cancer, skin health	Aquaculture feed, cosmetics, nutraceuticals	[97]
	Tetradesmus obliquus (as *Scenedesmus obliquus*) (Chlorophyceae)	Lutein and zeaxanthin	Eye health, macular degeneration prevention	Eggs, fortified cereals, dietary supplements	[98]
	Tetradesmus almeriensis (as *Scenedesmus almeriensis*) (Chlorophyceae)	Proteins, carotenoids, vitamins B1, B2, B3, B6, and minerals-like iron, magnesium, andphosphorus; phycocyanin	Nutritional enhancement	Aquaculture feed, functional foods	[99, 100]
	Ankistrodesmus falcatus (Chlorophyceae)	Omega-3 fatty acids, polyphenols, chlorophyll	Cardiovascular health, antioxidant, anti-inflammatory	Omega-3 supplements, functional foods, nutraceuticals	[101]
	Botryococcus braunii (Trebouxiophyceae)	Hydrocarbons, proteins, polysaccharides	Biofuel production, protein-rich, antioxidant	Biofuel feedstock, algae-based protein production	[102]
	Ecdysichlamys minuta (as *Oocystis minuta*) (Trebouxiophyceae)	Lutein, zeaxanthin, polysaccharides	Eye health, antioxidant, prebiotic	Functional foods, nutraceuticals, prebiotic supplements	[103]
	Monoraphidium contortum (Chlorophyceae)	Omega-3 fatty acids, phycocyanin, polysaccharides	Lipid-lowering, antioxidant, immune-modulating	Nutritional supplements, functional foods, algae-based proteins	[104]
	Chlorococcum infusionum (Chlorophyceae)	Carotenoids, proteins, lipids, chlorophyll	Antioxidant, protein-rich, lipid source	Functional foods, algae-based protein production, biofuels	[105]
	Kirchneriella lunaris (Chlorophyceae)	Polyunsaturated fatty acids, proteins, chlorophyll	Cardiovascular health, protein-rich, antioxidant	Nutritional supplements, functional foods, algae-based proteins	[106]
	Monoraphidium minutum (as *Selenastrum minutum*) (Chlorophyceae)	Beta-carotene, proteins, lipids	Provitamin A, protein-rich, lipid source	Food coloring, algae-based protein production, biofuels	[10]
	Desmodesmus subspicatus (Chlorophyceae)	Omega-3 fatty acids, chlorophyll, polysaccharides	Cardiovascular health, antioxidant, prebiotic	Omega-3 supplements, functional foods, prebiotic supplements	[107]
	Aphanizomenon flos-aquae (Cyanophyceae)	Phycocyanin, lavonoids and phenolic acids, aphanizomezon	Antioxidant, anti-inflammatory, immune-boosting, cholesterol-lowering, neuroprotective	Dietary supplements, food fortification, functional foods, algae-based foods	[108]
	Coelastrella striolata var. *multistriata* (Chlorophyceae)	Mannitol, fucoidan, phlorotannins	Anti-inflammatory activity, antimicrobial activity, immunomodulatory activity, gastrointestinal health benefits	Dietary supplement, thickener, emulsifier, stabilizer, and texturizer	[109]

Table 2 Advantages and challenges of microalgae cultivation and harvesting for food applications

Aspect	Advantages	Challenges	Reference
Environmental sustainability	- Utilizes non-arable land and low-quality water - Absorbs CO_2 during growth, contributing to greenhouse gas mitigation. - Potential for wastewater treatment through nutrient uptake - Recovery of nutrients from wastewater - Low freshwater usage compared to traditional crops	High energy consumption in closed photobioreactors - Environmental factors (temperature, pH, salinity, inorganic carbon, oxygen, light intensity, and CO_2)	[110–112]
Biomass yield and productivity	- Rapid growth rates compared to terrestrial plants - High oil and bioactive compound production potential - Closed systems offer controlled environments for optimized yields - Higher biomass yield compared to terrestrial crops - Rich source of bioactive compounds with potential health benefits - Nutritional profile suitable for food and nutraceutical applications	- Nutrient limitation impacting yield - Harvesting methods (centrifugation, flocculation, filtration, and screening) - High harvesting costs due to small cell size. - Biomass yield and quality fluctuations based on species and conditions - The mass fractions in culture broth are generally low	[110, 113–115]
Versatility and adaptability	- Wide range of bioactive compounds produced depending on species and cultivation conditions - Amenable to manipulation through genetic engineering for improved yields and quality - Can be integrated with aquaculture systems for mutual benefit - High adaptability to diverse environmental conditions	Contamination risks in open pond systems. - Downstream processing efficiency depends on biomass quality and composition	[116]
Bioreactor design flexibility	- Open pond systems are low-cost but susceptible to contamination. - Closed photobioreactors offer precise control over growth conditions but are energy-intensive - Hybrid systems combine advantages of both approaches - Scalability and flexibility in bioreactor systems - Integration with wastewater treatment for nutrient recycling	Development of cost-effective and scalable harvesting methods for closed systems - Risk of biofilm formation in photobioreactors	[117]
Economic viability and scalability	- Potential for cost-competitive production with technological advancements and optimized processes	Current production costs often exceed traditional sources. - Scaling up requires infrastructure development and innovative business models	[118]

Table 3 Different extraction methods for extracting bioactive compounds from marine algae

Applications of Enzyme assisted extraction (EAE) for bioactive compounds from marine macroalgae

Marine algae	Conditions	Bioactive compound	Key advantages	References
Undaria pinnatifida (Phaeophyceae)	Alginase, lyase enzymes (37 °C, pH 6.2)	Fucoxanthin	Increased yield, improved purity, reduced solvent usage	[119, 120]
Sargassum horneri (Phaeophyceae)	Carbohydrases, proteases	Antioxidant extracts	Enhanced extraction of phenolics and flavonoids, reduced thermal degradation	[121, 122]
Brown seaweed species	Carbohydrases, proteases	Antioxidant extracts	Improved recovery of polysaccharides and antioxidant activity compared to conventional methods	[57, 123]
Ultrasound-assisted extraction (UAE) for bioactive compounds from marine algae				
Sargassum spp. (Phaeophyceae), *Hypnea spinella* (Florideophyceae), *Porphyra* sp., *Undaria pinnatifida* (Phaeophyceae), *Chondrus crispus, Halopithys incurva* (Florideophyceae)	Sonication 30 min	Isoflavones	Enhanced yield, improved purity, reduced solvent usage	[124, 125]
Porphyra (Bangiophyceae), *Palmaria* (Florideophyceae), *Undaria pinnatifida, Himanthalia elongata, Laminaria ochroleuca* (Phaeophyceae)	17 kHz frequency, 65 °C	Minerals	Efficient mineral extraction, reduced processing time, preservation of heat-sensitive minerals	[126, 127]
Applications of Microwave assisted extraction (MAE) for bioactive compounds from marine algae				
Dunaliella tertiolecta (Chlorophyceae)	56 °C, atmospheric pressure	Carotenoids	Efficient extraction, improved purity, shorter extraction time compared to conventional methods	[128, 129]
Fucus vesiculosus (Phaeophyceae)	200–800 kPa pressure, 1–31 min extraction, 1/25–5/25 g/mL alga/water ratio	Fucoidan	Selective fucoidan extraction, enhanced yield, reduced solvent dependence	[130, 131]
Red and brown algae (Nori, Dulse, Wakame, Sea spaghetti, Kombu, Sea Lettuce)	200 °C, 1000 W power, 0–5 min holding time	Iodine	Rapid and efficient iodine extraction, improved purity, reduced extraction waste	[132, 133]
Supercritical fluid extraction (SFE) for bioactive compounds from marine algae				
Haematococcus lacustris (as *Haematococcus pluvialis*) (Chlorophyceae)	Ethanol as solvent	Astaxanthin	Enhanced yield and purity compared to conventional methods	[134, 135]
Tetradesmus almeriensis (as *Scenedesmus almeriensis*) (Chlorophyceae)	40 MPa pressure, 60 °C temperature	Carotenoids	Selective extraction of specific carotenoids, high purity, reduced solvent usage	[136, 137]
Dunaliella salina (Chlorophyceae)	Methanol as solvent	Chlorophyll	Efficient chlorophyll extraction, preservation of bioactivity	[136, 138]
Hypnea charoides (Florideophyceae)	40–50 °C temperature, 24.1–37.9 MPa pressure	Polyunsaturated fatty acids	Selective extraction of PUFA profiles, improved stability	[139, 140]
Botryococcus braunii, Chlorella vulgaris (Trebouxiophyceae), *Dunaliella salina* (Chlorophyceae)	30 MPa pressure, 40 °C temperature	β-carotene	High purity β-carotene extraction, reduced processing time	[2, 141]
Sargassum muticum (Phaeophyceae)	CO_2 with 12% ethanol, 15.2 MPa pressure, 60 °C, 90 min	Polyphenols	Targeted polyphenol extraction, improved antioxidant activity, solvent-free process	[142, 143]

3.1 Extraction technologies to effectively extract bioactive compounds from microalgae

The collection of marine algae consists primarily of harvesting from coastal areas or beaches. The collected algae then undergo a rigorous cleaning process to remove salt residues, impurities, and epiphytes. This typically involves multiple washing steps with seawater or freshwater, depending on the specific protocol. To optimize extraction, the cleaned algae are often dried and milled to increase their surface area and allow for uniform distribution. This enhances the efficiency of subsequent extraction processes and facilitates a more accurate analysis of the target bioactive compounds. Furthermore, these pretreatments play a crucial role in selectively isolating specific compounds by minimizing the coextraction of other algal components with similar properties. For example, pretreatment of algae with a solvent mixture such as methanol/chloroform/water (4:2:1 ratio) has been shown to be effective in preventing the co-extraction of unwanted compounds during aqueous isolation of fucoidan [15].

3.1.1 Conventional approaches to extracting

The extraction with solvents and hydrodistillations extraction is one of the most commonly used methods for extracting bioactive compounds from microalgae [16]. It involves the use of organic solvents, such as ethanol, methanol, and acetone, to dissolve and extract the desired compounds. The choice of solvent is crucial because it affects the selectivity, efficiency, and safety of the extraction process. Various factors, including solvent polarity, temperature, extraction time, and solvent-to-sample ratio, influence the extraction efficiency. Additionally, optimization techniques, such as response surface methodology and Box-Behnken design, are often used to enhance the extraction yield and bioactivity of the extracted compounds.

These methods are based on the differential solubility of desired compounds in specific solvents [17]. Although effective, they can suffer from limitations such as long extraction times, high solvent consumption, and potential damage to delicate bioactive molecules [18]. While established, Soxhlet extraction faces inherent limitations. One concern is mass transfer resistance, arising from the multiphase nature of the process. This can lead to prolonged extraction times, influenced by the diffusion rate of the chosen solvent [19]. In addition, conventional techniques are often energy-intensive [20] and involve manual steps, which affects reproducibility and consistency. Furthermore, the extraction process itself can affect the bioactivity of delicate compounds, such as fucoxanthin, potentially reducing yields. Similarly, the chosen pH, temperature, and pressure significantly influence the structure and activity of the extracted molecules. Finally, the reliance on organic solvents raises environmental concerns. For example, n-hexane, a common choice, is listed as a hazardous air pollutant by the US Environmental Protection Agency [21].

With these limitations and the growing demand for sustainable algae bioactives in mind, the development of alternative extraction technologies becomes imperative. These methods should prioritize selectivity, efficiency, cost effectiveness, and environmental responsibility. Ideally, they would facilitate rapid extraction with high yields while complying with the relevant regulations.

3.1.2 Advanced approaches to extraneous extraction

3.1.2.1 Enzymatic hydrolysis Microalgae boast a treasure trove of bioactive compounds, but their cell walls act like sturdy vaults, composed of intricate polysaccharides, proteins, and tightly bound ions [22]. Enzymatic hydrolysis is a technique that uses enzymes to break down complex biomolecules into smaller fragments, facilitating the extraction of bioactive compounds from microalgae. Enzymes, such as cellulases, proteases, and lipases, are employed to specifically target the cell walls and membranes of microalgae, releasing intracellular bioactive compounds. Enzyme-assisted extraction, a sustainable and gentle technique that replaces harsh solvents with the precision of these natural catalysts, offers several advantages, including high selectivity, mild reaction conditions, and reduced environmental impact. Under optimal temperature and pH conditions, enzymes such as carbohydrases and proteases (eg Viscozyme, Termamyl, Neutrase) selectively dismantle unnecessary cell wall components while releasing the desired bioactives [23].

This “biounlocking” method offers several advantages. Firstly, it overcomes the solubility limitations of certain compounds in water, making extraction more efficient. Second, its environmentally friendly nature eliminates the need for hazardous solvents, reducing the environmental impact and improving food safety [24]. Additionally, the cost-effectiveness of readily available food-grade enzymes adds to its appeal. Perhaps the most crucial benefit is the ability to preserve the potency of the bioactives. Unlike harsh extraction methods, enzymes work with gentle precision, maintaining the original efficacy of the extracted compounds [25]. This opens the door to various applications, from functional food formulations to nutraceuticals, and beyond. However, choosing the right enzyme is crucial. Different enzymes possess specific catalytic properties and target specific substrates. Careful consideration of these factors, along with optimal treatment time and temperature conditions, ensures maximum extraction yield [25].

3.1.2.2 Unlocking microalgal treasures: chemical modification and membrane filtration The bounty of bioactive compounds locked within microalgae holds immense potential to enrich food products with health-promoting properties. However, accessing this bounty often requires overcoming challenges related to stability, solubility, and bioavailability [26]. Chemical modification offers a transformative approach by, altering the molecular structure of bioactive compounds to enhance their suitability for food applications. Techniques such as acylation, esterification, glycosylation, and derivatization can improve solubility, making them easier to incorporate into food formulations [27]. For example, [28] acknowledges that esterifying *Chlorella* beta-glucan increases its solubility in water, allowing its inclusion in beverages and baked goods. In addition, bioavailability, the ease with which our bodies absorb and utilize these compounds, can be improved through chemical modifications. This can be achieved by masking functional groups that may interact with digestive enzymes, delaying breakdown and ensuring greater absorption in the intestine.

However, chemical modification requires careful consideration. Choosing the right reagents and reaction conditions is crucial to avoid undesired side reactions and to maintain the integrity of the bioactive compound. In addition, residual chemical traces raise concerns about safety and consumer acceptance in food products. Therefore, the discovery of natural, food-grade modifiers and optimizing reaction conditions remain ongoing challenges in this field. Membrane filtration, on the other hand, takes a physical approach to unlocking the bounty of microalgal [29]. This technique utilizes porous membranes that selectively allow bioactive compounds of specific sizes to pass through, effectively concentrating and purifying them from the extract. Depending on the size of the pores, membranes can be classified as microfiltration, ultrafiltration, nanofiltration, and reverse osmosis, offering various levels of selectivity [30]. This method boasts several advantages for food applications:

- High selectivity: membranes can be customized to isolate specific bioactive compounds from the complex mixture present in the extract, ensuring targeted enrichment of desired nutritional properties in food products [31].
- Continuous operation: unlike batch processes often used in chemical modification, membrane filtration allows for continuous processing, increasing efficiency and scalability [32].
- Low energy consumption: compared to other extraction methods, membrane filtration requires minimal energy, making it an environmentally friendly option [33].

However, this technique also faces challenges. The presence of impurities in the extract can cause membrane fouling, clogging pores and hindering filtration efficiency [34]. To address this, pretreatment steps such as centrifugation or enzymatic clarification are often used [35]. Additionally, optimizing membrane materials and cleaning protocols play a crucial role in maintaining long-term performance and ensuring food-grade purity of the extracted compounds. Both chemical modification and membrane filtration offer valuable tools for unlocking the vast potential of microalgal bioactive compounds for food applications. While each approach presents distinct advantages and challenges, its successful integration and optimization hold the key to enriching our food landscape with the health-promoting treasures hidden within these microscopic marvels.

3.1.2.3 Ultrasound-assisted extraction (UAE) Ultrasound-assisted extraction (UAE) provides a powerful and versatile tool to unlock the vast library of bioactive compounds stored within microalgae [36]. However, unlocking these treasures often requires overcoming the formidable barrier of their robust cell walls. UAE emerges as a potent weapon in this battle, utilizing inaudible sound waves (frequencies exceeding 20 kHz) to breach these defenses and facilitate efficient extraction. Unlike conventional extraction methods, UAE operates on a purely mechanical principle. The propagation of ultrasound waves induces rapid fluctuations in pressure, leading to the formation and violent collapse of cavitation bubbles within the extraction medium. This phenomenon generates immense localized shear forces and microjet streams, effectively disintegrating microalgal cell walls and liberating entrapped bioactives [37].

Unleashing the microalgal bounty with UAE requires a multi-pronged attack. First, cavitation's turbulent battlefield fosters rapid diffusion of liberated bioactives within the solvent, akin to soldiers swiftly traversing a chaotic landscape. This significantly accelerates the extraction process, ensuring valuable molecules reach the front lines faster. Second, intense shear forces act as microscopic battering rams, weakening and breaching the cell walls, much like siege towers toppling a castle. This increased permeability facilitates the release of previously locked intracellular treasures within. Moreover, by carefully tuning the ultrasonic parameters, we can become strategic treasure hunters. By exploiting the differential susceptibility of bioactives to cavitation, we can selectively unlock specific targets, similar to discovering hidden gems amidst a trove of riches. Finally, unlike traditional extraction methods that leave an environmental scar, UAE is a champion of sustainability. It rejects harsh chemicals, opting for a more green and ethical approach that perfectly aligns with the growing demand for eco-friendly practices in the functional food industry [38]. In essence, the UAE offers a powerful and environmentally conscious siege, not on microalgae themselves, but on the barriers that hold their treasures captive, paving the way for a bountiful harvest of bioactive compounds for functional food formulations.

Furthermore, UAE boasts remarkable versatility, adapting to various experimental and industrial scales [39]. From small-scale laboratory setups to large-scale biorefinery operations, the adaptable nature of UAE allows efficient extraction in diverse production capacities. Furthermore, researchers are actively exploring the synergistic integration of UAE with other extraction technologies, such as microwave-assisted extraction (MAE) and supercritical fluid extraction (SFE) [40]. These combined approaches potentially unlock even greater extraction prowess, maximizing the yield and purity of targeted bioactives.

3.1.2.4 Pressurized liquid extraction (PLE) For decades, pressurized liquid extraction (PLE), also known as accelerated solvent extraction, high pressure solvent extraction, pressurized fluid extraction, or enhanced solvent extraction, has offered a promising solution for extracting bioactive compounds from microalgae [41]. Unlike conventional Soxhlet extraction, PLE operates under controlled high temperatures (50–200 °C) and pressures (3.5–20 MPa), pushing solvents beyond their boiling points. This "superheated" state dramatically increases their extraction power. The magic lies in the interaction of elevated temperature and pressure. High heat enhances solubility and mass transfer, effectively breaking down intracellular bonds and liberating valuable compounds. Gleichzeitig, reduced viscosity and surface tension of the solvent improve its penetration into the algal matrix, leading to faster and more complete extraction.

PLE shines in its solvent efficiency. Compared to traditional methods, it significantly reduces the required solvent volume, minimizing environmental impact and cost [42]. Moreover, PLE caters to a broader range of solvents compared to that of supercritical fluid extraction (SFE), offering greater flexibility in tailoring the process to specific target compounds. However, PLE has its own limitations. High temperatures and pressures can degrade heat-sensitive bioactives, making them unsuitable for certain microalgal components. Additionally, PLE, while offering improved control over selectivity compared to Soxhlet, still falls short of the targeted precision achievable with SFE [43].

3.2 Microalgal bioactive compounds in functional food

Bioactive microalgal compounds, such as those found in *Spirulina* (Cyanophyceae), *Chlorella, Dunaliella*, and *Haematococcus* (Chlorophyta), have numerous applications and benefits in functional food formulation (Table 4). These compounds can enhance the nutritional value, functional properties, sensory attributes, and health effects of food products. For example, *Spirulina* contains carotenoids, phenolic compounds, phycocyanin, and chlorophylls, which can be used as natural antioxidants and coloring agents. *Spirulina* also has anticancer, neuroprotective, probiotic, anti-inflammatory, and immune system stimulating effects [44]. *Chlorella* contains nutrients and bioactive compounds that promote human health and prevent certain diseases, such as antioxidant, anti-inflammatory, and immunomodulatory properties [45]. *Dunaliella* is easily digestible and contains quality essential amino acids, polysaccharides, and phenolic compounds, making it a promising alternative source of prebiotics and a potential functional nutrition supplement [46, 47]. *Haematococcus lacustris* (formerly *Haematococcus pluvialis*) is rich in astaxanthin, a powerful antioxidant that supports eye, skin, immune and cardiovascular health, and reduces inflammation. These bioactive compounds of microalgae can be used to develop innovative functional food products that provide specific health benefits.

Consuming microalgae has been associated with several health benefits due to their diverse bioactive compounds [48]. Some of the reported health benefits include antihypertensive effects [49], anti-obesity properties [50], antioxidant properties [51], anticancer effects [47], cardiovascular protection [52], nutritional properties such as high levels of protein [53], carbohydrates [54], polyunsaturated fatty acids [55] and vitamins [56]. These health benefits make microalgae a promising candidate for the development of functional foods with potential positive impacts on human health.

Two distinct peptides isolated from *Nannochloropsis oculata* (Eustigmatophyceae), Gly-Met-Asn-Asn-Leu-Thr-Pro and Leu-Glu-Gln, showed antihypertensive activity through inhibition of the angiotensin-converting enzyme (ACE) with IC_{50} values of 123 µM and 173 µM, respectively [49]. These findings suggest that microalgal peptides possess potential for

Table 4 Key impacts for algae-infused functional foods

Food product	Microalgae	Formulation	Key impacts	References
Smoothie bowl	*Spirulina* (powder)	2–3 g blended with banana, berries, spinach, plant-based milk, topped with chia seeds & granola	Boosts protein, antioxidant profile (phycocyanin), gut health (prebiotics)	[82]
Spirulina tablets for immunity	Whole *Arthrospira platensis* (as *Spirulina platensis*) powder	Compressed tablets from dried & pulverized biomass	Concentrated protein, antioxidants, phycocyanin (Spirulina), immune support, natural energy boost	[144]
Dehydrated soup	Astaxanthin-rich microalgae	Microalgae are extracted using solvents like water, ethanol, methanol, chloroform, acetone, and others. The extract is then used to enrich the soup	High antioxidant capacity	[145]
Functional beverages	*Spirulina*	Whole Spirulina biomass or extracted purified compounds are used in the formulation of these beverages	Nutritional value, health benefits	[47]
Dietary supplements	*Spirulina, Chlorella*, Astaxanthin, DHA, EPA	Microalgae are extracted using GRAS solvents: acetone (A), ethanol (E), water (W), and a mixture of ethanol:water (1:1, v/v) (EW). The extracts are then used as whole ingredients or supplements	Nutrient-dense, health promotion	[146]
Vegetarian food gels	*Limnospira maxima* (as *Arthrospira maxima*) (Cyanophyceae), *Diacronema vlkianum* (Pavlovophyceae)	0.1–1% w/w	Improved gelation, texture, ω-3 PUFA enrichment	[147, 148]
Dairy products	*Arthrospira platensis* (Cyanophyceae)	3 g/L	Enhanced protein, vitamins, and minerals	[149–152]
	Spirulina	Whole *Spirulina* biomass is added to dairy products during formulation	Nutritional value, health benefits	[47]
Omega-3 enhanced milk	*Chlorella* oil	Microencapsulated & homogenized into milk	Increased omega-3 (DHA) for brain health, slight nutty flavor from chlorella	[153, 154]
Probiotic cheese spread	*Schizochytrium* sp. oil (DHA)	DHA oil, probiotic cultures, seaweed flakes	Added DHA for brain development, probiotic benefits, savory umami flavor (seaweed)	[79, 150]
Natural & probiotic yogurt	*A. platensis*	0.1–0.8% w/w	Texture modification, antioxidant & probiotic potential	[155, 156]
Fortified yogurt drink	Astaxanthin (*Haematococcus lacustris* as *H. pluvialis*)	Microencapsulated astaxanthin mixed with yogurt base, fruit purees, probiotics	Enhanced skin health, exercise recovery (astaxanthin), gut health (probiotics), natural pink color, antioxidant boost	[157]
Biscuits	*A. platensis,* Phycocyanin extract	A. platensis: 0.3–0.9%, Phycocyanin: 0.3% w/w	Nutritional boost (protein, iron), improved color	[44, 158]
Bread	*A. platensis*	11% w/w in flour	Enhanced dough properties, increased protein & minerals	[159, 160]
	Spirulina	Whole Spirulina biomass is mixed into the bread dough during the formulation process	Nutritional enhancement	[47]

Table 4 (continued)

Food product	Microalgae	Formulation	Key impacts	References
Multigrain bread	Astaxanthin extract	Added to dough with whole grains, seeds, nuts	Antioxidant richness (astaxanthin), extended shelf life, subtle pink hue	[161, 162]
Bakery products	*Nannochloropsis oculata, Porphyridium purpureum*	Microalgae biomass is extracted using a mixture of methanol:water:acetic acid as the extracting solvent. The extract is then used in the formulation of bakery products	Antioxidant activity, higher carotenoid and polyphenol content	[163]
Pasta	*Chlorella vulgaris* (green, orange), *L. maxima*	0.5–2.0% w/w in flour	Textural improvement, nutritional enrichment (protein, carotenoids)	[164, 165]
	Spirulina	Whole Spirulina biomass or extracted purified compounds are incorporated into the formulation of these products	Nutritional value, health benefits	[47]
Food supplements and nutraceuticals	Microalgae extracts	Microalgae are extracted using GRAS solvents: acetone (A), ethanol (E), water (W), and a mixture of ethanol:water (1:1, v/v) (EW). The extracts are then directly applied in the formulation of these products	Antioxidant and anti-inflammatory compounds	[146]
Protein bars	*Chlorella* powder	Mixed into bar base with nuts, seeds, dried fruit, sweetener	Increased protein & essential amino acids (chlorella), enhanced iron & B12, slightly earthy flavor, green flecks	[82]
Vegan omega-3 capsules	*Crypthecodinium cohnii* (Dinophyceae) oil (DHA & EPA)	Oil encapsulated in vegan-friendly soft gels	Plant-based Omega-3 source for heart & brain health (DHA & EPA), avoids fish oil contaminants	[166]

blood pressure regulation through two key mechanisms: (i) direct inhibition of ACE, a key enzyme responsible for vasoconstriction, and (ii) stimulation of the endothelial nitric oxide synthase (eNOS) pathway, thus promoting vasodilation and nitric oxide production. Preclinical and clinical evidence suggests that several microalgae possess anti-obesity properties [57]. Species such as *Euglena gracilis* (Euglenophyceae), *Phaeodactylum tricornutum* (Bacillariophyceae), *Limnospira maxima* (formerly *Spirulina maxima*), *Arthrospira platensis* (formerly *Spirulina platensis*) (Cyanophyceae), and *Nitzschia amabilis* (formerly *Nitzschia laevis*) (Bacillariophyceae) exhibit these effects through various mechanisms. Primarily, they inhibit preadipocyte differentiation and suppress de novo lipogenesis and triglyceride assembly, reducing fat accumulation. In addition, they stimulate lipolysis and fatty acid oxidation, further promoting fat breakdown. In particular, microalgae can activate thermogenesis in brown adipose tissue and induce browning in white adipose tissue, leading to increased energy expenditure. Beyond reducing body fat, microalgae treatment also improves other markers associated with obesity, including elevated plasma lipids, insulin resistance, diabetes, and low-grade systemic inflammation. The anti-obesity effects and improvements in comorbidities observed in preclinical studies have been further validated in clinical trials [58]. Levasseur et al. [56] identified a hydrogen atom donation mechanism as the underlying principle behind the antioxidant activity of specific microalgal pigments, including phenolic compounds.

Jaagichlorella luteoviridis (formerly *Heterochlorella luteoviridis*) (Trebouxiophyceae), rich in β-carotene (0.19 mg/g), suppresses tumor growth via its antioxidant and anti-inflammatory properties. *Chaetoceros* sp. (Mediophyceae), containing 5.13 mg/g fucoxanthin [59], induces apoptosis in colon cancer cells. *Haematococcus lacustris*, with astaxanthin levels ranging from 1.95 to 2.75%, exhibits potent antitumor activity through multiple mechanisms, including DNA damage and cell cycle arrest. *Chromochloris zofingiensis* (formerly *Chlorella zofingiensis*) (Trebouxiophyceae), with 1.5% astaxanthin, shows similar antiproliferative effects against various cancer cell lines [60]. Large-scale research suggests a link between the consumption of marine algae and improved cardiovascular health. For example, a study involving more than 86,000 participants linked increased flavonoids intake with a decreased risk of heart disease [61]. Furthermore, animal studies have shown that diets enriched with specific types of marine algae, such as the polysaccharide-rich red microalga *Dixoniella grisea* (formerly *Rhodella reticulata*) (Rhodellophyceae), can lead to lower cholesterol levels [62].

The wonders of nature, phycobiliproteins, are colorful proteins found in cyanobacteria, red algae, and other aquatic species [63]. These water-loving proteins capture light energy across a rainbow of wavelengths (470–660 nm), categorized into four distinct groups according to their maximum absorption: vibrant pink/red phycoerythrins, blue phycocyanins, reddish allophycocyanins, and orange phycoerythrocyanins. Their remarkable light-grabbing abilities stem from their clever architecture. Each phycobiliprotein is built from two different protein chains, each tightly linked to a pigment molecule called a chromophore. The specific amino acid sequence and the number of double bonds within these chromophores are like a secret code, dictating the unique colors and light-absorbing properties of each type. This precise design not only fuels photosynthesis in their host organisms, but also, interestingly, grants them potent antioxidant capabilities.

A survey by Mourelle et al. [54] revealed a treasure trove of various polysaccharides hidden within six key microalgal genera: *Porphyridium* (Porphyridiophyceae), *Phaeodactylum* (Bacillariophyceae), *Chlorella* (Trebouxiophyceae), *Tetraselmis* (Chlorodendrophyceae), *Isochrysis* (Coccolithophyceae), and *Rhodella* (Rhodellophyceae). These versatile molecules play several roles in the microalgal world, acting as shields against environmental threats, as fuel stores for energy, and building blocks for structure. They come in various forms, from pectins and protein-linked sugars to intriguing, sulfated polysaccharides (SPS) and simpler homo- and heteropolysaccharides. In particular, the research spotlight shines brightest on the sulfated family, with its impressive anti-inflammatory potential attracting considerable attention [64]. Human metabolism lacks complete biosynthetic pathways for essential polyunsaturated fatty acids (PUFAs), which require their dietary acquisition. These PUFAs are classified into two primary groups: omega-3 fatty acids (including α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA)) and omega-6 fatty acids (including arachidonic acid (ARA), linoleic acid (LA), γ-linoleic acid (GLA) and conjugated linoleic acid (CLA). The health benefits associated with the consumption of microalgae can be partially attributed to their rich PUFA content. These fatty acids have shown neuroprotective and ophthalmic benefits, in addition to their protective roles against cardiovascular diseases, obesity, diabetes, and arthritis. *Crypthecodinium* (Dinophyceae), *Schizochytrium* (Fungi), and *Ulkenia* sp. (Labyrinthulea) are recognized as potent PUFA producers within the microalgal realm. However, other genera such as *Phaeodactylum, Monodus* (Xanthophyceae), *Nannochloropsis* (Eustigmatophyceae), and *Porphyridium* also exhibit significant levels of DHA and EPA, highlighting the diverse potential of microalgae as dietary sources of these PUFAs that promote health [65].

Microalgae offer a promising avenue to diversify and potentially exceed established sources of essential vitamins in the human diet. In particular, although microalgae themselves may not directly produce vitamin A, they have considerable potential as reservoirs of precursor molecules like α- and β-carotenes and retinol, known for their protective effects against certain cancers [66]. Demonstratively illustrates this potential, showing *Nannochloropsis oceanica* capable of accumulating up to 1 µg/g dry weight of vitamin D3 under optimized ultraviolet B exposure. This exceeds baseline levels by more than 250 times, highlighting the inducible capacity of certain microalgae for vitamin D3 biosynthesis. Similarly, studies by [67, 68] have shed light on the exceptional vitamin B9 and K1 content of *Chlorella* spp. and cyanobacteria, respectively. Chlorella's reported vitamin B9 concentration surpasses that of soy flour, a commonly recognized source, by more than 100%. Cyanobacteria, meanwhile, boast a vitamin K1 level six times higher than parsley, a well-established dietary source. In particular, [67] also reported a notable vitamin B12 content in Chlorella spp., exceeding daily requirements by a factor of five. However, it is crucial to acknowledge the scarcity of data on the bio-accessibility and bioavailability of microalgal vitamins. Addressing this knowledge gap through focused research is vital for health regulatory agencies and the food industry to consider microalgae as a viable source of vitamins for functional food formulations.

4 Future perspectives and challenges

Microalgae offer a stunning glimpse into the future of sustainable food, but their consumption presents potential safety challenges that required careful consideration. One of the major challenges in the utilization of bioactive microalgal compounds is the safety and regulatory issues associated with their use in functional foods. Due to the novelty of these compounds, there is a lack of comprehensive safety assessments and regulatory guidelines. It is crucial to conduct thorough toxicological studies to ensure the safety of these compounds before they can be incorporated into food products. In addition, the establishment of clear regulatory frameworks will facilitate the commercialization and acceptance of microalgal-based functional food products. Unique proteins and polysaccharides within certain strains raise concerns about allergic reactions [69]. Although data on human allergic responses are limited, research is crucial for identifying potential allergenic compounds and assessing their risks.

The presence of toxin-producing strains requires robust identification and exclusion measures to ensure safety. Toxins such as microcystins, anatoxins, and saxitoxins pose serious health threats [70], highlighting the importance of controlled cultivation environments and rigorous monitoring. Furthermore, the high content of nucleic acid in the biomass of microalgae can increase uric acid, potentially leading to gout and kidney stones [71]. The exploration of strategies like genetic manipulation or optimized processing methods to reduce nucleic acid content is vital. Another limitation is the stability and bioavailability of bioactive compounds of microalgae. Many of these compounds are sensitive to environmental conditions such as light, temperature, and oxygen, which can lead to degradation and loss of their beneficial properties [72]. Moreover, their poor bioavailability in the human body limits their effectiveness. Therefore, it is essential to develop effective encapsulation and delivery systems to enhance the stability and bioavailability of these compounds in functional food products.

Consumer acceptance and preference are critical factors that determine the success of any functional food product. Although microalgal bioactive compounds offer numerous health benefits, their distinct taste, odor, and appearance may pose challenges in terms of consumer acceptance [73]. Therefore, it is necessary to conduct consumer studies to understand their preferences and develop strategies to mask or improve the sensory attributes of microalgal-based functional food products.

Furthermore, the economic feasibility and sustainability of bioactive microalgal compounds must be considered. The microalgae cultivation and extraction processes can be resource-intensive and costly, making it challenging to scale up production [74]. Furthermore, the environmental impact of large-scale microalgal cultivation must be evaluated to ensure sustainability. Exploring cost-effective cultivation methods and developing efficient extraction techniques will play a crucial role in making bioactive microalgal compounds economically viable and sustainable. Despite these challenges, the future of microalgae remains promising. Cultivating these organisms in controlled environments minimizes contamination risks and allows for targeted selection of high-nutrient strains. Optimization of extraction and modification methods is another important area of focus. Developing efficient and environmentally friendly extraction techniques will help maximize yield and preserve the bioactivity of microalgal compounds. Furthermore, exploring innovative modification methods such as enzymatic or microbial biotransformation can enhance the functionality and bioavailability of these compounds in functional food products. Establishing comprehensive regulatory frameworks for the production and processing of microalgal foods is crucial, including mandatory labeling to inform consumers about potential allergens, nucleic acid content, and the processing methods used. Continuous research on microalgal biology, toxicology, and processing technologies holds the key to addressing safety concerns and unlocking the full potential of these versatile organisms as a sustainable resource for future generations.

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Declarations

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