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REVIEW

The Impact of Microalgae in Food Science and Technology

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Abstract Microalgae (including cyanobacteria) are promising organisms for sustainable products for use as feedstocks for food, feed, fine chemicals, and biofuels. They can synthesize a broad range of products with medium- to high-value market price such as β-1,3-glucan polysaccharide, single-cell-protein, carotenoids and phycobilin pigments, and long-chain polyunsaturated fatty acids that are commercialized in the food industry as dietary supplements and functional foods, in the pharmaceutical and chemical industries as cosmaceuticals and flavorants, and in the therapeutic field as nutraceutical compounds. These microorganisms are also exceptional producers of omega-3 and omega-6 fatty acids such as eicosapentaenoic, docosahexaenoic, and arachidonic acids that have been linked to several human health benefits. The aim of this paper is to review the main existing high-value products that can be derived from microalgae with a particular focus on food science and technology applications. It also describes the gross and fine chemical composition of various algal species and details the nutritive importance of selected constituents. Finally, nutritional quality standards and legislative provisions to ensure food safety in the use of algal biomass are presented.

Keywords Microalgae · Chemical composition · Polyunsaturated fatty acids · Pigments · Cosmaceuticals · Nutraceuticals · Functional foods · Vegan food · Regulations · GRAS

Introduction

One of the major current challenges for the planet is to provide enough food for its population. As predictions of the world population will have increased by another 2 billion by 2050, current estimations have indicated that sufficient water and arable land is not available to support such demand [1]. Microalgae, therefore, have several advantages over traditional land plants that include: (1) efficient CO_2 conversion via photosynthesis; (2) high growth rate productivity in a few days (3–5 days) coupled with a very short harvesting cycle (8–10 days) compared with other feedstocks (which are harvested once or twice a year); (3) being able to grow in non-agricultural land (*e.g.,* seashore lands, desert or semiarid regions) using salt water (saline or brackish waters) or marginal wastewater (agricultural/domestic/municipal/industrial wastewaters); and (4) compatibility with integrated production of biofuels and co-products within biorefineries [2, 3]. These advantages allow microalgae to be excellent candidates for supply of commodities, including both food and non-food products [4, 5].

The microalgae (*i.e.,* the prokaryotic cyanobacteria and the eukaryotic microalgae) are an extremely diverse collection of microorganisms able to accumulate macromolecules such as protein, carbohydrates, and lipids through the efficient use of solar energy, CO_2 , and nutrients [6–8]. In addition, microalgae are considered perfect candidates for contemporary “nutraceutical” or “functional food” due to their ability to synthesize valuable products like carotenoids,

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long-chain fatty acids, sugar, essential and non-essential amino acids, enzymes, vitamins, and minerals useful for human nutrition [9–12].

The green algae (Chlorophyceae) *Chlorella vulgaris*, *Haematococcus pluvialis,* and *Dunaliella salina* and the cyanobacterium (also known as blue-green algae) *Arthrospira platensis* (Spirulina) are the most biotechnologically relevant microalgae that are widely commercialized as nutritional supplements for humans and as animal feed additives [13]. Algae also acts as a chemical platform for cosmetic purposes (*e.g.,* coloring pigments for skin cream), pharmaceutical and therapeutic applications [9]. The chlorophyceae *Haematococcus* and *Dunaliella* shall be highlighted as powerful pigment factories, and *Spirulina platensis* with outstanding capacity for protein accumulation [6, 9, 10].

Many algae accumulate substantial amounts of long-chain fatty acids (LC-PUFAs) in the form of triacylglycerols (TAG), such as omega-3 (ω-3): α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) and omega-6 (ω-6): linoleic acid (LA), γ-linolenic acid (GLA) and arachidonic acid (AA) that have been linked to human health effects (*i.e.*, mitigation effects of hypertension, anti-inflammatory effects, macular degeneration, rheumatoid arthritis, osteoporosis) [11]. Some of the algae reported to produce edible oil, normally commercialized as single cell oil (SCO) containing LC-PUFAs, include *C. vulgaris* (ALA-producer), *S. platensis* (GLA-producer), *Isochrysis galbana*, *Nannochloropsis oculata* and *Phaeodactylum tricornutum* (EPA-producers), *Crypthecodinium cohnii* and *Schizochytrium limacinum* (DHA-producers) and *Porphyridium cruentum* (AA-producer) [5, 6, 9]. Thus, SCO have attracted attention for their commercial development to provide superior nutritional supplement through consumption of LC-PUFAs [11].

Microalgae-derived compounds and products are shown in Fig. 1, which presents a schematic overview of potential microalgal "cell factory" production such as pigments, lipids, protein, polysaccharides, and bioactive compounds correlated to its areas of final application, such as food technology, fine-chemicals, pharmaceutical, nutritional purposes, and agriculture.

Products from algae have been reviewed in several papers (*e.g.,* [6, 9, 14, 15]). To underline the significance of the products synthesize and extracted from microalgae, this paper aims to provide up-to-date and comprehensive information on the potential commercial high-value products from microalgae, with a particular focus on the use of these high-value products in food science and technology. In addition, toxicological aspects in terms of nucleic acids, heavy metals, and algal toxins as well as the nutritional quality standards of algae for human consumption are discussed.

Market Potential of Microalgal Products

Important considerations while investing in algae products business include the potential market for algal products, the possible competition with non-algae sources, the time and cost of achieving approval for new products and more importantly their acceptance by the consumer [16]. Market

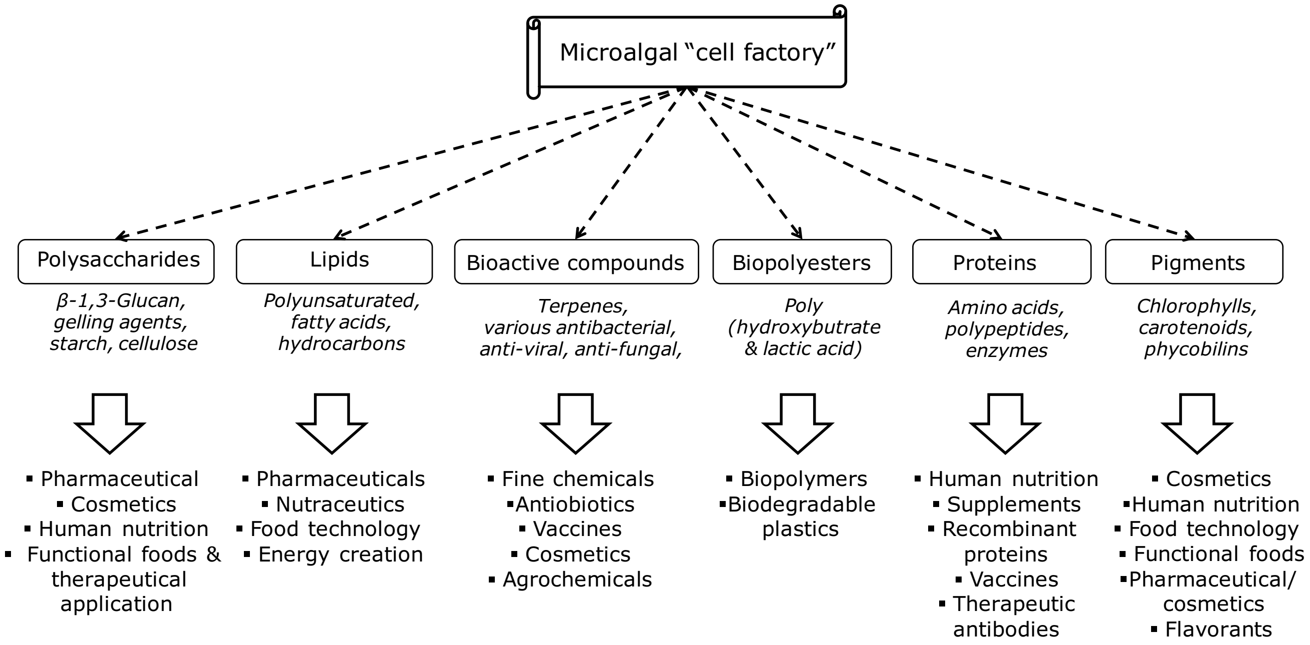

Fig. 1 Products synthesized by microalgae and potential area of application. Source: Modified [9]

prices for algal biomass and its valued components are strongly fluctuating globally at the production site, in the actual market situation, and especially the production purity [9]. According to Transparency Market Research (accessed on http://www.algaeindustrymagazine), a new market report entitled "*Algae market, by application, by cultivation technology, and geography—global industry analysis, size, share, growth, trends, and forecast—2016–2024*", has reported that the global algae market was value at US\$608.0 million in 2015 and is projected to reach US\$1.143 billion with a volume of 27,552 tons by 2024 [17].

In general, the market price of algal product (*e.g.,* astaxanthin or algal oil) is measured according to the market size of synthetic or traditional fish oil alternatives, and attentions of algal high-value products are focused on long-chain polyunsaturated fatty acids (DHA/EPA) and carotenoids (β-carotene, astaxanthin and lutein).

Traditionally, microalgae such as *Spirulina* and *Chlorella* have significant benefits as potential single cell protein (SCP) sources and are directly sold as dietary supplements; *Spirulina* was sold at a price of US\$20/kg and *Chlorella* at a price of US\$44/kg in 2010 [16], and these microalgae still have by far the largest production volumes. *Spirulina* production is concentrated in Asia and the USA, while *Chlorella* is mostly produced in Asia [18]. China has become the biggest country in *Spirulina* production in the world, with a total production of about 3500 t (dw) in 2009, and a special attempt is given to the *Spirulina* (*Arthrospira*) industry in Inner Mongolia of China, most of which are located at Wulan Town in the Ordos Plateau, where the *Spirulina* production cost is estimated at US\$3–4/kg, with total annual production of 700 t (dw) in 2009 [19].

The hydrocolloids, which are considered a medium–high value product from microalgae, are mostly used in the food industry as gelling and thickening agents. Some relevant algal carbohydrates that are important for technical applications are soluble fiber β-1,3-glucan (market value of US\$20–25/kg), agar (prices between US\$20–23/kg), carageenan (prices between US\$10–12 kg) and emulsifiers (prices between US\$3–8/kg), while gelling agents are worth US\$5–22/kg in 2012 [16].

According to Borowitzka [6], the market for carotenoids in 2010 was about US\$1.2 billion, with the bulk of the carotenoids being produced by chemical synthesis. In the pigment sector, the market value for β-carotene, based on *D. salina*, is estimated at US\$300–3000/kg depending upon the product purity, and its global market size of US\$285 million and production volume of 1200 t per year [9]. The astaxanthin carotenoid extracted from the alga *H. pluvialis*, which contains the highest amount of astaxanthin of any natural source, the average market price of astaxanthin is US\$2500/kg, and current global market size of natural pigment for the human market is estimated at US\$200 million annually [18]. For the high-prized phycobiliproteins from *Spirulina* and *Porphyridium* (phycoerythrin, phycocyanin and allophycocyanin), US\$3000 up to US\$25,000/kg is projected [9]. According to Borowitzka [6] the current total market value for phycobiliprotein products (including fluorescent agents) is estimated to be greater than US\$60 million. It is important to note that *C*-phycocyanin can be sell between US\$500 and 100,000/kg depending on purity, with the purity normally determined by the A_{620}/A_{280} absorbance ratio with food grade with a ratio of ≥ 0.7, reagent grade ~ 3.9 and analytical grade ≥ 4.0 [20].

Algal oil represents a significant segment within the omega-3 polyunsaturated fatty acid (PUFA) ingredients market. The current wholesale market price for algae omega-3 oil is about US\$140/kg (range US\$80–160/kg) mainly produced by *C. cohnii* and *Schizochytrium* sp. In 2012, the global market for microalgae based DHA + 30% oil was about 4614 metric tons, estimated to be nearly US\$350 million, being that infant formula represents 48.9% (microalgae based DHA + 30% oils), followed by dietary supplements (28.4%) and food/beverages (19.4%) of the total volumes sold [21].

Chemical Composition of Microalgae

Microalgae chemical composition is normally determined with the objective to provide the necessary nutritional information for the consumer. There is a general agreement that the biochemical composition of microalgae varies with species (type of strain), light, temperature, and growth stage/conditions [13], and their proportion can be modified to a certain extent by varying the culture conditions, for example provide nitrogen limitation in the algal culture medium promotes lipid accumulation [22], and excess temperature or light intensity induce carotenoid synthesis [23].

Many analyses of gross chemical composition of microalgae have been published in the literature (*e.g.,* [4, 24–26]). In general, protein is the major organic component, followed by carbohydrate + fiber or by lipid depending upon growth culture conditions [24]. It is not surprising that varying culture conditions for the same strain, different compositions is achieved.

For food applications, the harvested and concentrated algal biomass to be further utilized, a product with a water content of less than 10% is required. Moisture affects spoilage of the dried algal product by supporting the growth of bacteria, mould, and fungi [13].

Protein

Several microalgae can synthesize high protein content, for example *S. platensis* (60–65%) and *C. vulgaris*

(51–58%) of dry matter, and this outstanding capacity has been one of the main reasons to consider these organisms as a source of food [10]. It is important to note that the estimation of protein content from microalgae is frequently measured by multiplying the so-called crude nitrogen by the factor N × 6.25, and according to Tibbetts *et al*. [25], this calculation involves certain errors, because algae contain additional nitrogen-bearing constituents, for example, amines, glucosamides, nucleic acids, and cell-wall materials. For a correct purpose of N-to-P factor for algal protein content, a conversion factor of N × 4.78 is recommended [27].

Since protein is one of the most valuable algal components, four important parameters of protein quality are used to determine the appropriate nutritive value of algal protein, *i.e.,* protein efficiency ratio (PER), biological value (BV), digestibility coefficient (DC) or true digestibility and net protein utilization (NPU) [13]. The nutritive value of the alga-protein depends on the type of post-harvesting process and mostly of the microalgae have relatively thick cell wall, which makes improperly treated algal biomass indigestible for humans. Hence, to make the algal protein nutritionally accessible a variety of disruption methods (mechanical or non-mechanical) have been currently available for algal cell wall disruption. For instance, mechanical forces such as solid-shear forces (*e.g.*, high speed homogenization, bead mill), liquid-shear forces (*e.g.*, microfluidization, high pressure homogenization), energy transfer through waves (*e.g.*, microwave, ultrasonication), currents (e.g., pulsed electric field) or heat (*e.g.*, autoclaving, thermolysis), and non-mechanical methods such as enzymatic cell lysis and chemical cell disruption [12].

The growing world population and consequential deficiency in protein supply for human nutrition lead to increased activities exploring novel and alternative protein sources such as single cell proteins (SCP). Microorganisms such as algae, bacteria, fungi, and yeast/filamentous can be used as a source of SCP, but due to their high level of essential amino acids coupled with low nucleic acid content, algae are preferred over fungi and bacteria as a source of SCP for human consumption [28].

An interesting algal manufacturer, TerraVia™, has recently announced that Health Canada has issued and classified as Generally Recognized as Safe (GRAS) in compliance with the US FDA, a regulatory approval for its portfolio of AlgaVia® Whole Algae Ingredients, including AlgaVia Whole Algal Protein that contains approximately 65% vegan protein plus amino acids, fiber, and microelements and AlgaVia Whole Algal Flour making it an option for fortifying food due to its neutral flavor profile and texture [29]. With regard to a protein-cosmetic product, a relevant cosmaceutical derived from a protein-rich *Spirulina* extracts is "Protulines®-trade name" by Exsymol S.A.M. (Monaco) that has been used in cosmetic industry with skin properties, such as anti-aging, forming face and body benefits [30].

Amino Acids

Amino acids are subunits that make up proteins and, therefore, the nutritional quality of a protein is determined by the content, proportion, and availability of these organic compounds. Nine proteinogenic amino acids are called "essential" for humans and must be acquired through food. Because of their biological importance, amino acids are important in nutrition and are commonly used in nutritional supplements, fertilizers, and food technology. Industrial uses include the production of drugs, biodegradable plastics, and chiral catalysis [13, 30].

According to Becker [13], the amino acids pattern of almost all algae are comparable with that of the Food and Agriculture Organization (FAO) requirement, with minor deficiencies among the sulfur-containing amino acids methionine and cystein. Special attention is given during prolonged storage or excessive heat treatment (140–165 °C) of algal biomass that can induce the so-called Maillard reaction between amino acids and reducing carbohydrates, resulting in the non-availability of essential amino acids.

In order to estimate the quality of a given protein by its amino acid composition, two methods are commonly designated, namely, the chemical score (CS) and the essential amino acid index (EAAI). Deficiencies in certain amino acids can be compensated by supplementing with proteins from other sources or by consuming limiting amino acid directly [13].

According to Henrikson [10], *S. platensis* offers rich protein, about 60% by weight, which contains 10 of the 12 non-essential amino acids, and has been extensively used as a source of SCP by astronauts during space travel. In addition, *Spirulina* foods provide superior nutrition and have decades of history with chefs and household cooks that deliver high nutralence with colorful and tasty forms.

As microalgae are able to synthesize a wide range of amino acids, an interesting cosmetic active product based on algal-derived amino acid is "Exsy-Algine®-trade name" (Exsymol, Monaco) that has been marketed with the allegation to have skin care properties due to a better polar alga peptide (derivative of arginine) (http://www.exsymol.com).

Carbohydrates

Carbohydrates perform numerous roles in living organisms. Polysaccharides, for example, serve for the storage of energy in the form of starch and glycogen and as structural components in the form of cellulose in plants/algae. Special carbohydrates produced by microalgae are of increasing interest due to their potential therapeutic application [9, 32, 33].

Here, β-1,3-glucan, a natural soluble fiber active as immune-stimulator, antioxidant and reducer of blood cholesterol has to be mentioned, which is accessible from the cultivation of *Chlorella* strains [9], *I. galbana* [32], and *Euglena* [31]. In addition to the therapeutic use, β-1,3-glucan can be also used in food industry, mainly as fat substitute for texturing food products such as functional bread, ready-to-serve soups, functional snacks, and a variety of other products [9].

Extracellular polysaccharide (exopolysaccharides—EPS) is another carbohydrate-derived by-product that can be extracted from *Botryococcus braunii*. This microalga has a typical colonial organization embedded in an extracellular matrix composed of hydrocarbons and exopolysaccharides. A recently paper [34] has shown the extraction of EPS-derived from *B. braunii* in order to prepare a cryogel structure helping in capture nutrients that can act as anti-corrosive agent or bioflocculant in the food industry.

Exceptional attention is given to sulfated polysaccharides of red microalgae extracted from *P. cruentum*. The cells of the red microalgae are encapsulated within sulfated polysaccharides, and during growth of the algae in a liquid medium, the viscosity of the medium increases due to dissolution of the polysaccharide from the cell surface into the medium (soluble polysaccharide) [35]. Hence, one of the main characteristics of the red microalgal polysaccharides is their dynamic behavior in aqueous solution (*i.e.*, rheological properties comparable with those of industrial polysaccharides—carrageen) that makes them suitable for nutrition, cosmetic and pharmaceutical purposes [9]. Sulfated polysaccharides form thermally reversible gels similar to agar and carrageenan, which are used in food technology as thickeners, stabilizers, and emulsifiers. Furthermore, sulfated polysaccharides produced by microalgae can be even applied in anti-adhesive therapies against bacterial infections and as coadjuvant in vaccines where the polysaccharide protect protein-antibodies [35].

Microalgae extracts are recently intended for cosmaceutical products with biologically active ingredients purporting to have medical or drug-like benefits. This is the case of a marketed cosmaceutical product with trade name “Catalyst Alguronic Acid” sold by Algenist/Solazyme (CA, USA) [30]. This cosmaceutical product contains a mix of exopolysaccharides derived from extracts of algae (http://www.algenist.com).

Lipids and Fatty Acids

Lipids are constituents of all microalgae cells and the main biological functions include storing energy, signaling and acting as structural components of cell membranes. The lipid content of microalgae normally varies between 1 and 40% and, under certain conditions, it may be as high as 85% of the dry weight. In the case of *B. braunii*, one of the most scrutinized microalgal lipid producers, a total of 75% (w/w) of hydrocarbons in cell mass were reported [36, 37]. A large number of monounsaturated, polyunsaturated and even branched hydrocarbons are produced by *B. braunii*. These compounds can be chemically converted into biodiesel by the well-established alkaline transesterification with alcohols such as methanol [36]. Glycerol phase is the main by-product of this transesterification process and can be commercialized in the food industry as humectant (E422) or can be applied for manufacturing cosmetic products [9].

Algal lipids are typically composed of fatty acids with carbon numbers in the range of C_{12}–C_{22}. In general, saturated fatty acids (SFAs) in algae are mainly composed of myristic ($C_{14:0}$) and palmitic ($C_{16:0}$) acids; this last one is also naturally present in animal products (milk, meat, butter, and cheese) and plant products (cocoa butter, soybean oil, and sunflower oil). Palmitic acid is used to incorporate soaps, cosmetics, and industrial mold release agents [37]. Oleic acid ($C_{18:1}$) is the major monounsaturated fatty acid (MUFA) usually found in algae and is frequently included in the daily human diet as a component of animal fats and vegetables oils. It is also used as an emollient in cosmetic preparations, emulsifier in food, and solubilizer in aerosol products. A good example of commercial culinary algae oil is AlgaWise® Ultra Omega-9 Algae Oil (cooking and high stability oils) from Solazyme and Bunge Limited Company with operational facility in São Paulo state, Brazil. With high level of MUFA (> 90%), this unique oil is a clean-tasting oil ideal for healthier alternatives to saturated fat oils with excellent culinary performance [38].

Many algal oils contain special, often polyunsaturated, fatty acids with high market values, such as omega-3 (ω-3) including α-linolenic (ALA, $C_{18:3}$), eicosapentaenoic (EPA, $C_{20:5}$) and docosahexaenoic (DHA, $C_{22:6}$) acids and omega-6 (ω-6) including linoleic (LA, $C_{18:2}$), ɣ-linolenic (GLA, $C_{18:3}$) and arachidonic (ARA, $C_{20:4}$) acids. These PUFAs can be commercialized for nutraceutical, pharmaceutical and therapeutic applications, and yield much higher prices than after converting the lipids to biofuels [9]. The proportion of PUFA differs greatly depending on the species. For example, *C. cohnii* microalgae possess only DHA and no practically other PUFA; *N. oculata* and *P. tricornutum* contain predominantly EPA, whereas *P. cruentum* contains predominantly ARA.

PUFAs encompass ω-3 and ω-6 fatty acids that are well-known components in fish oil. However, fish do not produce PUFAs themselves; few fish actually directly consume plankton. Most fish get their PUFA by consuming small fish that have consumed zooplankton rather than have consumed phytoplankton. Consequently, algae are the primarily source of these essential nutritional components that display important functions in the human metabolism. For human health, PUFA ω-3 and ω-6 are entirely derived from the diet. Since the Western diet contains massive quantities of ω-6, ω-3/ω-6 fatty acids ratio of up 1:25 have been reported in the literature [39, 40], and nutrition experts have recommended that an ω-3/ω-6 fatty acids of ≤ 1:5 is desirable [41]. Therefore, nutritionists emphasize the need to consume seafood (notably fish) and oleaginous seeds (linseed, Brazil nut, walnut, hazelnut, among others) in order to supply PUFAs [42]. As global fish stocks are declining due to over-fishing, the production of PUFAs in the form of SCO from microalgae is an alternative approach [11].

The subsequent paragraphs, together with Table 1, provide an insight into potential applications of high-value microalgal fatty acids. Figure 2 shows the chemical structures of selected high-value fatty acids.

Omega-3 Fatty Acids

The ω-3 PUFAs ingredients market include EPA and DHA omega-3s from marine oils, such as fish oil, krill oils, squid oils, and algal oils. The end use application markets include dietary supplements, food and beverages, pet and aquaculture nutrition, infant formulation, pharmaceutical and clinical nutrition. Consumption worldwide of ω-3 PUFAs was estimated at 134 thousand metric tons valued at US$ 2.5

Table 1 Microalgal long chain fatty acids useful in food application

Fatty acid fraction	Microalgae source	Application of the fatty acid	Daily intake recommendation for human (mg)	References
Omega-3				
Alpha-linolenic acid (ALA)	*Chlorella vulgaris*	Nutritional supplement (single cell oil)	1000–2000	[57, 72]
Eicosapentaenoic acid (EPA)	*Nannochloropsis oculata, Phaeodactylum tricornutum, Monodus subterraneus, Isochrysis galbana*	Nutritional supplement, Psycotherapeutic medication, brain development for children, cardiovascular health	250–500	[44, 57, 73]
Docosahexaenoic acid (DHA)	*Schizochytrium limacinum, Crypthecodinium cohnii, Pavlova lutheri*	Food supplement, important for brain and eye development at fetus and for children, significant for cardiovascular health, adult dietary supplement in food	250–500	[9, 74–76]
Omega-6				
Gamma-linolenic acid (GLA)	*Arthrospira platensis*	Nutritional supplements, anti-inflammatory, auto-immune diseases	500–750	[10, 77]
Arachidonic acid (ARA)	*Porphyridium cruentum, Mortierella alpina, Parietochloris incisa*	Nutritional supplements, anti-inflammatory, muscle anabolic formulations (body buider)	50–250	[24, 45, 76]

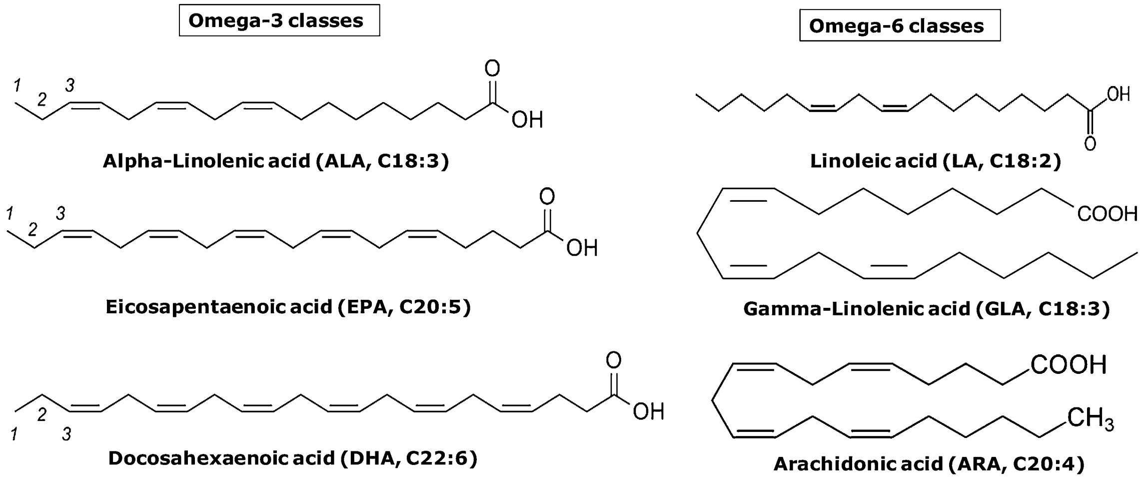

Fig. 2 Chemical structures of the most commonly polyunsaturated fatty acids found in microalgae

billion in 2014 and is projected to 2020 that the demand will reach 241 thousand metric tons of ω-3 PUFAs with a value of US$4.96 billion [43].

The human body converts α-linolenic acid to eicosapentaenoic acid, a ω-3 fatty acid that plays an important role in human body because act as a precursor for prostaglandins, thromboxane, and leukotriene eicosanoids, which is succession are responsible for various functions in immune system, blood clotting, vascular pressure, and cancer prevention [44]. This is based on various positive health effects related to this compound as stated by the Food and Agriculture Organization (FAO), which recommends a daily intake of 250–500 mg ω-3 for human nutrition. Eustigmatophyceae strains of *Nannochloropsis* sp. and diatom *P. tricornutum* are the most prominent microalgal EPA-producers [9]. EPA-rich oils are mainly used in combination with DHA for infant formulation or dietary supplements (single cell oils). Additionally, EPA is used in aquaculture for fish-farming (Salmon farming) that plays a crucial role in their juvenile development [13, 45].

Functionally, EPA is a precursor to DHA in human body, which is linked to primary structural component of the human brain and eye retina. It is important for infantile brain and eye development. Anti-inflammatory effects of DHA, and its importance for the development human fetus and healthy breast milk are also evidenced by recent scientific studies [40, 46]. The popularity of DHA allows including it in infant formula fortifications, which is recommended by various health and nutrition organizations for example, FAO [41].

Microalgae such as *C. cohnii* and *S. limacinum* are exceptional producers of DHA and became the major source alternative for production of ω-3 PUFAs. The former Martek Biosciences Corporation based in Columbia, MD USA, has become one of the most successful microalgae companies, and they developed a patented DHA-rich oil product for food, feed and pharmaceutical applications. In addition to algal-based nutraceutical formulations, there is an increasing demand for so-called vegan health food rich in PUFAs for which microalgal biomass could act as the raw material of choice [6, 9].

Omega-6 Fatty Acids

Spirulina platensis is one of the best sources of ɣ-linolenic acid (GLA), an ω-6 fatty acid [10]. GLA is as a precursor of C_{20} eicosanoids to synthesize prostaglandin and has been associated with beneficial health effects, anti-inflammatory effects and for auto-immune diseases. A dairy intake dosage of 500–750 mg GLA (Table 1) is recommended by FAO [41]. In addition, GLA-rich supplements are promoted to help people suffering from diabetes, obesity, rheumatoid arthritis, heart disease, multiple sclerosis, and neurological problems related to diabetes [9]. Exsymol S.A.M. (Monaco) is a good example of manufacturer that sells anti aging skin cosmetic product based on GLA-lipids from *S. platensis* (http://www.exsymol.com).

Arachidonic acid (ARA), a four-fold unsaturated ω-6 fatty acid, is an essential component of membrane phospholipids and is abundant in the brain, muscles, and liver. It also acts as a vasodilator, shows anti-inflammatory affects and is, therefore, used for nutrient supplements. A commercial source of ARA has been mainly derived by *Mortierella alpina* fungus, although marine microalgae *P. cruentum* is also ARA-producer [6]. ARA is necessary for the repair and growth of skeletal muscle tissue and this role makes ARA an important dietary component in support of the muscle anabolic formulations, and supplementation of 50–250 mg daily for healthy adults has been recommended [40]. DSM Corporation, for example, produces an infant milk food supplement with a combination of ARA ω-6 + DHA ω-3 long-chain polyunsaturated fatty acids (LC-PUFAs) with trade name "DHASCO". Besides this food product, an example of a commercial cosmetic active product from microalgae is ARCT'ALG® (Exsymol, Monaco) that formulates an extract from red alga containing ARA-ω-6-rich offering energetic strength for the human body with a series of skin benefits such as increasing skin's resistance to extreme conditions and improvings cutaneous metabolism through cytostimulation and lipolysis breakdown (http://www.exsymol.com).

Pigments

Algal pigments are associated with light harvesting, CO_2 fixation, protection of algal cells against damage by excessive illumination and, macroscopically, the coloration or pigmentation of the algal culture. Three major groups of pigments are found in microalgae, which include chlorophylls (greenish coloration), carotenoids (among them, carotenes provide an orange coloration, whereas xanthophylls are yellow pigments), and phycobilins (red and blue coloration) [9].

Novel extraction and separation techniques, such as supercritical CO_2 extraction, centrifugal partition chromatography, and pressurized liquid method have recently been employed in the extraction of pigments from algae [23, 46]. Because of the low productivities and high product recovery costs, the market penetration of microalgal pigments is still in a very early stage compared to the chemosynthetic production of the same compounds or their isolation from other natural sources [6]. In the case of β-carotene, the portion produced by *D. salina*-β-carotene amounts to about a quarter of the entire β-carotene market, because there was also an established market for synthetic β-carotene, which meant an easier and quicker path to the market for the algal product. This situation is even more notable in the case of astaxanthin, where the development of the "natural" astaxanthin market was more difficult than for β-carotene, where less than 1% of the commercialized quantity is allotted to the algal-derived pigment [9].

Various pigments are isolated from algae and thus have attracted much attention in the fields of food, cosmetic, and pharmacology. The pigment fraction of algae can be applied as nutrient supply due to their high contents of pro-vitamin A (E160a) and vitamin E (E306, E307, E308) [9], and for other veterinary, pharmaceutical medical purposes (antioxidant activity, anti-inflammatory effects, neuroprotective, cancer prevention [46], as well as in food technology and cosmetic industry). In addition, canthaxanthin pigment (E161g) is recommended for animal nutrition in feedstuffs for salmon and trout, laying hens and other poultry due to colouring matter [47]. These general facts of algal-pigments are collected in Table 2, whereas Fig. 3 presents the chemical structures of the most commonly pigments found in microalgae.

Chlorophylls

Chlorophylls are greenish lipid-soluble pigments, which contain a porphyrin ring and found in all algae, higher plants, and cyanobacteria. Structurally, chlorophylls are substituted tetrapyrrole with a centrally coordinated magnesium atom. Chlorophylls are modest absorbers of green and near-green portions of the spectrum, and this causes the typical green coloration of the green algae (*Chlorophyta*) that harbor chlorophylls as the predominant pigments.

Chlorophyll is registered and approved as a colorant additive (E140) and is mostly used in the food pigmentation and dietary supplement industries. Famous "chefs de cuisine" use chlorophyll to provide a green coloring of foodstuffs and beverages, such as pasta, pesto, or absinthe [9]. In general, most chlorophyll available on the market is in the form of the derivative sodium copper chlorophyllin, which makes these structural changes favorable to convert fat-soluble chlorophyll into a water-soluble compound and this derivative

Table 2 Microalgal pigments and potential fields of application

Pigment	Color of pigment	Microalgal representative	Application of the pigment	References
Chlorophylls	Green	All phototrophic oxygenic algae	Pharmaceutical and cosmetics (deodorant)	[9]
Carotenoids (Carotenes)				
β-Carotene	Yellow	*Dunaliella salina, D. bardawil*	Pro-vitamin A, antioxidant food, additive E160a, coloration of egg yolk	[9, 30, 77]
Bixin	Yellowish to peach-color	*Dunaliella salina*	Food additive E160b (colorant), cosmetics	[9, 78]
Carotenoids (Xanthophylls)				
Astaxanthin	Reddish-salmon	*Haematococcus pluvialis, Botryococcus braunii*	Food additive E161j, antioxidant, farming of salmon and trout (color, immune-response)	[23]
Canthaxanthin	Golden-orange	*Haematococcus pluvialis, Chlorella zofingiensis*	Food additive E161 g, farming of salmonids and chicken, tanning pills	[47, 79]
Fucoxanthin	Brown to olive	*Phaeodactylum tricornutum*	Anti-adipositas	[80]
Lutein	Yellow-orange	*Chlorella salina, C. zofingiensis, D. salina*	Food additive E161b, yellow coloration of egg yolk (feed additive), pigmentation of animal tissues, pharmaceutical (anti-macular degeneration), cosmetics (coloration)	[53, 81]
Violaxanthin	Orange	*Botryococcus braunii, Dunaliella tetriolecta*	Food additive E161e (approved in Australia & New Zealand)	[9]
Zeaxanthin	Orange-yellow	*Phaeodactylum tricornutum*	Food additive E161 h, animal feed, pharmaceutical (anti-colon cancer, eye health)	[53, 82]
Phycobilins				
Phycocyanin	Blue-green ("cyano")	*Arthrospira, Spirulina* (cyanobacteria)	Food colorant (beverages, ice cream, sweets), cosmetics, fluorescent marker in histochemistry, antibody labels, receptors and other biological molecules	[10, 52]
Phycoerythrin	Red	*Porphyridium,* cyanobacteria	Immunofluorescence techniques, labels for antibodies	[46, 55, 58]
Tocopherol (α-Tocopherol)	Brown	*Chlorella sp., Nannochlropsis oculata, Euglena gracilis*	Vitamin E, food additive E306, E307, E308 antioxidant in cosmetics and foods	[46, 83]

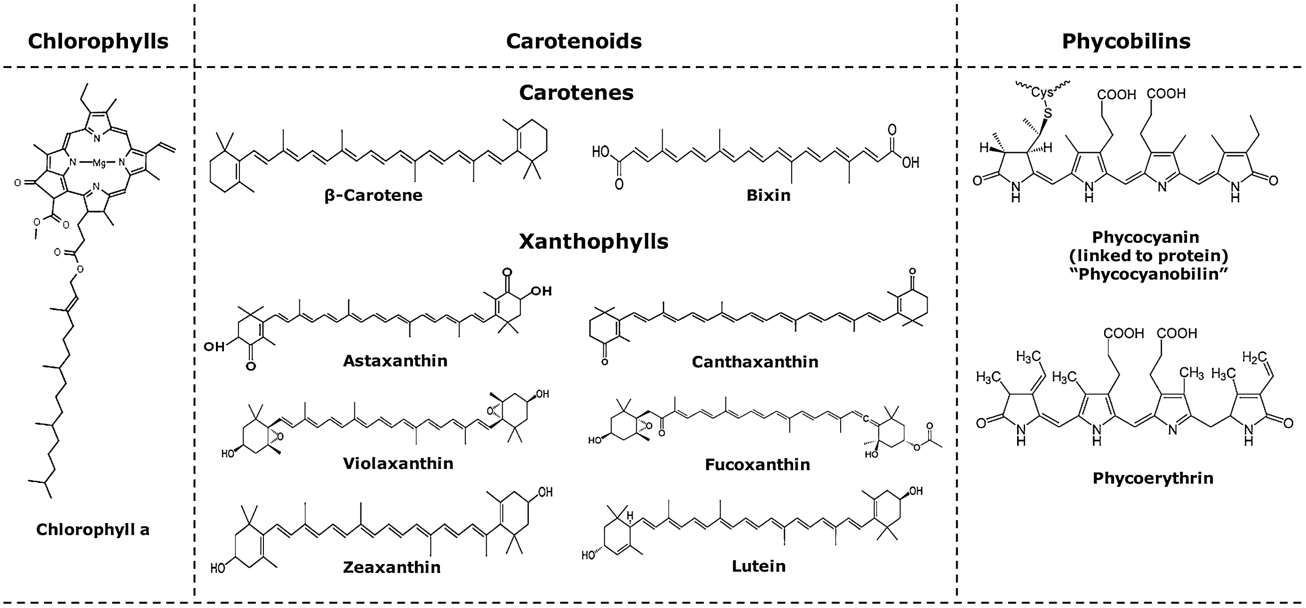

Fig. 3 Chemical structures of some microalgal pigments. Source: [9]

molecule (chlorophyllin) has shown antimutagenic effects to various polycyclic procarcinogens such as aflatoxin-B_1, polycyclic aromatic hydrocarbons and some heterocyclic amines, demonstrating potential chemo-preventive agent [48]. Because of its high deodorant capacity, chlorophyll *a* is also used as an ingredient in items of personal hygiene products (deodorants, pastilles, and bad breath formulations) [9].

Carotenoids

Carotenoids are organic lipophilic compounds that are produced by plants and algae. Their color is directly linked to their structure and ranging from pale yellow through bright orange to deep red [49]. Most carotenoids share a common C_{40} backbone structure of isoprene units (termed terpenoid), and are divided into two groups: carotenes, which are unsaturated hydrocarbons; and xanthophylls, which present one or more functional groups containing oxygen. Carotenoids display so-called secondary light harvesting pigments, supporting the "primary pigment" chlorophyll in capturing light energy. They also act as antioxidants that inactivate reactive oxygen species (ROS) formed by exposure to excessive solar radiation. As microalgae and cyanobacteria are excellent source of carotenoids [9, 46, 49], the major carotenoids of market interest are astaxanthin, β-carotene, canthaxanthin, fucoxanthin, and lutein in combination with zeaxanthin [50].

With production starting in the 1980s mainly in Israel and Australia, β-carotene was the first high-value product commercialized from the microalga *D. salina*, with reported intracellular β-carotene contents of up to 14% [6]. This green alga (Chlorophyceae) is characterized by its preference for extremely saline habitats like salt evaporation ponds, a fact that facilitates its cultivation in open raceway ponds without risking microbial contamination [3]. In humans, one molecule of β-carotene can be cleaved by the intestinal enzyme β,β-carotene 15,15′-monooxygenase into two molecules of vitamin A, and thus β-carotene has been used for human vitamin supplement with a daily intake of 2–7 mg/day (Table 3) and for food coloring (E160a). In addition, β-carotene is of outstanding importance in human metabolism for retinal synthesis needed for generation of rhodopsin. Besides this, it plays a certain role for the prevention of toxin build-up in the liver, potentially improves the immune system, and may have a preventive role in eye diseases like night blindness and cataract [49].

The second carotenoid from algae traditionally commercialized is astaxanthin (E161j) extracted from the green alga *H. pluvialis*, which is characterized to synthesize up to 3.0% by dry mass of astaxanthin [9]. Algatechnologies® is a good example of established industry for *H. pluvialis* cultivation and astaxanthin production, which is produced in a closed

Table 3 Recommended dosages of pigments useful for human consumption mainly due to antioxidant activity Sources: [10, 46, 49]

	β-Carotene (carotenoid) (mg)	Astaxanthin (carotenoid) (mg)	Phycobilins (phycocyanin, phycoerythrin) (mg)
Daily intake recommendation	2–7	6–12	200–400

system of glass photobioreactors energized by the abundant natural sunlight of the Arava desert in Israel [6]. Astaxanthin has been mainly used as feed supplement for salmon, shrimp, chickens, and egg production and exhibits several-fold stronger anti-oxidant activity than β-carotene and vitamin E [49, 51], with a daily intake recommended dosage of 6–12 mg/day (Table 3). In human metabolism, it has the potential to enhance antibody production, anti-aging, alleviating sports fatigue, and promoting hair growth. It is also used in sunscreen creams due to its UV-protecting activity that by far surmounts the activity of vitamin E [6, 9, 23, 52].

Two other carotenoids xanthophylls, lutein and zeaxanthin are also becoming increasingly important in the nutraceutical market since they play a significant role in eye heath, which is clinical proven to prevent cataract and macular degeneration [52, 53]. Lutein is traditionally used in chicken feed in order to fortify yellow egg yolk and food additive (E161b) approved for use in the EU, Australia, and New Zealand [47]. In general, a daily intake of 20 mg/day of these two carotenoids is relatively safe for human consumption.

Fucoxanthin is another xanthophylls carotenoid that can be produced by microalgae. Diatom *P. tricornutum* is a good example of fucoxanthin-rich algae marketed as an anti-obesity functional food and anti-inflammatory activities [49, 54]. A further example of the carotene group of carotenoids is bixin (E160b) that provides a yellowish to peach-color shade and is mainly used as food additive in dairy products (cheese, butter, and margarine) and colorant in cosmetics [9].

Violaxanthin and canthaxanthin are additional members of the xanthophylls. Violaxanthin has an orange coloration that makes it another food colorant (E161e) approved in Australia and New Zealand. *Dunaliella tertiolecta* and *B. braunii* are good ones algae producers. Canthaxanthin is approved in United States and in EU for food coloring agent (E161g), colouring agent for medical products (tanning pills) and as colour additive in animal feed for poultry and fish feeds [47]. Eustigmatophyceae species such as *Nannochlorospsis* sp. (*N. salina*, *N. gaditana* or *N. oculata*) are typical algal strains of canthaxanthin synthesis [9, 30].

Phycobilins

Phycobilins (phycocyanin and phycoerythrin) are light-capturing bilins found in the stroma of chloroplasts of cyanobacteria, rhodophyta (red algae), Cryptophyta, and Glaucophyta. Chemically, they constitute open-chain tetrapyrroles molecules that typically act as chromophores and structurally related to the pigments of mammalian bile and pigments of hemoglobin and chlorophyll, which make them colored. Phycocyanin is a blue pigment primarily found in cyanobacteria, for example *S. platensis* and *Aphanizomenon flos-aquae* species, while phycoerythrin is a pigment occurring predominantly in red algae, for example *P. cruentum* and *Gracilaria gracilis* species, and responsible for its characteristic red coloration [6, 9]. Phycobilins are unique among all known photosynthetic pigments because they are bonded to certain water-soluble protein, building a pigment-protein complex known as phycobiliproteins. Similar to carotenoids, they serve as "secondary light harvesting pigments" and, therefore, forward the energy of the harvested light to chlorophylls for photosynthesis [9].

Phycobilins, in the form of C-phycocyanin and R-phycoerythrin, are useful in a wide range of applications including fluorescent tag for use in flow cytometry and immunology, as photosensitisers in photodynamic therapy for treatment of cancers and fluorescent dyes in microscopy techniques [55]. Besides these highly sophisticated applications as chemical-pigment tag; such phycobiliproteins are the algal-derived products revealing the by far highest market values [9]. Furthermore, phycobilins are also used as natural coloring agent in food industry acting as a component of functional foods (sweets, ice cream, and beverages), as possible antioxidant in personal care skin products (*e.g.*, phycocyanin cosmetic "Line Blue" sold by DIC Lifetec Corp., Japan) and hydrating skin product (e.g., phycoerythrin from *P. cruentum* sold by Soliance®, France) [6].

Vitamins

Vitamins are essential for the normal growth and development of a multicellular organism and microalgae represent a valuable source of several important vitamins, especially from B complex, a class of water-soluble vitamins. In general, the concentrations of the various vitamins are comparable between the different algae as well as in higher plants. An important exception seems to be vitamin B_{12} and various vitamins B complex such as cobalamin and cyanocobalamin. In addition to the vitamin B_{12}, other vitamins such as vitamin K, vitamin A, isomers of tocopherol (vitamin E), as well as metabolic intermediates can be found in almost all algae. The usual dietary sources of vitamin B_{12} (also called cobalamin) are from animal source (eggs and milk, fish and shellfish) and from bacteria (including cyanobacteria) [13]. The recommended dietary intake of vitamin B_{12} for adults is 2.0 µg/day and especially *S. platensis*, which is largely sold as a vitamin supplement contains 127–244 µg vitamin B_{12} per 100 g weight [56]. Thus, *Spirulina* is far good source of vitamin B_{12} and it could be an excellent source of vitamin B complex for vegetarian consumers.

Other Valuable Compounds

Aside from essential compounds of basic physical structure, algae also produce a myriad of secondary metabolites that assist in the survival of the organism. The ecological function of secondary metabolites in microalgae or cyanobacteria may play some roles from defense against predation. For example, the brevetoxins produced by some of marine dinoflagellates have an ecological role as a feeding deterrent, which are also responsible for neurotoxic shellfish poisoning (NSP) [48]. The cyanotoxin lyngbyatoxin-*a*, which is produced by certain cyanobacterium species, notably in *Moorea producens,* has an ecological role as a defense mechanism to ward off any eventual predator [51].

It is this rich chemical and biological diversity that gives the secondary metabolites of algae and cyanobacteria potential value as commercial products. Some of these molecules retain their natural functions when used for anthropogenic purposes; for example, polyunsaturated fatty acids found in many algae are used in the nutraceutical industry for their health benefits [57], as discussed later in this paper. Cyanobacteria have been identified as source of bioactive compounds with interesting biological activities; this is the case for example of the allelopathic compound named noscomin, a diterpenoid compound isolated from *Nostoc commune* that has shown potential antibacterial activity, while some engineered cyanobacteria have the ability to synthesize mycosporine-like amino acid (MAA) that has been incorporated in human sun-screen products; this small secondary metabolite has important role to deal with UV radiation and protection from oxidative damage [58].

There are a variety of algal-derived biopolymers that have been used for commercial purposes (agarose, alginate and carageenan and polyhydroxyalkanoates). Of these biopolymers, polyhydroxyalkanoates (PHAs) are the only ones that have been applied to plastic applications, and this chemical class has been shown to be produced by a number of cyanobacterial genera including *S. platensis* and *Spirulina maxima*, *Synechococcus* sp. MA19 and *Synechocystis* sp. 6803 [6, 9, 48]. As microbial storage compounds with plastic-like properties, PHAs are of increasing significance to replace well-established plastics of fossil origin on the market [9].

Other valuable compounds from secondary metabolism of microalgae and cyanobacteria are terpenoids molecules that are derived from isoprene units with chemical formula C_5H_8. A common example of a terpene-derived metabolite is the plant steroid β-sitosterol, where microalgae are able to synthesize a wide range of phytoesterols including brassicasterol, sitosterol, and stigmasterol depending on the taxonomic affiliation of the alga [6]. These algal phytoesterols may have pharmaceutical applications or in functional foods, but the potential of microalgae as sources of phytoesterols remains to be fully explored.

The hydrocarbons produced by the microalga *B. braunii* are one class of terpenoid currently being developed for their biofuel potential. Squalene, a linear triterpene natural product is traditionally isolated from the liver oils of deed-sea shark, but it is also synthesized and produced by the microalga *B. braunii*, although in relatively low amounts. *Aurantiochytrium* strains (thaurostrochytrid) are the most promising microalgal source of squalene, which have been shown to accumulate a level of 198 mg/g squalene [6]. Squalene has found many applications in medicine, food, and cosmetic industries due to its antioxidant, antistatic and anti-carcinogenic properties [51], and special attempts have been given for the production of squalene by metabolic engineering of cyanobacterium *Synechocystis* sp. PCC 6803 [59].

Microbial production of isoprene has been recently demonstrated by an engineered *Synechocystis* sp. PCC 6803 strain [60]. In cyanobacteria, isoprene is a common structural molecule important for synthesis of terpenes compounds (also known as isoprenoids) required for many cell functions included photosynthesis, membrane stability and cellular production of carotenoids. To produce isoprene in *Synechocystis*, the isoprene synthase gene from kudzu vine (*Pueraria montana*) was expressed under regulation of the photosystem component *psbA2* promoter [61]. According to the same authors, isoprene is highly volatile hydrocarbon that microorganisms such as cyanobacteria offers the advantages to be cultivated in enclosed bioreactors, which permits collection and sequestration of the volatile isoprene.

Some non-natural chemicals synthesized by engineering cyanobacteria typically not produced by these organisms in nature can be obtained by the advancement of synthetic biology and genetic manipulation [62]. This is the case of engineering cyanobacteria for production of high-value chemicals including higher alcohols (Table 4) from renewable sources. The production of isobutyraldehyde by the cyanobacterium *Synechpcoccus elongatus* PCC7942 has been reported [62] where the 2-ketoacid pathway was selected for engineering and expression in a mutant *S. elongatus* strain to synthesize isobutyraldehyde, which is used as a chemical intermediate to produce polymers, flavor and fragrance, glycols, insecticides and isobutanol [61]. Isobutanol is a another higher alcohol used as a solvent in the manufacture of flavor and fragrances as well as flavoring agent in food industry, and has been synthesized by engineered *S. elongatus* PCC7942 using a keto acid pathway [63].

Further higher-chain alcohol of interest as a chemical feedstock is 1-butanol that has been produced by engineered *S. elongatus* using a modified CoA-dependent pathway [64]. 1-Butanol is normally produced industrially from the petrochemical feedstock propylene, this primary alcohol have been used as an intermediate in the production of butyl esters. Butyl esters also known as butyl ethanoate has impart flavor characteristic and associated with banana or apple

Table 4 Chemical products synthesized by engineered cyanobacteria Source: Modified from [61]

Product	Potential applications in food science	Host organism
1-Butanol	Used as an ingredient in processed and artificial flavorings, for the extraction of lipid-free protein from egg yolk, and for the manufacture of hop extract in beer making	*S. elongatus* PCC7942
Isobutanol	Flavoring agent in food industry, ink ingredient	*S. elongatus* PCC7942
Isobutyraldehyde	Used primarily as a chemical intermediate to produce glycols, essential amino acids, flavor and fragrance, polymers, insecticides and isobutanol	*S. elongatus* PCC7942
Isoprene	Used as a synthetic version of natural rubber. Isoprene-derived several biological molecules such as phytol, retinol (vitamin A), tocopherol (vitamin E), and squalene	*Synechocystis* sp. PCC6803

smell. It is used as a synthetic fruit flavoring in foods such as candy, ice cream, cheeses, and baked goods. 1-Butanol can also be produced as a by-product of microbial fermentation processes using *Clostridium acetobutylicum* [65].

Toxicological Aspects

Microalgae, which are considered as unconventional food, have to undergo a series of toxicological tests to prove their harmlessness. Several recommendations and toxicological evaluations have been published by different international organizations, but it has to be assumed that additional national regulations exist from country to country, which specify the recommended analyses.

As part of the toxicological characterization, the algal material has to be mainly analyzed in terms of two aspects [*i.e.*, compounds synthesized by the alga itself (biogenic toxins) or compounds that are accumulated from the environment (non-biogenic toxins)]. The biogenic toxins include nucleic acids and algal toxins, whereas non-biogenic products comprise environmental contaminants, such as heavy metals [13].

Nucleic Acids

Nucleic acids are biopolymers essential to all known forms of life, and these include RNA and DNA, which are sources of purines. There is a general association between purine-rich diet and possible increased plasma urate concentration that causes gout disease [66, 67]. This is mainly because uric acid is the end-product of purine degradation and the avoidance of purine-rich foods is highly recommended for gout patients [67]. As microorganisms are source of single-cell-protein that may contain purines, they are sometimes considered as limitation for food application. In general, algae contain 4–6% of nucleic acid, while yeast contains 8–12% and bacteria contains up to 20% of nucleic acid in dry matter [13]. Because of a possible health hazard, the Protein Advisory Group of the United Nations (Nutrition Bulletin) has recommended a maximum daily intake of 4.0 g/day nucleic acid for unconventional food source. As mentioned earlier, single-cell protein from algae sources are preferred over fungi and bacteria sources due to their low nucleic acid content [28], and according to Becker [13] the safe level of algal biomass consumption is 20 g of algae per day or 0.3 g of algae per kg of body weight.

Algal Toxins

Algal toxins are produced by various algae and are found both in seawater and fresh water; they have no taste or smell and are not eliminated by cooking or freezing. Algal toxins occur from blooms of algae with considerable frequency—sometimes unpredictably—in several regions of the globe and can lead to poisoning of humans, fish die-offs or states having to close fisheries. Certain marine algae (*Anabaena* sp., *Mycrocystis* sp., *Dynophysis* sp., and *Pseudo-nitzschia*) produce potent toxins (saxitoxins, brevetoxin, domoic, and okadaic acids) that have an impact on human health through consumption of contaminated fish, oysters, scallops, clams, shellfish, and mussels, which filtered these toxins from the water accumulating to levels that can be lethal to consumers, including humans [68]. Algal toxins are capable of causing widespread different poisoning syndromes that are: (1) amnesic shellfish poisoning (ASP), (2) ciguatera fish poisoning (CFP), (3) diarrhoeic shellfish poisoning (DSP), (4) neurotoxic shellfish poisoning (NSP), and (5) paralytic shellfish poisoning (PSP); and all have generally been shown to harm human health [30].

Algae such as *Spirulina* (*Arthrospira*), *I. galbana*, *H. pluvialis, C. vulgaris, D. salina, P. tricornutum*, and *P. cruentum*, among others that are commercially used in aquaculture and food supplements do not produce toxin. Even within the same species, large differences exist between toxic and non-toxic algae. For example, dinoflagellates and diatoms are best known for their production of toxins that can affect humans, but for instance the dinoflagellate strain *C. cohnii* has a GRAS (Generally Recognized as Safe) status by FDA for ω-3 DHA human food consumption [30] (Table 5). As such, it is very important to know the safety of algae at strain level that will be eventually applied for food or feed application.

Table 5 Safety aspect of relevant microalgae for food application Source: [30]

Organism	Species	Safety aspect	Organism	Species	Safety aspect
Chlorophyta	*Chlamydomonas reinhardtii*	NT	Haptophyta	*Isochrysis galbana*	NT
	Chlorella vulgaris	GRAS		*Pavlova* sp.	NT
	Dunaliella salina	NT	Heterokontophyta	*Nitzschia dissipata*	NT
	Haematococcus pluvialis	NT		*Nannochloropsis* sp.	NT
	Scenedesmus sp.	NT		*Phaedactylum tricornutum*	NT
	Tetraselmis sp.	NT		*Skeletonema* sp.	NT
Cyanobacteria	*Spirulina* (*Arthrospira*)	GRAS		*Schizochytrium*	GRAS
	Synechococcus sp.	NT		*Thalassiosira pseudonoma*	NT
Dinophyta	*Crypthecodinium cohnii*	GRAS	Rhodophyta	*Porphyridium cruentrum*	GRAS

NT no toxins known, *GRAS* Generally Recognized As Safe by the Food Drug Administration (FDA)

Heavy Metals

Heavy metals are often assumed to be highly toxic or damaging to the environment, while prolonged eating of foods contaminated with heavy metals can lead to long-term human health problems. According to WHO/FAO guidelines, an adult person of 60 kg body weight should not incorporate more than 0.3 mg of mercury, 0.5 mg of cadmium, 3 mg of lead, and 20 mg of arsenic per week through beverages and food. As microalgae are capable of accumulating heavy metal ions by biosorption, traces of heavy metals may be found in algal food products. At present, some official standards have estimated the content of heavy metal in foods, which is also estimated for algal biomass intended for human consumption (Table 6) [13].

Nutritional Quality Standard and Regulations

Products from microalgae that are intended for human or animal nutrition must be subject to a range of regulations and standards. There exist international testing programs for unconventional foodstuffs such as single-cell protein, which have to be or should be performed. There are only few countries that have so far stipulated legislative standards for *Spirulina*. It is not known whether official regulations also exist for other types of microalgae, that is, *Chlorella*, *Dunaliella*, etc. These official requirements, as well as suggested quality criteria found in the literature and elsewhere are summarized in Table 6 [13].

According to Becker [13] several steps need to be necessarily evaluated for the approval of algal biomass for human and animal consumption. It seems useful to have the following specifications to become available:

Table 6 Some quality standards for microalgae Source: Adapted from [13]

	IUPAC (1974)	WHO (1972)	Earthrise Algal Farm (USA)	Indian Standard (1990)	Japan (Jassby, 1988)	France (Becker 1994)	Brazil (ANVISA, [69–71])
Total protein (%)				> 55.0		> 45.0	
Total ash (%)				< 9.0			
Moisture (%)			< 7.0	< 9.0	< 7.0		< 10.0
Standard plate count (x10^6/g)			< 0.2		< 0.005	< 0.1	
Mould (number/g)			< 100		< 100		
Coliformes (number/g)			Absent		Absent	< 10	< 10
Salmonella sp.			Absent			Absent	Absent
Staphylococcus sp. (number/g)			Absent			< 100	< 500
Filth (insect fragment) (number/g)			< 10				Absent
Pb (ppm)	< 5.0	< 0.1	< 0.2				< 0.30
Hg (ppm)	< 0.1	< 0.001	< 0.025				< 0.50
Cd (ppm)	< 1.0	< 0.01	< 0.2				< 0.10
As (ppm)	< 2.0	< 0.05					< 1.00

- Proximate chemical composition;
- Evaluation of protein quality and quantity of essential amino acids;
- Biogenic toxic substances (phycotoxins, nucleic acids, other toxicants);
- Non-biogenic toxic compounds (heavy metals, residues from harvesting, and processing);
- Sanitary analyses (microbial analyses for contamination);
- Toxicological and safety evaluations (feeding trials with experimental animals).

Food safety at international level is governed by Codex Alimentarius Commission (CAC), which was created by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO). The Codex Alimentarius is a collection of internationally recognized standards, codes of practice, guidelines and other recommendations relating to food production and food safety. In the case of food additives and novel foods (including nutraceuticals and functional foods), laws and regulations may vary from country to country. For example, in the USA the FDA has the primary responsibility for regulating new food ingredients, whereas the EU through the European Food Safety Authority (EFSA) has regulations for food additives, novel foods, and genetically modified organisms [6]. In January 2011, the FDA Food Safety Modernization Act was signed into law in the USA. This means that the manufacturer may seek approval for a new ingredient by filing a food additive petition with the FDA to (a) making a GRAS determination or (b) request a formal pre-market review. This petition needs to be supported by clinical and nonclinical studies [6, 30].

In Australia and New Zealand, novel foods and novel food ingredients, including products from microalgae are regulated under Standard 1.5.1—Novel Foods—of the Australian and New Zealand Food Standards Code administered by Food Standards Australia New Zealand [6]. In Brazil, the ANVISA (Agência Nacional de Vigilância Sanitária) has estimated some limits for microbial and heavy metal contaminants in tablets/capsules of *Spirulina* sold as dietary supplement [69–71] (see Table 6).

For many markets, including algae industry, Good Manufacturing Practice (GMP) certification and ISO 9001/2000 are essential in order to conform to the guidelines recommended by agencies that control authorization and licensing for manufacture products. In the case of food and related industries, a Hazard Analysis and Critical Control Points (HACCP) methodology is highly recommended. However, specific markets may require additional certifications, for example, organic certification by a recognized certifying authority, for example USDA Organic in the USA and IBD Accreditation in Brazil.

Challenges Surrounding Algal Usage

New market food products from microalgae or products that include algal-derived substances are subject to food safety regulations that apply to all food products. This implies that novel foods and food ingredients must be safe for consumers (*i.e.*, not being dangerous and properly labeled so as not to mislead consumers) [30]. This is the case, for example, in the extraction of polyunsaturated fatty acids (*e.g.*, EPA, DHA, and ARA) from microalgae, where organic solvents have been widely applied to the extraction of microalgal lipid-rich for biodiesel production [84, 85]; however, such use for the pharmaceutical and nutraceutical sector may lead to contamination of the final product in the form of residues. Because the solvents used in the extraction will be removed by evaporation, solvents with low boiling points should be chosen to avoid prolonged heating. Thus, the lower boiling fractions of petroleum ether (35–60 °C) could be used instead of the higher boiling fractions (*e.g.,* hexane 65–69 °C) [86]. Nevertheless, the use of petroleum ether for large-scale industrial solvent extraction may have limited permission in many countries, while *n*-hexane is ranked on the list of substances prohibited in cosmetic products (Regulation (EC) no. 1223/1999 of the European Parliament [88].

When extracting carotenoids from biological samples, such as microalgae, a water-miscible organic solvent (*e.g.*, acetone, methanol, or ethanol) that allow better solvent penetration can be used. Acetone is widely used for carotenoid extraction; however, with increasing use of high performance liquid chromatography (HPLC), tetrahydrofuran (THF) may become a popular extraction solvent [86]. Carotenoid-astaxanthin from *H. pluvialis* is an example of high-value product that has been habitually extracted with organic solvent. In such case, supercritical fluid extraction (SFE) based on carbon dioxide and coupled with ethanol as a cosolvent can greatly increase the extraction efficiency [23, 87] and has emerged as a “green” technology due to its non-flammable characteristics, pressure relatively low and chemically inert [85]. In addition to carbon dioxide, other compressed solvents can be used to extract polyunsaturated fatty acids and carotenoids from microalgae, including ethane, propane, *n*-butane, and dimethyl ether [88–90]. In fact, a recent study (*e.g.*, [91]) has evaluated the efficiency of using subcritical *n*-butane in comparison to supercritical CO_2 for extraction of lipids, polyunsaturated ω-3 and ω-6 and carotenoids from three marine microalgae (*N. oculata*, *P. tricornutum,* and *P. cruentum*) for food and pharmaceutical applications. The choice of *n*-butane as alternative solvent was motivated by its gentle vapor pressure, close chemical structure, low price and its classification as authorized solvent for foodstuff production without limitation (Directive 2009/32/EC). Nevertheless, the economic viability of these new approaches for extraction of high-value products from microalgae for food application will have to be demonstrated.

Another important issue regarding to algal usage is the products containing genetically modified (GM) algae that has two main aspects related to biosafety [30]: potential environmental risks and potential toxicity or allergenicity to human health, which may depend on the relative quantity of GM in the product [30]. In general, all research—including microalgae research—is governed by principles of Good Laboratory Practice (GLP), which is defined as a set of rules and criteria for a quality system. In the case of USA and Europe, the FDA and the EFSA are responsible for the biosafety evaluation, respectively [30]. For potential human health risks, the biosafety evaluation of GM algal products need to be tested on a case-by-case basis before introduction in order to guarantee that they are safe and they do not produce toxic substances or allergens [92]. In the case of environmental risks, Henley *et al*. [93] have published a comprehensive study of GM from microalgae towards to biofuel production with a focus on environmental risk assessment. In particular for GM food applications, GM-algae that would have a history of safe use over a longer period of time imply that production has proven to be safe. Nevertheless, the process of bringing a GM microalgae product to market can, therefore, be a long and complicated issue.

Conclusions and Outlook

The variety products accessible from the primary and secondary metabolism of diverse algal species clearly demonstrates the importance of these versatile microorganisms as cellular factories. With a well-established marketplace, products from microalgae have grown, and there are clear opportunities for new products. In this view, microalgae are interesting for products that protein, lipids, starch and pigments are pivotal of cellular storage. Cyanobacteria are promising host organisms for the production of small biological molecules such as isoprene and high-chain alcohol (1-butanol). Considering recent progress in the field of genetic engineering, implementation of improved algal genes is very likely to understand algal intracellular activities with a broad industrial application. Besides the genetic engineering, the development of algal cultivation process from laboratory conditions to large scale production will require substantial progress towards a cost-efficient algal-based technology in order to encourage manufactures for new products to be developed and marketed in the next decade.

Useful Definitions

Nutraceutical can be defined as a food or food product that reportedly provides health and medical benefits, including the prevention and treatment disease. Carotenoids and long-chain polyunsaturated fatty acids are good examples.

Functional foods can be broadly defined as foods that are either enriched or fortified, a process called nitrification, which has been satisfactorily demonstrated to improved state of health and well-being. An example of microalgal "functional food" are biscuits enriched with ω-3 fatty acids by the inclusion of *I. galbana* biomass.

Vegan foods can be ethical defined as avoidance of animal food products and their derivates. In this context, food products from algae are considering alternative food source for vegan consumers.

Cosmaceuticals are cosmetics products with bioactive ingredients purported to have medical or drug-like benefits. For example, phycobilin- and carotenoid-algae pigments.

Dietary supplement is a product taken by mouth that contains a "dietary ingredient" intended to supplement the diet. For examples, pills of β-carotene (pro-vitamin A), softgels of EPA + DHA ω-3 and capsules/tablets of single cell protein (SCP).

Single cell protein (SCP) refers to edible unicellular microorganism containing protein-derived from algae or yeast or fungi or bacteria source.

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References

1. Smithers GW (2016) Food science—yesterday, today, and tomorrow. Ref Modul Food Sci:1–11
2. Chen CY, Zhao XQ, Yen HW, Ho SH, Cheng CL, Lee DJ, Bai FW, Chang JS (2013) Microalgae-based carbohydrates for biofuel production. Biochem Eng J 78:1–10
3. Varshney P, Mikulic P, Vonshak A, Beardall J, Wangikar PP (2015) Extremophilic microalgae and their potential contribution in biotechnology. Bioresour Technol 184:363–372
4. Tibbetts SM, Whitney CG, MacPherson MJ, Bhatti S, Banskota AH, Stefanova R, McGinn PJ (2015) Biochemical characterization of microalgal biomass from freshwater species isolated in Alberta, Canada for animal feed applications. Algal Res 11:435–447
5. Draaisma RB, Wijffels RH, Slegers (Ellen) PM, Brentner LB, Roy A, Barbosa MJ (2013) Food commodities from microalgae. Curr Opin Biotech 24:169–177
6. Borowitzka MA (2013) High-value products from microalgae – their development and commercialisation. J Appl Phycol 25:743–756
7. Matos AP, Feller R, Moecke EHS, Sant'Anna ES (2015) Biomass, lipid productivity and fatty acids composition of marine *Nannochloropsis gaditana* cultured in desalination concentrate. Bioresour Technol 197:48–55
8. Wijffels RH, Kruse O, Hellingwerf KJ (2013) Potential of industrial biotechnology with cyanobacteria and eukaryotic microalgae. Curr Opin Biotech 24:405–413
9. Koller M, Muhr A, Braunegg G (2014) Microalgae as versatile cellular factories for valued products. Algal Res 6:52–63
10. Henrikson R (2009) Earth food spirulina. Ronore Enterprises, Hawaii

11. Armenta RE, Valentine MC (2013) Single-cell oils as a source of omega-3 fatty acids: an overview of recent advances. J Am Oil Chem Soc 90:167–182
12. Gunerken E, D'Hondt E, Eppink MHM, Garcia-Gonzalez L, Elst K, Wijffels RH (2015) Cell disruption for microalgae biorefineries. Biotechnol Adv 33:243–260
13. Becker EW (2013) In: Richmond A, Hu Q (eds) Handbook of microalgal culture: applied phycology and biotechnology, vol 25, 2nd edn. Wiley, UK, pp 461–503
14. Pulz O, Gross W (2004) Valuable products from biotechnology of algae. Appl Microbial Biotechnol 65:635–648
15. Spolaore P, Joannis-Cassan C, Duran E, Isambert A (2006) Commercial applications of microalgae. J Biosci Bioeng 101:87–96
16. Oilgae (2016) Emerging algae product and business opportunities. Comprehensive report on attractive algae product opportunities. www.oilgae.com/ref/report/non-fuel-algae-products.html. Accessed 07 Mar 2017
17. Algae Industry Magazine (2016) $1.1B global algae market projected by 2024. www.algaeindustrymagazine.com. Accessed 12 Mar 2017
18. Voort MPJ, Vulstake E, Visser CLM (2015) Macro-economics of algae products. Public output report WP2A7.02 of the EnAlgae project, Swansea, June, p 47. http://www.enalgae.eu/public-deliverables.htm
19. Lu YM, Xiang WZ, Wen YH (2011) *Spirulina* (*Arthrospira*) industry in Inner Mongolia of China: current status and prospects. J Appl Phycol 23:256–269
20. Rito-Polamares M, Nunez L, Amador D (2001) Practical application of aqueous two-phase systems for the development of a prototype process for c-phycocyanin recovery from *Spirulina maxima*. J Chem Technol Biotechnol 76:1273–1280
21. Shanahan C, Frost & Sullivan. Algae Industry Magazine (2014) The global algae oil omega-3 market in 2014. http://www.algaeindustrymagazine.com. Accessed 10 Feb 2017
22. Franz AK, Danielewicz MA, Wong DM, Anderson LA, Boothe JR (2013) Phenotypic screening with oleaginous microalgae reveals modulators of lipid productivity. ACS Chem Biol 8:1053–1062
23. Panis G, Carreon JR (2016) Commercial astaxanthin production derived by green alga *Haematococcus pluvialis*: a microalgae process model and a techno-economic assessment all through production line. Algal Res 18:175–190
24. Matos AP, Feller R, Moecke EHS, Oliveira JV, Junior AF, Derner RB, Sant'Anna ES (2016) Chemical characterization of six microalgae with potential utility for food application. J Am Oil Chem Soc 93:963–972
25. Tibbetts SM, Milley JE, Lall SP (2014) Chemical composition and nutritional properties of freshwater and marine microalgal biomass cultured in photobioreactors. J Appl Phycol 27:1109–1119
26. Tibbetts SM, Melanson RJ, Park KC, Banskota AH (2015) Nutritional evaluation of whole and lipid-extracted biomass of the microalga *Scenedesmus* sp. AMDD isolated in Saskatchewan, Canada for animal feeds: proximate, amino acid, fatty acid, carotenoid and elemental composition. Curr Biotechnol 4:530–546
27. Lourenço SO, Barbarino E, Lavín PL, Marquez UML, Aidar E (2004) Distribution of intracellular nitrogen in marine microalgae: calculation of new nitrogen-to-protein conversion factors. Eur J Phycol 39:17–32
28. Anupama Ravindra P (2000) Value-added food: single cell protein. Biotechnol Adv 18:459–479
29. Algae Industry Magazine (2017) TerraVia's whole algal protein gets Canadian approval. http://www.algaeindsutrymagazine.com. Accessed 14 Mar 2017
30. Enzing C, Ploeg M, Barbosa M, Sijtsma L (2014) Microalgae-based products for the food and feed sector: an outlook for Europe. Joint Research Centre Scientific and Policy Reports, European Commission. Publications Office of the European Union, Luxembourg, p 82. doi:10.2791/3339
31. Shibakani M, Tsubouchi G, Sohma M, Hayashi M (2016) Synthesis of nanofiber-formable carboxymethylated *Euglena*-derived β-1,3-glucan. Carbohyd Polym 152:468–478
32. Sadovskaya I, Souissi A, Souissi S, Grard T, Lencel P, Greene CM, Duin S, Dmitrenok PS, Chizhov AO, Shashkov AS, Usov AI (2014) Chemical structure and biological activity of a highly branched (1 → 3,1 → 6)-β-d-glucan from *Isochrysis galbana*. Carbohyd Polym 111:139–148
33. Turu IC, Kayhan CT, Kazan A, Ozturk EY, Akgol S, Celiktas OY (2016) Synthesis and characterization of cryogel structures for isolation of EPS from *Botryococcus braunii*. Carbohyd Polym 150:378–384
34. Arad SM, Moppes DV (2013) In: Richmond A, Hu Q (eds) Handbook of microalgal culture: applied phycology and biotechnology, vol 21, 2nd edn. Wiley, UK, pp 406–416
35. Atobe S, Saga K, Hasegawa F, Furuhashi K, Tashiro Y, Suzuki T, Okada S, Imou K (2015) Effect of amphiphilic polysaccharides released from *Botryococcus braunii* Showa on hydrocarbon recovery. Algal Res 10:172–176
36. Tasic MB, Pinto LFR, Klein BC, Velikovic VB, Filho RM (2016) *Botryococcus braunii* for biodiesel production. Renew Sustain Energ Rev 64:260–270
37. Keng PS, Basri M, Zakaria MRS, Rahman MBA, Ariff AB, Rahman RNZA, Salleh AB (2009) Newly synthesized palm esters for cosmetic industry. Ind Crop Prod 29:37–44
38. Algae Industry Magazine (2015) Bunge, Solazyme expand JV for healthier foods. http://www.algaeindustrymagazine.com. Accessed 14 Mar 2017
39. Martin CA, Almeida VV, Ruiz MR, Visentainer JEL, Matshushita M, Souza NE, Visentainer JV (2006) Ácidos graxos poliinsaturados ômega-3 e ômega-6: importância e ocorrência em alimentos. Rev Nutr 19:761–770
40. Kleiner AC, Cladis DP, Santerre CR (2014) A comparison of actual versus stated label amounts of EPA and DHA in commercial omega-3 dietary supplements in the US. J Sci Food Agric 95:1260–1267
41. FAO/WHO (2008). Fats and fatty acids in human nutrition—report of an expert consultation. Food and Agriculture Organization of the United Nations—FAO
42. Lemahieu C, Bruneel C, Termote-Verhalle R, Muylaert K, Buyse J, Foubert I (2013) Impact of feed supplementation with different omega-3 rich microalgae species on enrichment of eggs of laying hens. Food Chem 141:4051–4059
43. Algae Industry Magazine (2014) Omega-3: A global market overview. http://www.algaeindustrymagazine.com. Accessed 16 Jan 2017
44. Nelson JR, Wani O, May HT, Budoff (2017) Potential benefits of eicosapentaenoic acid on atherosclerotic plaques. Vasc Pharmacol 91:1–9
45. Carlson SE, Colombo J (2016) Docosahexaenoic acid and arachidonic acid nutrition in early development. Adv Pediatr 63:453–471
46. Pangestuti R, Kim SK (2011) Biological activities and health benefits affects of natural pigments derived from marine algae. J Funct Foods 3:255–266
47. European Commision (2002) Opinion of the scientific on animal nutrition on the use of canthaxathin in feedingstuffs for salmon and trout, laying hens, and other poultry. C2—Management of Scientific Committees; Scientific Co-Operation and Networks, p 29
48. Coates RC, Trentacoste E, Gerwick WH (2013) In: Richmond A, Hu Q (eds) Handbook of microalgal culture: applied phycology and biotechnology, vol 26, 2nd edn. Wiley, UK, pp 516–543

49. Gong M, Bassi A (2016) Carotenoids from microalgae: a review of recent developments. Biotechnol Adv 34:1396–1412
50. Business Communications Company (2015) The global market for carotenoids—FOD025E. http://www.bccresearch.com/market-research/food-and-beverage/carotenoids-global-market-report-fod025e.html Accessed 03 Mar 2017
51. Mao X, Liu Z, Sun J, Lee SY (2017) Metabolic engineering for the microbial production of marine bioactive compounds. Biotechnol Adv. doi:10.1016/j.biotechadv.2017.03.001
52. Vo TS, Ngo DH, Kim SK (2012) Marine algae as a potential pharmaceutical source for anti-allergic therapeutics. Process Biochem 47:386–394
53. Nwachukwu ID, Udenigwe CC, Aluko RE (2016) Lutein and zeaxanthin: production technology, bioavailability, mechanisms of action, visual function, and health claim status. Trends Food Sci Tech 49:74–84
54. Foo SC, Yusoff FM, Ismail M, Basri M, Yau SK, Khong NMH, Chan KW, Ebrahimi M (2017) Antioxidant capacities of fucoxanthin-producing algae as influenced by their carotenoid and phenolic contents. J Biotechnol 241:175–183
55. Tang Z, Zhao J, Ju B, Li W, Wen S, Pu Y, Qin S (2016) One-step chromatographic procedure for purification of B-phycoerythrin from *Porphyridium cruentum*. Protein Expres Purif 123:70–74
56. Watanabe F, Katsura H, Takenaka S, Fujita T, Abe K, Tamura Y, Nakatsuka T, Nakano Y (1999) Pseudovitamin B_{12} is the predominate cobamide of an algal health food, *Spirulina* tablets. J Agric Food Chem 47:4736–4741
57. Guedes AC, Amaro HM, Barbosa CR, Pereira RD, Malcata FX (2011) Fatty acid composition of several wild microalgae and cyanobacteria, with a focus on eicosapentaenoic, docosahexaenoic and α-linolenic acids for eventual dietary uses. Food Res Int 44:2721–2729
58. Han D, Deng Z, Lu F, Hu Z (2013) In: Richmond A, Hu Q (eds) Handbook of microalgal culture: applied phycology and biotechnology, vol 23, 2nd edn. Wiley, UK, pp 445–456
59. Englund E, Pattanaik B, Ubhayasekera SJK, Stensjo K, Bergquist J, Lindberg P (2014) Production of squalene in *Synechocystis* sp. PCC 6803. PLoS One 9:e90270
60. Lindberg P, Park S, Melis A (2010) Engineering a platform for photosynthetic isoprene production in cyanobacteria, using *Synechocystis* as the model organism. Metab Eng 12:70–79
61. Machado IMP, Atsumi S (2012) Cyanobacterial biofuel production. J Biotechnol 162:50–56
62. Atsumi S, Higashide W, Liao JC (2009) Direct photosynthetic recycling of carbon dioxide to isobutyraldehyde. Nat Biotechnol 27:1177–1180
63. Atsumi S, Hanai T, Liao JC (2008) Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels. Nature 451:86–89
64. Lan EI, Liao JC (2011) Metabolic engineering of cyanobacteria for 1-butanol production from carbon dioxide. Metab Eng 13:353–363
65. Lee SY, Park JH, Jang SH, Nielsen LK, Kim J, Jung KS (2008) Fermentative butanol production by *Clostridia*. Biotechnol Bioeng 101:209–228
66. Kelley RE, Andersson HC (2014) Chapter 55—disorders of purine and pyrimidines. Handb Clin Neurol 120:817–838
67. Liu S, Perez-Ruiz F, Miner JN (2017) Patients with gout from health subjects in renal response to changes in serum uric acid. Jt Bone Spine 84:183–188
68. Proença LAO, Fonseca RS, Pinto TO (2011) Microalgas em área de cultivo do litoral de Santa Catarina. RiMa Editora, São Carlos, p 90p
69. BRASIL (ANVISA) Agência Nacional de Vigilância Sanitária. Regulamento técnico sobre padrões microbiológicos para alimentos. RDC No. 12 de 02/Janeiro/2001, Brasília-DF, p 37
70. BRASIL (ANVISA) Agência Nacional de Vigilância Sanitária. Dispõe sobre matérias estranhas macroscópicas e microscópicas em alimentos e bebidas, seus limites de tolerância e dá outras providências. RDC No. 14 de 28/Março/2014, Brasília-DF, p 9
71. BRASIL (ANVISA) Agência Nacional de Vigilância Sanitária. Dispõe sobre o regulamento MERCOSUL sobre limites máximos de contaminantes inorgânicos em alimentos. RDC No. 42 de 29/Agosto/2013, Brasília-DF, Diário Oficial da União-Seção 1, pp 33–35 **(ISSN 1677-7042)**
72. Matos AP, Ferreira WB, Torres RCO, Morioka LRI, Canella MHM, Rotta J, Silva T, Moecke EHS, Sant'Anna ES (2014) Optimization of biomass production of *Chlorella vulgaris* grown in desalination concentrate. J Appl Phycol 27:1473–1483
73. Rezanka T, Lukavsky J, Nedbalová L, Sigler K (2014) Production of structured triacylglycerols from microalgae. Phytochemistry 104:95–104
74. Raposo MFJ, Morais RMSC, Morais ACMB (2013) Health applications of bioactive compounds from marine microalgae. Life Sci 93:479–486
75. Sun GY, Simonyi A, Fritsche KL, Chuang DY, Hannink M, Gu Z, Greenlief CM, Yao JK, Lee JC, Beversdorf (2017) Docosahexaenoic acid (DHA): an essential nutrient and a nutraceutical for brain health and diseases. Prostag Leukotr Ess. doi:10.1016/j.plefa.2017.03.006
76. Lewis KD, Huang W, Zheng X, Jiang Y, Feldman RS, Falk MC (2016) Toxicological evaluation of arachidonic acid (ARA)-rich oil and docosahexaenoic acid (DHA)-rich oil. Food Chem Toxicol 96:133–144
77. Turak EJ (2017) Carotenoids microencapsulation by spray drying method and supercritical micronization. Food Res Int. doi:10.1016/j.foodres.2017.02.001
78. Hosseini SRP, Tavakoli O, Sarrafzadeh MH (2017) Experimental optimization of SC-CO_2 extraction of carotenoids from *Dunaliella salina*. J Supercrit Fluid 121:89–95
79. Esatbeyoglu T, Rimbach G (2017) Canthaxanthin: from molecule to function. Mol Nutr Food Res 61:1–17
80. Loredo AG, Benavides J, Polomares MR (2016) Growth kinetics and fucoxanthin production of *Phaeodactylum tricornutum* and *Isochrysis galbana* cultures at different light and agitation conditions. J Appl Phycol 28:849–860
81. Gayathri S, Rajasree SRR, Kirubagaran R, Aranganathan L, Suman TY (2016) Spectral characterization of β-ε-carotene-3-3′-diol (lutein) from marine microalgae *Chlorella salina*. Renew Energy 98:78–83
82. Eilers U, Dietzel L, Breitenbach J, Buchel C, Sandmann G (2016) Identification of genes coding for functional zeaxanthin epoxidases in the diatom *Phaeodactylum tricornutum*. J Plan Physiol 192:64–70
83. Millao S, Uquiche E (2016) Antioxidant activity of supercritical extracts from *Nannochloropsis gaditana*: correlation with its content of carotenoids and tocopherols. J Supercrit Fluid 111:143–150
84. Halim R, Danquah MK, Webbey PA (2012) Extraction of oil from microalgae for biodiesel production: a review. Biotechnol Adv 30:709–732
85. Cheng CH, Du TB, Pi HC, Jang SM, Lin YH, Lee HT (2011) Comparative study of lipid extraction from microalgae by organic solvent and supercritical CO_2. Bioresour Technol 21:10151–10153
86. Amaya DBR, Kimura M (2004) HarvestPlus handbook for carotenoid analysis. HarvestPlus technical monograph series 2, p 63
87. Golmakani MT, Mendiola JA, Rezaei K, Ibánez E (2012) Expanded ethanol with CO_2 and pressurized ethyl lactase to obtain fractions enriched in ɣ-Linolenic acid from *Arthrospira platensis* (*Spirulina*). J Supercrit Fluid 62:109–115
88. Rapinel V, Rombuat N, Rakotomanomana N, Vallageas A, Cravotto G, Chemat F (2016) An original approach for lipophilic

natural compounds extraction: use of liquefied *n*-butane as alternative solvent to *n*-hexane. Food Sci Technol. doi:10.1016/j.lwt.2016.10.003

89. Capeletto C, Conterato G, Scapinello J, Rodrigues FS, Copini MS, Kuhn F, Tres MV, Magro JD, Oliveira JV (2016) Chemical composition, antioxidant and antimicrobial activity of guavirova (*Campomanesia xanthocarpa* Berg) seeds extracts obtained by supercritical CO_2 and compressed *n*-butane. J Supercrit Fluid 110:32–38
90. Goto M, Kanda H, Wahyudiono Machmudah S (2015) Extraction of carotenoids and lipids from algae by supercritical CO_2 and subcritical dimethyl ether. J Supercrit Fluid 96:245–251
91. Feller R (2017) Microalgae biomass as a source of natural compounds: chemical characterization and new approaches for lipid extraction and culture harvesting. (Thesis in Chemical Engineering), Federal University of Santa Catarina, Florianópolis, Brazil, p 146
92. Song Q, Lin H, Jiang P (2012) Advances in genetic engineering of marine algae. Biotechnol Adv 30:1602–1613
93. Henley WJ, Litaker RW, Novoveská L, Duke CS, Quemada HD, Sayre RT (2013) Initial risk assessment of genetically modified (GM) microalgae for commodity-scale biofuel cultivation. Algal Res 2:66–77