Importance of Polyunsaturated Fatty Acids from Marine Algae

9

Rafael Zárate, Nabil el Jaber-Vazdekis, and Raquel Ramírez-Moreno

Introduction

Marine algae, appeared on Earth sometime 3–4 billion years ago, these include marine cyanobacteria, marine eukaryotic microalgae, and seaweeds which appear widely spread in the oceans, going from the polar regions to tropical areas, covering many ranges of environments, such as nutrient-rich coastal seas or oligotrophic open oceans [1]. They are photoautotroph, and unlike "plants," they do not possess roots, leaves, and other organs that characterize higher plants, but they do possess chlorophyll. Marine algae range in size from microscopic individual cells of microalgae to huge seaweeds that are greater than 30–50 m long and are called macroalgae *Macrocystis*, the genus that includes kelp. Marine algae are responsible for approximately 40–50 % of the photosynthesis that occurs on Earth annually [2]. These species have been used by humans to obtain abundant valuable compounds, with interest for human health and nutrition, or cosmetics, oils (e.g., triglycerides), polysaccharides (e.g., algin, agar), pigments (e.g., phycobili proteins, carotenoids), including also biodiesel, for the chemical industry, and often the discovery of potent new pharmaceuticals [3–6].

This vast group of organisms is defined as the primary producers in the marine environment, and their presence is essential for the maintenance of the food chain and life. Regarding that, it should be highlighted their capacity to produce high valuable omega-3 polyunsaturated fatty acids (PUFAs), which have been demonstrated to possess great importance in human health, since the consumption of EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) has been reported to participate in the optimal functioning of the cell membrane, and thus in literally all possible physiological and biochemical events within the cells and bodies, participating also in reproduction, many other mechanisms, and to prevent cardiovascular, obesity, nervous systems, inflammatory conditions, and numerous pathological disorders [7, 8]. Accordingly, the global demand for omega-3 fatty acids has significantly increased over the years, but on the other hand, there is also a growing concern about how to manage and achieve a sustainable exploitation of their major source, i.e., wild fish (fish oils), which are being seriously depleted [9].

Therefore, a different scenario emerges a few decades ago, in which algae appear to be the natural takeovers due to their ability, not only to produce high amounts of PUFAs, particularly omega-3, but also the ability to be cultured and grown in controlled and optimal conditions. Numerous autotrophically and heterotrophically grown microalgae species have been studied for their high-EPA and/or high-DHA contents, such as *Nannochloropsis*, *Phaeodactylum*, *Schizochytrium,* and *Thraustochytrium* [10]. In the last few years, biotechnology approaches have also been applied to improve the quality of PUFA profiles, as well as boosting the amounts of these desirable compounds, together with the optimization of culture techniques and strategies [11]. Many advances have been attained, and progress is being achieved at a regular pace, with many interesting outcomes being reported. All these considerations make algae, particularly microalgae, a very attractive group to be exploited and in the near future to likely become one of the main sources of omega-3 PUFAs, and more importantly to offer a continuous and sustainable source and supply of these important metabolites to thus satisfy the world demand.

In this chapter, references to the marine environment and the food chain in which these organisms participate have been addressed, making also some comparisons with terrestrial plants and their fatty acids; furthermore, the

R. Zárate (✉) · R. Ramírez-Moreno
Canary Islands Cancer Research Foundation (FICIC), Ave. La Trinidad 61, Torre A. Arévalo, 38204 La Laguna, Tenerife, Spain
e-mail: rzarate@icic.es

R. Ramírez-Moreno
e-mail: raquelramirezmoreno@gmail.com

N. el Jaber-Vazdekis
Laboratory of Photosynthesis, Institute of Microbiology CAS, Novohradska 237, Opatovicky mlyn., 379 81 Trebon, Czech Republic
e-mail: nabil@alga.cz

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appearance and profiles of fatty acids from both microalgae and seaweeds are also presented, together with updated information on the biotechnological approaches conducted for boosting fatty acid yields with microalgae. For the optimal exploitation and consumption of fatty acids from these natural sources, comments on the regulatory processes and their constrains were also treated. Finally, under concluding remarks, references are made to the expected impacts of new or more comprehensive approaches, the expected results for boosting production and thus establish algae as an attractive and effective source of fatty acids for human health in the near future.

The Marine Environment and Algae

The sea, home to the marine environment, covers around 71 % of the Earth's surface and represents about 97 % of its waters, corresponding to approximately 1370×10^6 km^3. Given the current state of continental drift, the seawater is not distributed evenly between the two hemispheres. In the Northern Hemisphere, the sea ratio is approximately equal to the ground (61 %), while the Southern Hemisphere is significantly oceanic (80 %). The sea is an interconnected system with five large areas called oceans (Pacific Ocean, Atlantic Ocean, Indian Ocean, Antarctic Ocean, and Arctic Ocean) and smaller sections known as seas (Mediterranean Sea, Baltic Sea, Red Sea, etc.). The Pacific Ocean is the most extensive, almost as large as the rest all together. Life in our planet is dependent upon the oceans, being the sea the main stabilizer of the world climate. In addition, the oceans are the main highway for international trade, as well as providing us water, food, energy, and sustaining the livelihoods of millions of people. Actually, marine biotechnology is being largely focused on marine biomolecules for medicine or engineering uses [12].

The presence of seawater is common in all marine habitats. The seawater is distinctly salty due to the nature of water and materials dissolved therein. Only six ions comprise 99 % of the dissolved solids. The most common are sodium and chloride, representing almost 85 %. In oceanic areas, the salinity has little variation, generally is between 33 and 37 ‰ depending mainly on the balance between precipitations and evaporation. However, in other regions, the salinity levels can change more conspicuously caused by many factors, such as the currents, the incoming of freshwater from rivers and glaciers, or the high evaporation levels in shallow areas. The sunlight penetrates only about 200 m, depending on water turbidity and angle of the sun, so at greater depths there is darkness. In addition to salinity and light, others physicochemical factors such as temperature, wind, currents, waves action, tides, acidity, dissolved gases, pressure, and substrate have influence on the functioning and diversity of the varied marine ecosystems. Also, these ecosystems depend on dissolved nutrients washed down from the land and sediments of the seabed. Rock composition, waves, tides, and currents are the principal agents responsible for erosion and deposition along coastlines and seabed. Besides, the currents are important in the transport of essential nutrients for the development of marine life, from the north to the south and vice versa and from deep to superficial areas.

Marine habitats can also be modified by their inhabitants. Some organisms such as coral, kelp, mangroves, and sea grasses are ecosystem engineers capable of reshaping the marine environment to the point where they even can create further habitats for other organisms [12].

The geological structure of the seabed is fairly similar worldwide. Marine habitats are distributed horizontally from the coasts, over the continental shelf, until the deep seabed. Alternately, the marine habitats can be vertically divided, and the entire area of the open water column is the pelagic environment; pelagic organisms live in open sea away from the seabed. The demersal environment is just above the seabed, and the benthic environment corresponds to the sea bottom, with 3.8 km of average depth. A third division is by latitude, from temperate tropical waters to polar waters. Depending on the distance from the coast, the pelagic environment is horizontally subdivided into the neritic zone on the continental shelf, which extends 68 km on average, and the oceanic zone. Progressing vertically and according to depth, the pelagic environment can be subdivided into different zones. The epipelagic, synonymous to photic or lighted region, is the zone of primary production in the ocean and with a major importance. The transition area between light and dark, called the disphotic region, is equivalent to mesopelagic. This area has enough light for vision but not enough for photosynthesis and extends down to about 700–1000 m. Next is the bathypelagic, which cover until 2000–4000 m. Overlying the plains of the major ocean basins is the abyssal pelagic, which has its lower boundary at about 6000 m. The open water of the deep oceanic trenches between 6000 and 10,000 m is called the hadalpelagic.

Corresponding to the last three pelagic zones are three bottoms or benthic zones. The bathyal is the area of seabed encompassing the continental slope and down to about 4000 m. The abyssal zone includes the broad abyssal plains of the ocean basins between 4000 and 6000 m. The hadal is the benthic zone of the trenches between 6000 and 10000 m. The benthic zone underlying the neritic pelagic zone on the continental shelf is named the sublittoral. It is illuminated and a permanently submerged area. The intertidal or mesolittoral zone includes shore areas lying between the extremes of high and low tide. It represents the transitional area from marine to terrestrial conditions. The most elevated area corresponds to supralittoral zone, called the splash.

Seawater penetrates in these areas only during storms with high tides [13].

Most marine life is found in coastal habitats, even though the shelf area occupies only 7 % of the total ocean area. Many sceneries such as sandy and rock shores, estuaries, and mangroves are colonized by great quantity of organisms constituting several different communities, such as sea grass beds, kelp forests, and coral reefs. In contrast, only about 10 % of marine species live in the open ocean.

Marine organisms have very different eating ways. The food is not only vital for their survival, but determines their trophic position and their relationships with other organisms in the ecosystem. Food chains show the flow of matter and energy, from primary producers or from traces of organic matter (called detritus chain), then to consumers until decomposer organisms. However, the reality in the ecosystems is that a species can eat different things and can be consumed by various types of organisms, so at the ecosystem level, food chains have a network aspect (food web). These networks are often complex and include further the route of microscopic decomposers and recyclers of organic matter [14].

The primary producers or autotrophs are organisms that synthesize organic matter from inorganic matter. In the marine environment, this role is played by algae and marine plants, as well as archaea and bacteria, phototrophs, and chemotrophs. Other primary producers referred to as mixotrophs can behave as both, autotrophs or heterotrophs, or combine both strategies depending on the environmental conditions, such as dinoflagellates. In addition, other autotroph organisms can establish symbiotic relationships with heterotrophs, for example, the case of the corals. Heterotrophs that feed directly from autotroph organisms are called herbivores or primary consumers. Benthic herbivores, such as sea hedgehogs, eat algae that are attached to the substrates. Others in the pelagic environment, such as copepods, feed on phytoplankton, separating them from the water through filtering structures. Secondary consumers eat primary consumers. In this group, there are carnivores, parasites, omnivores, etc. The large predators like sharks or orcas represent the upper trophic level. Ultimately, in the marine environment, there are also decomposer organisms, mostly microscopic such as bacteria and fungi, and also macroscopic such as polychaete, that feed on detritus or inert organic matter, returning to the medium some of the original molecules, which can be used by primary producers.

Food webs connect the pelagic and benthic systems, for example, the remains of organisms and organic matters, called marine snow, that fall to the seabed from the most productive surface layers provide food for numerous sessile benthic organisms that feed by filtering water and capturing food particles. Besides, while marine snow is deposited, it decomposes and enriches by decomposer microorganisms that can also serve as fresh food for benthic animals. It is known that only around 10 % of organic matter and energy pass from one trophic level to the next, and the rest is degraded for to obtain energy. Some parameters evaluate the transfer of matter and energy within an ecosystem; an example is the biomass that is the mass of all organisms that form a trophic level or ecosystem per unit area or volume. One measure of the relative importance of different marine habitats is the rate at which they produce biomass. Another parameter is the productivity, which allows to observe the rate of biomass renewal [12].

Marine ecosystems are home to a myriad of vegetable species (algae and plants) and animals, both invertebrates and vertebrates, ranging from microscopic phytoplankton and zooplankton, fungi, bacteria, and viruses, including marine bacteriophages, to large marine mammals of various sizes.

The first global Census of Marine Life (www.coml.org) has been completed recently. A consortium of around 2700 scientists from more than 80 nations has established a unique picture of the marine life diversity, distribution, and abundance. This global project has generated the most comprehensive inventory of marine life, from microorganisms to whales, from the icy poles to the warm tropics, and from shores to deep and dark seabed, as the basis for future research. In addition, this has been useful to forecast, measure, and understand changes in the global marine environment, as well as the management and conservation of the marine resources.

The number of marine species scientifically described has been around 250,000, but the census still could not reliably determine the total number of species from the sea. If the results were extrapolated, it would exist at least 1 million total marine species and tens or even hundreds of millions of kinds of marine microorganisms. In the last decade, the census found more than 6000 potentially new species and completed formal descriptions of more than 1200 of them. On the other hand, 170,000 cases of synonymy between previously known species, that is, a species described under two or more different names, have been found. Applying genetic analysis, i.e., DNA barcoding techniques, the number of species was expanded, especially the number of marine microorganism species. Moreover, on a dataset of 35,000 species from widely differing major groupings of marine life, the census identified the proximity and distance of relations among distinct species and observed organisms that had been mistakenly named separate.

Using new technologies and state-of-the-art equipments, the census found life in extreme marine habitats and was also able to register migratory routes of many species, establishing areas where they succeed and where they die. Coastal species showed maximum diversity in the tropical Western Pacific, whereas high diversity of species

frequenting the open ocean peaked across broad mid-latitudinal bands in all oceans. Regarding biomass, it is known that approximately 90 % of marine life is microbial, and the census database still has records for no more than 20 % of the sea. As an interesting data, the census has shown a global decrease of phytoplankton near the surface and the decline of large animals at the top of the food chain, but whether the total weight of life in the sea is changing remains unknown [15, 16].

Even though many types of seaweed are plant-like in appearance, in contrast plants show a very high degree of differentiation, with roots, leaves, stems, and vascular network, with their reproductive organs covered by sterile cells. Moreover, all plants have a digenetic life cycle with an alternation between a haploid gametophyte and a diploid sporophyte. Algae do not have any of these features; although some of them show differentiation of their vegetative cells, they do not form embryos; their reproductive structures consist of cells that are potentially fertile; parenchymatous development is present only in some groups and has both monogenetic and digenetic life cycles.

Historically, the major groups of algae are classified into divisions based on the types of pigments, the presence of reserve products like polysaccharides, structure of chloroplasts and cell wall, number, arrangement, and structure of flagella, reproduction cycles, and other special features. Recently, the sequence of some genes and the 5S, 18S, and 28S ribosomal RNA sequences are being used to confirm that these divisions are non-artificial, even though they were originally defined on the basis of morphology alone.

According to the most recently published classifications, the term algae refer to a highly diversified group of phototroph organisms. Prokaryotic members are grouped into the kingdom Bacteria and phylum Cyanobacteria with one class Cyanophyceae also called blue-green algae, where the members of the proposed division Prochlorophyta, considered artificial after phylogenetic analysis, are also included, while eukaryotic members are included in the kingdoms: Plantae, Chromista, and Protozoa. In general, the term algae refer to both macroalgae and a highly diversified group of microorganisms known as microalgae. The number of algal species has been estimated to be one to ten millions, and most of them are microalgae [17].

The algae are highly variable in dimensions. Among the unicellular algae, most are microscopic; nonetheless, others such as *Acetabularia acetabulum* have several centimeters (8 cm) despite being a unicellular organism. Similarly, the size of multicellular algae can range from microscopic forms (0.2–2 μm in diameter) to the giant kelp, a large brown alga that may grow up to 60–80 m in length.

They show a wide range of levels of organization and morphology, as unicellular structures with or without flagella, or colonies, aggregates of a variable number of cells with a growth by cell division of its components, without division of labor, and each cell capable of surviving on its own. Others, the filamentous algae, are the result of cell divisions in the plane perpendicular to the axis of the filament, so the cell chains are daughter cells connected to each other. Filaments can be simple and have false or true branching, other with a single layer of cells called uniseriate or with multiple layers called multiseriate. The siphonous or coenocytic algae consist of tubular filaments lacking transverse cell walls, created by repeated nuclear division without forming cell walls, and hence, they are unicellular and multinucleate. The parenchymatous and pseudoparenchymatous algae are mostly macroscopic whose overall body is called thallus. The thallus is formed by undifferentiated cells and originates from a meristematic tissue with cell divisions in three dimensions. In the parenchymatous structure, the cells of the primary filament are divided into all directions and any essential filamentous structure is lost. This structure is found, for example, in *Ulva* (Chlorophyta) and many of the brown algae. The pseudoparenchymatous thallus is made up of a filamentous construction with little or no internal cell differentiation. The branched filaments are intertwined and held together by mucilage, especially in red algae.

Methods of reproduction may be vegetative, by the division of a single cell or fragmentation of a colony, asexual by the production of motile spore, or sexual by the union of gametes. Vegetative and asexual modes provide a fast and economical means of increasing the number of individuals, but restrict genetic variability. In contrast, sexual mode involves genetic recombination and allows variation [12, 17, 18].

Algae are found almost anywhere because they can tolerate a broad range of temperatures, salinity, pH, O_2, and CO_2 concentrations. Accordingly, the algae can be planktonic, like most unicellular species, and also benthic, attached to seabed or living within sediments, limited to illuminated areas. Benthic algae can live in supralittoral zone, above the high tide level within the reach of waves and spray; in intertidal zone on shores exposed to tidal cycles or sublittoral zone in the benthic environment from the extreme low-water level to around 200 meters of deep. They can grow attached to stones (epilithic), on mud or sand (epipelic), on other algae or plants (epiphytic), on animals (epizoic), in symbiosis, as parasites, etc. It is possible to find algae in snow and ice (cryophilic) and in hot springs (thermophilic), and some of them can live on or in soil (edaphic).

The microscopic algae, the phytoplankton, are significantly involved in the accumulation of oxygen in the marine environment and also in the atmosphere. Besides, they represent carbon storage, fixing the atmospheric and seawater dissolved CO_2 through photosynthesis, helping to maintain cold the temperature of the planet and the seawater acidity at optimum levels for life. Sometimes, when the population of

these organisms is very large, due to pollution with nutrients, such as nitrogen and phosphate, the blooms can reduce the water transparency causing the death of other photosynthetic organisms. At the same time, these blooms contribute to the proliferation of other heterotrophs that consume elevated amounts of O_2, causing the depletion of this gas in the seawater so necessary for life resulting in the death of many organisms. In addition, some of them can also produce toxins able to kill other superior organisms (toxic blooms) [17].

Commonly, the prokaryote algae are called blue-green algae or cyanobacteria. These correspond exclusively to the Cyanophyta division and are non-motile Gram-negative eubacterias. They are among the first photosynthetic organisms on the planet. According to the endosymbiotic theory, the chloroplasts (photosynthetic organelles with circular DNA) found in plants and eukaryotic algae evolved from cyanobacterial ancestors via endosymbiosis. Currently, this group of algae is the most widely distributed and can tolerate wide ranges of salinity and temperature. The structure of cyanobacteria is diverse, from unicellular to colonial species appearing in planktonic and benthonic ecosystems. Cyanobacteria pigmentation includes chlorophyll-a, blue and red phycobilins (c-phycoerythrin, c-phycocyanin, allophycocyanin, and phycoerythrocyanin), and xanthophylls (myxoxanthin and zeaxanthin) that protect cells from excess sunlight and β-carotene. As reserve products, they present cyanophycin, an arginine and asparagine polymer, and cyanophycean starch. Some of them can also produce potent hepatic and neurotoxins. However, certain species have a high biotechnological potential, as well as being employed as dietary supplements [12, 17].

Within eukaryotes, algae are ordered into three major groups: red, brown, and green individuals. The red algae (Rhodophyta division) are essentially marine algae, of the 4100 species described, very few live in freshwater or terrestrial environments. This group includes different microalgae genera, but mostly consists of seaweeds. Their characteristic color is the result of chlorophyll-a masked by red or blue phycobilins (r- and b-phycoerythrin, r-phycocyanin, and allophycocyanin). Exclusive characteristics of this division are the absent of flagellate stages and the presence of hemiellipsoidal phycobilisomes and phycobilinproteins complexes anchored to thylakoid membranes into the chloroplasts. Moreover, they present α- and β-carotene, and the xanthophylls lutein. The polysaccharide floridean starch is the storage product more representative and abundant, located in the cytoplasm, unlike green algae that are located within the chloroplast. Some species of *Coralline* are directly involved in the formation and development of coral reefs. Others are recollected for different uses; for example, *Chondrus* species are useful as a gelatine substitute in food industry, and from *Gracilaria* and *Gelidium* species, agar is obtained, a gelatine-like substance, an important ingredient as solidifying agent in the preparation of culture media for bacteria and fungi. *Phorphyra* (Nori) is traditionally and widely consumed as a vegetable in Japan.

The brown algae or Phaeophyceae class (Heterokontophyta division) include approximately 1500 species, almost all of them are marine and common in cold waters along the coasts. Depending on the proportion of green pigment (chlorophyll) and brown pigment (fucoxanthin), they show a color palette from dark brown to olive green. As member of this division, the phaeophytes possess chlorophylls a and c, α-carotene, β-carotene, and ε-carotene, fucoxanthin, and vaucheriaxanthin and lack phycobilins. The polysaccharide chrysolaminarin is the principal storage product, located in special vacuoles inside the cytoplasm. They present a wide range of forms and sizes; for instance, the complex and giant kelps (*Laminaria*) are the biggest algae with a length of 80 m. Some seaweeds have a special gas-filled bladders, called pneumatocysts, which keep photosynthetic parts of the algal thallus floating on or near the surface of the water closer to sunlight. Brown algae are an important source of algin, a colloidal gel used as a stabilizer in the baking and ice cream industries, and also a major source of iodine and potash. Certain species are also used as fertilizer, and several are eaten as a vegetable such as *Laminaria* and *Undaria.*

The diatoms or basillariophytes (Heterokontophyta division) are other important and numerous groups of microalgae that contribute significantly to the total oceanic primary production [19]. About 16,000 species have been described; they are eukaryote organisms, most of them unicellular brown pigmented, with a pelagic life. As principal feature, diatoms are enclosed within a silica cell wall called frustule, usually with a bilateral symmetry. The yellowish brown chloroplasts of diatoms are typical of the Heterokontophyta division. They present chlorophylls a and c, β-carotene, and fucoxanthin as the major pigments, and chrysolaminarin as storage products and lipids. Thus, the diatoms serve as food for many animals, both directly and indirectly. Additionally, fossil diatoms, called diatomaceous earth, are used as base in dynamite, filters, insulation, abrasives, paints, and varnishes [17, 18].

Although most green algae (Chlorophyta division) are freshwater ones, there are also terrestrial and marine species. Nearly, 7000 species are described with only 10 % marine species, many of them unicellular. However, some species dominate certain marine environments with a wide salinity range. The photosynthetic pigments of chlorophytes are present at similar proportions as in higher plants, so it is believed that green algae are the ancestral form of land plant. The main pigments are chlorophylls a and b, and lutein and prasinoxanthin like xanthophylls. Chlorophylls are not masked by other pigments, so the chlorophytes show a brilliant green color. They also have carotenoids (α-, β-, and γ-carotene); phycobilins are absent in this division, and the

most important reserve polysaccharide is starch, which resides inside chloroplast as grains. Green algae vary in shape and size, including unicellular individuals as *Chlamydomonas* and macroalgae as *Caulerpa* or *Ulva.* The uses of green algae are diverse, in cosmetic industry, for human consumption (*Ulva*) and even employed for sewage purification (*Chlorella*) [12, 17, 18].

Other important members of marine phytoplankton are the dinoflagellates (Dinophyta division); approximately, half of the species are photosynthetic and significant in the food chain, but even among these, many are also predatory. They are unicellular and usually biflagellate organisms. Other morphologic characteristic is their covering with vesicles, which can be empty or filled with cellulose. Some of them are invertebrate parasites, others are endosymbionts (zooxanthellae) of tropical corals, and some can produce bioluminescence. Dinoflagellates have chlorophylls a, b, and c, β-carotene, and different xanthophylls (peridinin, fucoxanthin, diadinoxantin, dinoxanthin, and gyroxanthin); phycobilins are absent in this division. The main reserve polysaccharide is starch, located in grains in the cytoplasm, similar to green algae, but oil droplets are present in some genera. Some dinoflagellates produce toxins; consequently, under favorable conditions, dense algal blooms appear that can poison fish and other marine animals, even to humans by eating, for example, mussels that have ingested and processed large quantities of these dinoflagellates [17, 18].

Terrestrial Plants and Fatty Acids

Under this subheading, attention is drawn and some details are presented, on how the biosynthetic capability of terrestrial plants is regarding fatty acid formation, as well as showing some differences compared with the plant marine sources. Generally, a large interest exists on omega-3 fatty acids, also those taken from higher plants, mainly due to their importance in human nutrition and health, because of the relatively scarce sources of these types of compounds, and our intake of them, in comparison with the omega-6, which are much more abundant, and present in an excessive degree in our diets.

Terrestrial plants all are derived from the seas about 400–500 million years ago, in particular from green algae. This being one of the most important events in the evolution of life on Earth, affecting the development of landscapes, the appearance of new species colonizing this medium, and the atmospheric concentration of gases [20–22]. Following evolution, higher plants have developed different biochemical capabilities for fatty acid biosynthesis, and one would expect to encounter similarities between plants and marine species in relation to the synthesis and accumulation of fatty acids, but also some differences.

In general, it is known that in higher plants, the synthesis of fatty acids takes place in different cell compartments, participating in plastids and endoplasmic reticulum, involving also the release from different reservoirs and transport of precursors within the cell [23]. Predominantly, omega-3 fatty acids in higher plants display carbon chains up to 18 carbons in length, and in a few cases, the maximum number of double bonds reaches up to 4 (18:4n-3, SDA = stearidonic acid), but more commonly 3 (18:3n-3, ALA = α-linolenic acid) [24, 25]. This is where the biosynthetic capacity reaches, being unable to synthesize longer carbon chain omega-3 with larger number of double bonds, such as, for instance, EPA (eicosapentaenoic acid, 20:5n-3) and DHA (docosahexaenoic acid, 22:6n-3) of large nutritional interest [26]. Nonetheless, there are some examples, no omega-3, in which certain plants can produce longer carbon chain fatty acids, up to 22 or 24 carbons, such as erucic acid (22:1n-9) and nervonic acid (24:1n-9), but with only one double bond present at position 9 [27, 28]. Similarly, regarding the omega-6 fatty acids, plants display a reduced number of them, such as linoleic and γ-linolenic acids (18:2n-6; 18:3n-6); however, longer carbon chain ones, with a higher number of double bonds, such as dihomo-gammalinolenic and arachidonic acids (20:3n-6; 20:4n-6) are rarely encountered in higher plants, unlike other sources of fatty acids, such as fish and algae, which accumulate longer carbon chains and more unsaturated compounds, displaying almost all spectra of fatty acids. Thus, fewer plants possess the ability to accumulate Δ6-desaturated fatty acids, in particular SDA and mainly GLA. Within these fewer plants, the genus *Echium* (Boraginaceae), well represented in the Canary Islands with 23 endemic species, show attractive amounts of SDA and GLA [29–34]. Moreover, other plant families, i.e., Saxifragaceae, Scrophulariaceae, and Primulaceae, contain species accumulating these two 18 carbon fatty acids [35, 36]. However, beyond these fatty acids, higher plants do not possess the enzymatic machinery to undertake further elongation and desaturation steps to produce the more interesting omega-3, such as EPA and DHA.

Regarding the synthesis of highly unsaturated fatty acids, this can be conducted following several ways, either by anaerobic pathways (by polyketide synthase enzymes known as the polyketide pathway), which is only present in some microorganisms, or by aerobic metabolic routes, which are more universal as occurring in plants, fish, fungi, algae, etc., and consisting of successive elongation reactions (elongation of the carbon chain) and desaturation reactions (introduction of double bonds into the carbon chain). These reactions are governed by two types of enzymes, i.e., elongases and desaturases [37–40].

There exist variations in the order in which the desaturation and elongation steps occur. Thus, the "metabolic pathway 6" begins with a 6-desaturation reaction, followed

by chain elongation and desaturation of the carbon chain, leading to the formation of eicosapentaenoic acid (EPA, 20:4n-3), when the initial substrate is ALA, or to arachidonic acid (ARA, 20:4n-6), when the initial substrate is LA (linoleic acid, 18:2n-6). Next, EPA is elongated to docosapentaenoic acid (DPA, 22:5n-3), which again is desaturated at 4-position, to finally produce docosahexaenoic acid (DHA, 22:6n-3) (Fig. 9.1).

Furthermore, there is another variation called Sprecher's route, which describes the production of DHA by means of Δ6 and Δ5 successive desaturation of ALA through the formation of metabolic intermediates of C24 that are eventually shortened to DHA by one β-oxidation step taking place in the peroxisomes [41–43]. What characterizes this route is that the final product, DHA, is synthesized without the intervention of the enzyme 4-desaturase. This latter route has been characterized in mammals and fish [44–46] (Fig. 9.1).

Finally, the "alternative route 8," frequent in protists and algae, begins directly with an elongation step from C18 or C20 intermediates, followed by a desaturation at position 8, and an additional one at position 5, to eventually form dihomo-gammalinolenic acid (DHGLA, 20:3n-6) and ARA, or eicosatetraenoic acid (ETA, 20:4n-3) and EPA (Fig. 9.1) [47, 48].

Despite these apparent limitations of higher plants for producing the valuable omega-3 fatty acids, alternative approaches have been conducted employing them, such as attempts to modify the genetic makeup of higher plants, aiming at the production of these important metabolites. Furthermore, natural resources of fish oils and the omega-3 fatty acids they contain are diminishing, and are not maintained and exploited in a sustainable manner. Therefore, alternative sources of these important compounds are needed. Thus, much efforts have been applied in the genetic engineering of higher plants by introducing multiple genes of this pathway derived from other organisms, such us fungi, microalgae, bacteria, and diatoms, which have resulted in the establishment of different transgenic plants, i.e., soybean, Arabidopsis, Brassica, tobacco, and others, capable of producing DHA and EPA with attractive yields [26, 49–51].

Many obstacles appear to be solved, particularly the control of the biosynthetic intermediates which should be released from different reservoirs and transported to and

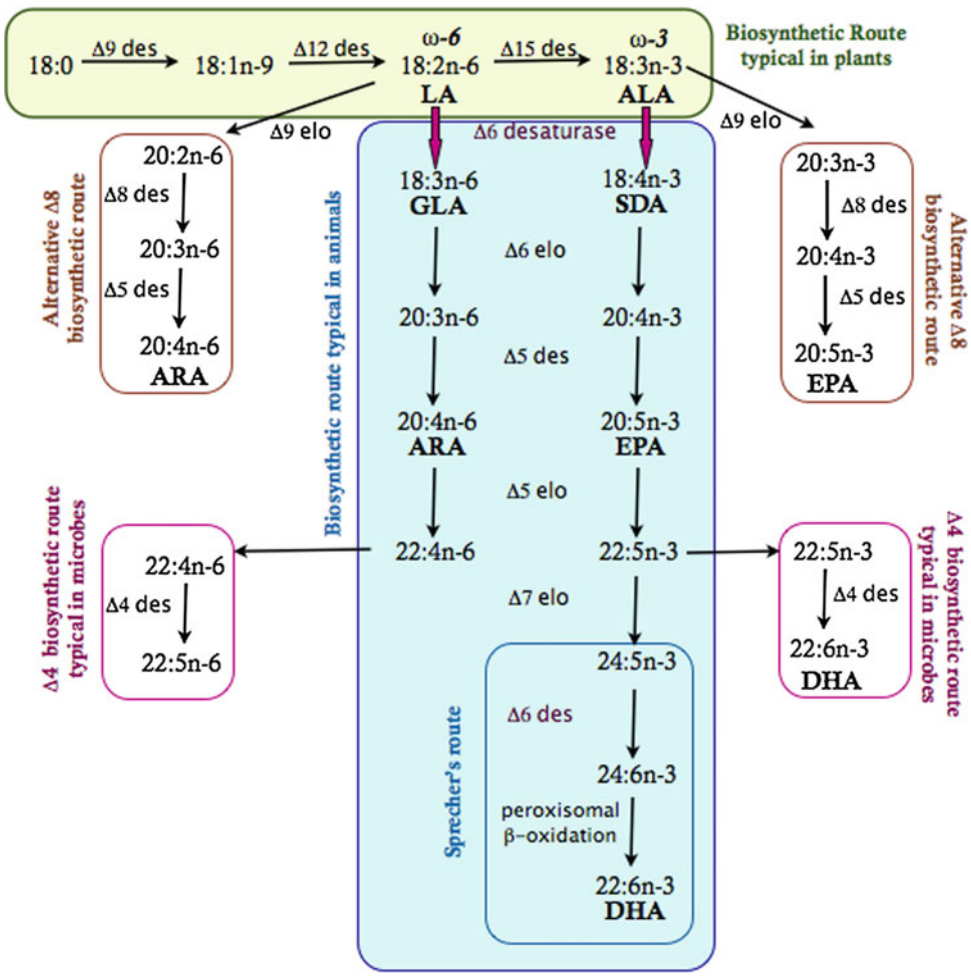

Fig. 9.1 Aerobic metabolic pathway for the formation of long-chain polyunsaturated fatty acids (PUFAs) n-3 and n-6 from short-chain essential fatty acids n-3 and n-6, respectively (*des* = desaturase, *elo* = elongase)

from different organelles; furthermore, control of the primary synthesis and redirecting the flux of substrates and biosynthetic precursors have also been accomplished, particularly being able to halt to reduce the stimulation of the omega-6 branch of the pathway after characterizing and properly utilizing acyl CoA-dependent desaturases. Thus, following the insertion of 5–7 genes in the fatty acid pathway into *Camelina sativa* resulted in the high production of EPA and DHA in seeds, accumulating 12 and 14 % respectively, levels similar to fish [52].

Analogously, other authors have also engineered this species to convert native oleic acid, LA, and ALA into mainly DHA and EPA [53]. Their strategy also maintained at bay the amount of omega-6 fatty acids produced, with only small amounts of GLA (1.2–1.4 %), and no other omega-6 PUFA accumulated. More importantly, the levels of DHA recorded exceeded 12 %, whereas total omega-3, i.e., ETA, EPA, and DPA, reached 25 %.

These results demonstrate the effectiveness of the genetic engineering of oilseed crops, and how the advances made offer a real agronomic alternative for the sustained production of long-chain omega-3 fatty acid in higher plants. This approach has all the potential to exploitation and is an alternative source of production of these important PUFAs, although many issues related to the perception and opposition to the acceptance of GM crops have to be overcome before these genetically engineered species would be implemented as an agronomic crop for fatty acid production. Nonetheless, the scientific advancements have been achieved, showing the successful generation of a terrestrial source of long-chain omega-3 fatty acids employing higher plants, pending to solve regulatory issues before the commercial production of long-chain omega-3 fatty acid through oilseed plants becomes a reality.

Codex Alimentarius and Human Nutrition

Historical documents from the earliest civilizations as Assyrian, Egyptian, Greek, or Romans provide evidence that governing authorities were already interested in the regulation of food sale to protect consumers from fraud or bad produce. However, the first overall food laws were approved in mid-nineteenth century, coinciding with the period in which the food chemistry began to be a recognized scientific discipline.

The *Codex Alimentarius,* the Food and Drug Administration from USA (FDA), and the European Food Safety Authority (EFSA) are the three major authorities regulating food safety worldwide.

Nowadays, the *Codex Alimentarius* is a collection of internationally recognized standards, codes of practice, guidelines, and other recommendations relating to foods, food production, and safety, whose aim is consumer protection [54]. This food regulation body, which is relatively young, was established in 1963 by Food and Agriculture Organization of the United Nations (FAO) and World Health Organization(WHO). It develops standards, based on independent scientific data, which are taken as a world benchmark and often serve as the basis for drafting legislation. Its main goals are to protect the health of consumers and ensure reliable and equitable practices in the international food trade; it also promotes the coordination of all food standard work undertaken by international governmental and non-governmental organizations.

The *Codex* texts, the most important global reference for consumers, food producers, government agencies, food control, and international professional associations, are developed, maintained, and updated by the *Codex Alimentarius* Commission. In addition, this organization helps developing countries to apply Codex standards and strengthen national food control systems taking advantage of international food trade opportunities. The *Codex Alimentarius* Commission includes 186 members: 185 member countries and 1 member organization (European Union), and 225 observers: 52 intergovernmental organizations, 157 nongovernmental organizations, and 16 United Nations organizations, which can attend sessions of the commission and of its subsidiary bodies, as well as the meetings [54].

The Food and Drug Administration of the USA (FDA) was founded in 1906, and it is the oldest authority for regulation, representing a reference worldwide. The FDA is a division of the Department of Health and Human Services. This organization is divided into several centers; one of them is the center for the food safety and applied nutrition. However, the FDA is responsible not only for regulating food for humans but also for animals, besides drugs (human and veterinary), cosmetics, medical (human and animal) devices, biologics, and blood products, in order to ensure the safety and quality of products consumed within the country [55].

The European Food Safety Authority (EFSA) is very recent compared to the two previous authorities and was created in 2002. The main objective was to ensure consumer protection and restore confidence in the European market [56]. EFSA is funded by the EU budget, but it is an independent European agency that operates separately from the European Commission, European Parliament, and EU Member States. EFSA provides a forum for collaboration and exchange of information of 28 European members, Iceland, and Norway and has observers in Switzerland and the European Commission. This is the European authority that provides scientific and technical support on food and feed safety, nutrition, health and welfare animal, and plant health. Additionally, it assesses environment safety and is a support to agroinnovation. At the request of the European

Commission, EFSA experts frequently participate in meetings organized by the Codex Alimentarius Commission.

These agencies require the evaluation of the different parameters before granting authorization to exploit any product derived from these organisms. In brief, this includes risk assessment based on scientific evidence and information; evaluation of the production and processing methods of the final product; food and feed safety; traceability which shall be established at all stages of production, processing, and distribution; and presentation of the product.

Table 9.1 List of microalgae and seaweeds accepted for human use by any of the three regulatory authorities ESFA, FDA, and Codex Alimentarius

Microalga	Seaweed
Chaetoceros sp.	*Ascophyllum nodosum*
Chlorella sp.	*Ahnfeltia plicata*
Crypthecodinium sp.	*Alaria esculenta*
Dunaniella salina	*Caulerpa* sp.
Haematococcus pluvialis	*Chondrus crispus*
Isochrysis sp.	*Cladosiphon okamuranus*
Nannochloropsis sp.	*Durvillaea* sp.
Nitzschia laevis	*Ecklonia* sp.
Odontella aurita	*Enteromorpha* sp.
Pavlova sp.	*Eucheuma* sp.
Phaeodactylum sp.	*Fucus* sp.
Porphyridium sp.	*Gelidium* sp.
Schizochytrium sp.	*Gigartina* sp.
Skelotenma sp.	*Gracilaria* sp.
Spirulina (*Arthospira*) sp	*Gracilaria* sp.
Tetraselmis sp.	*Himanthalia elongata*
Thalassiosira sp.	*Hizikia fusiforme*
Thraustochytrium sp.	*Hypnea musciformis*
Ulkenia sp.	*Kappaphycus alvarezii*
	Laminaria sp.
	Lessonia sp.
	Lithothamnium sp.
	Macrocystis pyrifera
	Mazzaella laminaroides
	Monostroma sp.
	Palmaria palmata
	Phymatolithon calcareum
	Porphyra sp.
	Pterocladia sp.
	Sarcothalia crispata
	Sargassum sp.
	Ulva sp.
	Undaria pinnatifida

According to the current legislations and after all the evaluations and assessments performed, the consumption of several microalgae and seaweeds, as well as many of their products, is approved for human use, as well as other industrial applications different than for human use, which has boosted the commercial exploitation of many of these species and many more to come (Table 9.1) [57].

Fatty Acids from Marine Microalgae

Microalgae in general and marine microalgae in particular are considered important organisms acting as the natural primary producers of long-chain PUFAs in the food web and similarly being major sources of intermediates for the production or accumulation of these fatty acids by other organisms, particularly fish, higher in the food chain [58]. These are also natural producers of other important compounds, such as pigments, antioxidants, sterols, vitamins, polysaccharides, and many other bioactive compounds [59–61].

Furthermore, it is known that there is high increase in the demand for fish oils rich in PUFA, particularly EPA and DHA. These are mainly obtained from extractive fishing practices, or from aquaculture, which also depend directly on extractive fishing to manufacture fish feed, affecting global fish stocks and fish sustainability. According to FAO figures [9], fish stocks are being depleted at a fast pace, and rapid actions are being implemented in order to avoid environmental tragedies; nonetheless, the tendency of stock depletion is maintained despite all these efforts, and new approaches for producing these valuable metabolites have also been devised. Likewise, microalgae offer attractive advantages over fish oils since these possess high quantities and different balances of DHA and EPA depending on the particular species. More importantly, microalgae display simpler fatty acid profiles compared to fish oils, which simplifies their adaptation or nutritional modification and becomes more attractive from a commercial viewpoint. Microalgae also produce and accumulate important antioxidants that provide stability to the PUFA, avoiding their oxidation and bad odors. In general, these also display a low n6/n3 ratio making them more nutritionally attractive. On the other hand, independent quality control analyses of microalga oils have established the absence of heavy metals and other contaminants in these organisms, which emphasizes and demonstrates a major interest and attractive features for exploring and exploiting these resources, avoiding these issues often encountered when consuming fish oils [9, 62]. This PUFA source is also suitable for the vegan and vegetarian consumers who do not manage to intake appropriate omega-3 fatty acids from plant sources.

This scenario has ignited the evaluation, research, and development of new sources of PUFA and proteins to meet

the nutritional needs of the increasing human population. Thus, in order to find and establish other sources of production of PUFA, mainly EPA and DHA, including also the sustainable and effective production of these products, microalgae research has been conducted for several decades, showing their potential commercial exploitation, which is presented under this subheading, making an effort to unite the literature in relation to the production of EPA and DHA by these organisms.

Often the term microalgae is applied to and includes also different prokaryote organisms that are not strictly speaking microalgae (eukaryote). In this chapter, and assuming the lack of rigor but for a more ordered manner for presenting the scientific results, we have decided to consider under the term microalgae, either microalgae themselves, as well as other single cell organisms that are not necessarily microalgae, such as diatoms, dinoflagellates, cyanobacteria, and protists, which are equally able to produce and accumulate these important PUFA and in many instances have been exploited or showing attractive potential for commercial exploitation.

The microalga *Nannochloropsis* (Eustigmatophyte) has long been used as a source of n-3 PUFA to mainly supply larval fish and other markets. The predominant fatty acids in *Nannochloropsis* are palmitic acid (16:0), palmitoleic acid (16:1), and EPA (20:5n3), independently of the culture conditions [63]. It has also been shown how EPA content changes with different culture conditions. EPA as a percentage of dry mass was 3.2 and 3.1 % in low and high nitrogen (N) levels, which increased by 50 and 46 % compared with 2.1 % in middle N level. The total amount of PUFAs (20:4, 20:5, 22:6) showed also an increase of 12, 23, and 41 % in low, middle, and high N levels, respectively. However, the percentage of PUFAs decreased with increasing salt concentrations and temperature [63].

Similarly, the FA profile of *Nannochloropsis oculata* showed that the main fatty acids were palmitic, palmitoleic, eicosatrienoic, and eicosapentaenoic acids, the latest being also the major constituent, with a value of 48.86 % (w/w) [64]. In fact, the genus *Nannochloropsis* is currently the source of marketed oils due to its potential to produce high-EPA lipids with very low DHA and ARA content, which is considered advantageous for the manufacture of dietary supplements [65]. Contrarily, *Nannochloropsis limnetica*, an unusual freshwater species from this genus, produced 28 mg g^{-1} DW of EPA under aerated suspension cultures at the stationary phase of growth. More interestingly, this species was able to produce 55.5 mg g^{-1} DW of EPA when cells were grown in a non-aerated culture supplemented with dipotassium phosphate, together also with linoleic and arachidonic acids (22.2 and 10.5 mg g^{-1} DW) [66].

Another species feasible of being used as a source of omega-3 FA is the diatom *Phaeodactylum tricornutum*. This microalga produces high levels of EPA, appearing as the most abundant, with yields of 23.7 % with respect to the total fatty acids and lower amounts of DHA (2.5 %) [67]. It is known that glycerol is one of the most used substrates in microalga culture because it causes strong effect on fatty acids accumulation, and is added as a carbon source to mixotrophic cultures in order to change fatty acid profile and content in microalga, especially eicosapentaenoic acid (EPA) [68]. Using glycerol as a carbon source in semi-continuous cultures, the optimal dilution rate (0.143 mol L^{-1} glycerol) produced both the highest biomass production (25.4 g L^{-1}) and the highest EPA accumulation with a 3 % increase (50 mg L^{-1} day^{-1}). Similarly, an outdoor batch culture with the strain *P. tricornutum* UTEX-640 was able to produce maximum lipid productivity. EPA content was increased up to 3 % (DW) in mixotrophic growth, giving a productivity of 56 mg L^{-1} day^{-1}, a significant increase compared to the photoautotrophic control, which yielded a maximum EPA content of 1.9 % (DW) and a productivity of 18 mg L^{-1} day^{-1} obtained in semi-continuous mode, suggesting that these culture conditions might be an excellent choice for transferring to an outdoor pilot-scale plant [69]. A linear programming approach has also been described to simulate how the growth and storage of important compounds of *P. tricornutum* could vary, based on mass and energy balances under nutrient-limiting conditions. It was predicted that both carbohydrates and lipids are synthesized simultaneously but at different rates under nutrient limitations [70].

Odontella aurita is a microalga marine diatom used for human nutrition that is known to contain high levels of EPA (26 % of total fatty acids) and several bioactive compounds, such as pigments, fibers, and phytosterols, which have beneficial effects on human health [71, 72]. In one instance, *O. aurita* was cultured under high UV radiation which did not affect the fatty acid composition of the total lipids and lipid fractions of the cells with EPA levels remaining attractively high (27–28 % of total lipids) during the 8 days of treatment. Indicating that open cultures of *O. aurita* in medium or high UV irradiation latitudes could yield high-EPA algal biomass [71]. Based on its EPA profiles, together with its other bioactive components, this species has been evaluated as an attractive dietary supplement for dyslipidemia, platelet function, and oxidative stress in high-fat fed rats. The synergistic effect of these microalgal compounds displayed a beneficial effect in reducing the risk factors for high-fat-induced metabolic syndrome, i.e., hyperlipidemia, platelet aggregation, and oxidative stress, in the treated rats [73].

Following a different approach, *O. aurita* was cultured in cylindrical glass columns and flat-plate photobioreactors, altering different conditions, such as low light and nitrogen, high light and low phosphorus, high light and low silicon, or high light and low sulfur. Under optimal conditions,

O. aurita yielded a maximum biomass production of 6.7–7.8 g L^{-1} under high light. Furthermore, the protein content decreased, while carbohydrate, mainly β-1,3-glucan, increased remarkably to about 50 % of dry weight during the entire culture period. The highest lipid content was 19.7 % of dry weight, and 80 % of fatty acid profiles were C-14, C-16, and C20. ARA and EPA accounted for 1.6–5.6 % and 9–20 % of total fatty acids, respectively [74].

Another promising species is *Trachydiscus minutes*, a yellow-green alga that produces high amounts of EPA. This microalga was cultivated in a standard medium and also in media without sulfur and nitrogen. The best productivity of EPA was in excess of 35 % of total fatty acids; the productivity was thus 88 mg L^{-1} day^{-1}. So, this organism could be considered as an alternative source for EPA production [75]. In the same fashion, *T. minutes* was cultivated under different conditions varying different parameters, such as light intensity, salinity, or nitrogen source [76]. It was shown that for producing a considerable amount of EPA, urea was a very attractive nitrogen source, behaving similar to nitrate, producing in both cases 26 mg EPA L^{-1} day^{-1}. A NaCl concentration of 0.2 % slightly stimulated EPA content, reaching 24 mg L^{-1} day^{-1}, while higher salt concentration above 0.8 % was lethal. Regarding light and temperature conditions, the microalga grew best at high light intensities and a temperature of 28 °C, reaching EPA amounts of ca. 30 mg L^{-1} day^{-1}. So, taken into account all these data, it could be suggested that for an optimal EPA production, outdoor cultivation under conditions of a temperate climatic zone in summer, using urea as a nitrogen source, could be the best combination [76].

It is well known that the dinoflagellate *Crypthecodinium cohnii* is a very attractive producer of DHA. In fact, differences in productivity among four different strains, *C. cohnii* ATCC 30 556, ATCC 50 051, UTEX L 1649, and RJH, were studied. It was shown that all the four analyzed strains produced DHA in high amounts, but with the highest production found in ATCC 30 556 strain with 159.4 mg L^{-1} grown in Porphyridium medium with 5 g L^{-1} glucose at 25 °C during 96 h. These results demonstrated that it is also feasible to grow microalgae in heterotrophic conditions, with a concomitant great potential for the production of PUFAs [77].

It is generally considered that low temperature favors the formation of polyunsaturated FA including DHA, so further experiment with this high-DHA producer strain in which temperature was shifted from 25 to 15 °C, to promote the accumulation of cellular DHA, was studied. High temperature (30 °C) favoured the growth of the microalga; in contrast, low temperature boosted the accumulation of PUFAs. The highest DHA content was obtained at 15 °C in the early stationary phase, recording 6.21 % (of dry weight), and the highest DHA productivity was of 1.47 mg L^{-1} h^{-1} [78].

Recently, a screening of approximately 300 different microorganisms belonging to the genus *Crypthecodinium* was carried out, finding that only 34 were DHA producers. All these 34 strains showed different ranges and amounts of FA, containing, for instance, ARA, EPA docosatetraenoic acid, and DHA as PUFAs. Their productivities varied from 7.87 to 502 mg L^{-1} of total fatty acids production, and particularly, the range of DHA was 8.7–66.7 % of total fatty acids. Surprisingly, the isolated strain D31 identified as a related species of *C. cohnii* possessed a unique fatty acid composition, where DHA was the only PUFA. The amount of DHA produced by this strain grown in GPY liquid medium for 7 days was over 60 % of total fatty acids, amounting 124 mg L^{-1}. DHA accumulated mainly as a polar lipid (79.4 % of total DHA), especially as phosphatidylcholine (71.4 % of polar DHA), although most oleaginous microorganisms accumulate DHA as triacylglycerol [79].

Further modifications of various culture conditions (carbon sources, nitrogen sources, salinity, and initial pH of nutrient medium) were carried out in order to evaluate the effect on DHA production. D-Glucose, D-fructose, acetic acid, ethanol, and glycerol were better sources to promote cell growth, whereas other saccharides, organic acids, and sugar alcohols did not have effect on growth. Besides, ethanol and glycerol stimulated DHA productivity, reaching amounts comparable to that obtained with glucose. DHA production was the highest with glycerol as the carbon source (103 mg L^{-1}). Different sources of nitrogen and salinities in the medium were also studied. The mixture of polypeptone and yeast extract, used as the basic GPY medium, was preferable for growth and DHA production, while the optimal saline concentration was 50 % to that of seawater. The pH was also analyzed, reporting a constant DHA production at acidic pH range (pH 3.0–6.0). Therefore, cultures under the so-called optimal conditions (glycerol as the carbon source, a mixture of yeast extract, and polypeptone as the nitrogen sources, salinity at 50 % to that of seawater, and pH 5.5) yielded 375 mg L^{-1} of DHA [79].

Pavlova lutheri is another species which produces EPA and DHA at attractive levels [80], and it is widely used in aquaculture as live feed for marine invertebrates, for example, feeding oyster larvae *Crassostrea gigas* [81]. When cultured under different irradiance levels and with different carbon sources, it was observed that lipid composition was more sensitive to light intensity variation than carbon source. The highest EPA levels were achieved predominantly in the galactolipid fraction when the cells were cultured at low light, regardless of the carbon source, accumulation that could be related to facilitate thylakoid membrane fluidity. Furthermore, the highest DHA levels were observed under high light conditions. Thus, PUFAs corresponded to approximately 45–55 % of the total fatty acids [82]. Recently, cultivation of *P. lutheri* has been carried out on

large-scale, which demonstrates that this microalga is able to grow similarly in 300 L or 250 mL cultures. This fact makes this organism, together with the optimization of culture conditions, to be considered for its potential to yield EPA and DHA at a profitable large scale [83]. As fatty acids are generally considered to be sensitive to oxidation by UV radiation (UV-R), and in order to determine the possible deleterious effect of UV radiation on fatty acid profiles of *P. lutheri*, this was cultured under high UV radiation. Exposure to UV-R treatment led to a decrease in the proportions of PUFAs, such as EPA and DHA, especially into structural lipids (glycolipids and phospholipids). It caused a reduction of 20 % in EPA levels, from 22.6 % molar of control to 18.2 %, and 16 % in DHA levels, from 13.4 % molar of control to 11.2 %, after 8 days of UV-R treatment [71].

Several species belonging to the genus *Isochrysis* are amenable for the production of DHA. For instance, *I. galbana* produced attractive amounts of DHA with 15.8 mg g^{-1} dry weight. Nonetheless, for most of the microalgae studied, the most abundant fatty acid was palmitate [84]. The effects of the addition of sodium nitrate at different intervals as a nitrogen source have a marked effect in lipid production. Cell density of *I. zhangjiangensis* was improved significantly when sodium nitrate was supplied at an interval of 24 h, as well as the lipid productivity, reaching the maximum value of 140.9 mg L^{-1} day^{-1} [85]. This algal strain can accumulate lipids under nitrogen-repletion conditions and accumulate carbohydrates under nitrogen-depletion conditions. When cultured in a high nitrate concentration, the growth of algal cells was suppressed, but the highest lipid content of 53 % was attained. Furthermore, a two-stage cultivation model was also assessed; in the first stage (0–96 h), sodium nitrate was added into the medium at an interval of 24 h. In the second stage (96–240 h), three different nitrogen treatments, i.e., nitrate-repletion condition (75 mg L^{-1}, 24 h interval addition), nitrate-depletion condition (no added nitrate), and the intermediate condition (75 mg L^{-1}, 72 h interval addition) are carried out. After these treatments, DHA remained as the major PUFA with 13 % of total fatty acids amount and EPA with 1.5 % [85].

Recently, the optimization of different *I. galbana* strains for DHA production has been studied. Although all the strains possessed almost the same FA composition, DHA content varied from 6.8 to 17.0 % of total fatty acids, being *I. galbana* #153180 the strain showing the greatest DHA content of 16 % of total fatty acids, achieving the highest DHA productivity (6.13 mg L^{-1} day^{-1}). Different parameters were varied (light, nitrogen, phosphorus, and salinity) assessing growth and DHA yields. After optimization, this strain registered the highest DHA figures, up to 17.5 % of total fatty acids (13.6 mg L^{-1} day^{-1}) or 1.7 % of cell dry weight (0.72 g L^{-1} day^{-1}) [86].

It has been recognized that *I. galbana* is a rich source of PUFAs and has been used to enhance nutritional value of different foods, such as pasta, to produce high-value nutritional PUFA sources for human consumption. The enrichment of raw fresh pastas with *I. galbana* biomass led also to a significant increase of EPA (20:5n3) and DHA (22:6n3) that were absent in raw control pastas [87].

Another species that has received large attention is *Schizochytrium* sp., mainly for the production of DHA. In particular, *S. limacinum*, a thraustochytrid closely related to heterokont algae, first isolated from a mangrove area in the west Pacific Ocean [88], displays attractive DHA levels, more abundant than EPA. In one report, it was demonstrated that the culture temperature and percentage of glucose in the nutrient medium influence DHA and biomass productivity. Growth was better at 25 °C than at 20 or 30 °C. DHA yields were enhanced (874 mg L^{-1}), by increasing glucose from 1 to 5 %; nonetheless, when 6 % glucose was used, the biomass decreased clearly, resulting in an overall decline of DHA [89]. Employing *Schizochytrium* sp., other authors showed that DHA production ranged from a maximum of 204 mg g^{-1} with a biomass of 13.2 g L^{-1} to a minimum of 158 mg g^{-1} with a biomass of 10.8 g L^{-1}. The maximum yield was attained with an optimal salinity of 25–30 ‰ [90]. Oxygen supply also affects the production of DHA in *Schizochytrium* sp. Optimizing the level of oxygen together with glucose resulted in a high cell density (71 g L^{-1}), high lipid content (35.75 g L^{-1}), and high-DHA percentage (48.95 %) that were achieved using a stepwise aeration controlled strategy. Thus, DHA productivity reached 119 mg L^{-1} h, 11.21 % larger than the best results obtained by constant aeration rate [91]. This approach was further optimized following a two-stage oxygen supply control strategy based on oxygen transfer coefficient employing a 50-L fermenter with a working volume of 35-L. Maximum concentrations of lipid and DHA reached 46.6 and 17.7 g L^{-1} with a productivity of 111 mg L^{-1} h^{-1}. Both results were higher than those attained at constant oxygen transfer coefficient processes. This strategy resulted in a considerable improvement in lipid and DHA concentrations, but more importantly, in a clear increase in DHA productivity [92]. This species has been approved for human use and is being largely exploited by different manufacturing companies for the production of mainly DHA, which is used for the preparation of infant food, other human nutraceutical products, and also as feeding additive in egg-laying hens enriched in this FA.

Different species of the genus *Thraustochytrium*, taxonomically related to *Schyzochytrium*, both from the same order Labyrinthulales, possess interesting FA profiles, which have been largely studied, and some are also commercially exploited. A *Thraustochytrium* strain, named KK17-3, showed high-DHA content (52.1 % of total fatty acids) and

wide range of PUFA (76.1 %), comprising arachidonic, EPA, and docosapentaenoic acids, together with DHA. Glucose and tryptone were the optimal carbon and nitrogen sources, in a medium with salinity at 75 % that of seawater. The PUFA contents in polar lipids (22.1 % of total lipid), in which the DHA content was 39.3 %, were higher than those in neutral lipids and glycolipids [93]. Analogously, a native Labyrinthulomycetes strain, *Thraustochytriidae* sp. TN5 (Thraustochytrium) from Japan, was scaled from shaken flask to a laboratory fermenter. Different growth media, particularly the influence of two discrete—carbon and nitrogen sources—and six continuous—concentrations of the carbon and nitrogen sources, yeast extract and artificial seawater, incubation temperature, and time factors, were assessed for DHA and lipid production. In the flask experiments, the best lipidic content was 25.2 % w/w of the biomass, with a DHA concentration of 0.48 g L^{-1} and a biomass production of 5.1 g L^{-1}. Fed-batch bioreactor experiments increased biomass concentration to 14 g L^{-1}, with the lipidic fraction between 16.2 and 34.8 % w/w, with lipids and DHA productivities of 50 and 23 mg (L^{-1} h^{-1}), respectively [94]. *Thraustochytrium* sp. ATCC 20892 strain was also investigated. Glucose and sodium glutamate were the preferred carbon and nitrogen sources, respectively, and the optimum condition for growth and DHA production was at pH 7.0 at 25 °C with 40 g glucose L^{-1} for 4 days. Under these conditions, the maximum DHA yields were 67.6 mg L^{-1} or 35 % of total fatty acids, relatively low amounts of 16:0 (29 %) and 18:1 (13 %) and insignificant amounts of other PUFAs [95].

Currently, several companies already exploit different species of microalgae for large-scale production of these omega-3 FA, especially for the manufacture of functional foods for human consumption. Many of them have already obtained approval by the various authorities for marketing them to the broad market in human medicine. For example, a company based in New Zealand, Protonz, is one of the largest producers of EPA by scaling and optimization of the fermentation of the microalga *Nitzschia laevis*. It has also established state-of-the-art infrastructure and technology for an industrial scale reactor of 7.5 tons, to meet mainly the demand of the cardiovascular healthcare sector market with figures of around 60 billion dollar and for which there is a high demand for EPA. Analogously, the company Martek native to the USA, and recently acquired by the Dutch multinational DSM, has also developed a line of business and has obtained approval to market the production of DHA and ARA, by fermentation of the microalgae *Thraustochytrium* and *Schizochytrium* mainly for the preparation of infant milk and for the preparation of omega-3 capsules and supply preparations for making multiple enriched end products with these FA for human consumption, with annual business figures of over a billion dollar. Nutrinova–Celanese, originally a Germany-based company, also exploits microalgae for DHA and EPA production, and Solarvest from Canada also produces and commercializes DHA.

In Table 9.2, a short summary of the most frequent microalgae species cultured for the production of the nutritionally important omega-3 FA, i.e., DHA and EPA, is

Table 9.2 Summary of the most frequent microalgae species cultured for the production of the nutritionally important omega-3 fatty acids, i.e, DHA and EPA

Microalga	Fatty acid						
	EPA	DHA	ARA	GLA	SDA	LA	ALA
Crypthecodinium	−	++	−	−	−	−	−
Isochrysis	+−	++	−	−	−	+	+
Nannochloropsis	++	−	+	−	−	+	−
Nitzschia	++	+	−	−	−	+−	+−
Odontella aurita	++	+−	−	−	−	−	−
Oocystis	+-	−	−	−	−	++	++
Pavlova	+	+	−	−	++	+	+
Phaeodactylum	++	+−	+	−	+	+	−
Porphyridium	+	−	+	−	−	+	−
Spirulina (*Arthospira*)	+	−	−	++	−	+	+
Schizochytrium sp.	+	++	−	−	−	−	+−
Tetraselmis suecica	+	−	−	−	−	+	++
Thraustochytrium	+	++	+	−	−	−	−
Trachydiscus minutus	++	−	−	+	−	+	+
Ulkenia	−	++	−	−	−	−	−

The appearance of other fatty acids for the same listed species is also displayed

presented. The appearance of other fatty acids for the same listed species is also displayed [65, 84, 96–106].

Fatty Acids from Seaweeds

Macroalgae are distributed worldwide; it is estimated that there exist about 9000 species; nonetheless, approximately about 200–220 are economically important and exploited mainly for the presence of other compounds, i.e., alginates, agar, polysaccharides, and pigments. These are also a source of PUFA, but, in spite of their accumulation, they are not highly exploited. Their PUFA contents also change according to seasonal period and geographical location. In a very recent report, lipid and fatty acid compositions were analyzed in 100 marine macroalgae. Almost all the lipid contents in macroalgae were low (2.3–20 mg g^{-1} fr. wt.), but regarding PUFAs, they showed high amounts of nutritionally important compounds such as LA, ALA, STA, ARA, EPA, and DHA. Up to 90 % of the species showed nutritionally beneficial n6/n3 ratios [107]. For instance, many species from different groups from various locations of Diu and Saurashtra, coast of Gujarat, India, have been studied [108]. The study of 9 members of Chlorophyta showed that this group was an attractive producer of omega-3 fatty acids. Some members of the genus *Ulva* produced higher amounts of DHA than EPA, although *Caulerpa racemosa and Caulerpa veravalnensis* were better EPA producer, with almost negligible amounts of DHA. Generally, they produced less amounts of ARA, with the exception of *C. veravalnensis.* An important fact of this group was that the ratio n6/n3 was between 1.5 and 2 % for almost all species, with *Ulva linza* showing the best value of 1.42. Regarding Phaeophyta, all the analyzed species produced EPA as the major omega-3 fatty acid, but with very low amounts of DHA. On the contrary, they produced high amounts of ARA, 32 % of total FAME (fatty acid methyl esters) in *Cystoseira indica.*

It is clear that here the ratio n6/n3 was higher than Chlorophyta, reaching 5.15 in *Sargassum tenerrimum*. It was also shown in this study how different species of Rhodophyta contained EPA as the major omega-3 fatty acids, with almost no detectable amounts of DHA. However, ARA was produced in higher amounts recording 46 and 58 % of total FAME in *Gracilaria debilis* and *Gracilaria dura*, respectively. Again the ratio n6/n3 was higher than in Chlorophyta, especially in these two species, reaching 18 and 27. Surprisingly, in *Grateloupia indica* and *G. wattii,* EPA was produced in higher amounts compared with ARA, and therefore, the n6/n3 ratio achieved by these two species was one of the best in this study with values of 0.61 and 0.74, respectively [108].

In another study, many different species from Chlorophyta, Phaeophyta, and Rhodophyta from the Algarve coast (Portugal) were analyzed in order to determine their fatty acid composition [109]. In the Chlorophyta group, all members showed EPA (20:5n-3) being detected at medium concentrations, ranging from 1 to 4 % of the total fatty acid content, and DHA was only detected in *Cladophora albida* (0.8 %). The only exception of a good omega-3 producer was *Ulva* sp., in which ALA was detected at high percentages (16 %), although the important EPA and DHA were very minor. In this group, the n6/n3 ratio varied notably, with the worst ratio of 31 with *Chaetomorpha* sp., and the best ratio achieved by *Ulva* sp. with 0.31. Similarly, species belonging to Phaeophyta were also analyzed. Accordingly, in all Phaeophyta, EPA was produced at relatively high amounts (6–14 %), except for *Dictyota spiralis*, in which EPA was not detected. DHA was only detected in *Halopteris scoparia, Taonia atomaria, and Sargassum vulgare* at low concentrations (0.8–1.5 % of total fatty acids). In this group, ARA amounts (11–18 %) were slightly higher than EPA. Again, the n6/n3 ratio was between 2 and 4 in all cases. Rhodophyta are considered as very interesting EPA producer as shown by Graeve et al. [110]. Accordingly, in this study, all analyzed species from Algarve coast produced high amounts of EPA (4 out of the 5 species showed higher than 15 % of total FAME) with *Peyssonnelia* sp. being the only representative of this phylum in which DHA (22:6n-3) was also detected. These are also attractive producers of ARA with amounts ranging from 1.79 to 26.6 of total FAME. This group also showed the best n6/n3 ratio, almost always under 1, with *Bornetia secundiflora* being the best one (0.29) [109].

In another report, species from Ireland, The Netherlands, France, and Norway were analyzed [111]. The best EPA producers were *Laminaria hyperborean* (Phaeophyta) and *Palmaria palmata* (Rhodophyta) with amounts of 26 and 59 % of total fatty acids. On the contrary, *Sargassum natans* (Phaeophyta) was the only DHA producer with a 13 % of total fatty acid content. Regarding omega-6 series, the best ARA producer was *Undaria pinnatifida* (Phaeophyta) with 16 % of total fatty acids. Here, the n6/n3 ratio, in almost all the cases, was less than 1, which is a ratio recommended by the World Health Organization, which should be less than 10 in order to prevent inflammatory, cardiovascular, and nervous system disorders [111, 112].

Nevertheless, there are also other species of algae that were poor producers of EPA, DHA, or ARA. This was the case of *Laurencia filiformis and L. intricata* collected in Espírito Santo State, Brazil [113]. In these two species, DHA was not detected, while EPA reached amounts of 0.8 mg g^{-1} dry weight in *L. filiformis* and 1.7 mg g^{-1} dry weight in *L. intricate.* Amounts of ARA were slightly lower, with 0.4 and 1.5 mg g^{-1} dry weight in *L. filiformis* and *L. intricate*, respectively. On the other hand, these species were good producers of palmitic acid, reaching amounts of 4.1

and 3.1 mg g^{-1} dry weight in *L. filiformis* and *L. intricata,* respectively.

It is known that the fatty acid content analyzed in different seasons changes among species [114, 115] and also that temperature affects clearly the fatty acid profile [116–118]. Heavy metals [119] and light [120, 121] are also external factors able to induce changes in growth and fatty acid content.

Recently, the total fatty acid content and profiles of 16 common Irish macroalgae, as a potential source of PUFAs, collected at two different seasonal sampling times, have been studied [122]. The data revealed that in the group of Phaeophyta, the most commonly distributed PUFA was ARA, with values ranging from 4.7 % of total fatty acid content in *Saccharina latissima*, to 17.6 % in *Himanthalia elongata*. EPA was also produced by all the Phaeophyta species obtaining the maximum amounts of 13 % in *Laminaria digitata.* Regarding the other valuable omega-3 fatty acid, DHA was detected in lower amounts or even not detected according to the species. The most dramatic change in any of the FA analyzed was observed in *S. latissima* with EPA fluctuating from 1.8 % in November to 10.8 % in June. In all these species, the n6/n3 ratio was nearly 1.0 or even less. Regarding Rhodophyta, the two main PUFAs were ARA with 30.8 % of total fatty acids in *Gracilaria gracilis*, and EPA with 41.2 % in *P. palmata* in which DHA was almost not detected. The highest EPA shift was observed in *G. gracilis* with no production in June, to reach an amount of 30.8 % in November. In most of all the species examined, the ratio of n6/n3 was about 1 with the lowest ratio of 0.04 observed for *P. palmata* due to its high amounts of EPA. The last group studied was Chlorophyta, in which only two species were analyzed. The most common PUFA was the essential omega-3 ALA with levels up to 11.3 % in *Ulva lactuca* and 19.9 % in *Codium fragile.* Percentages of EPA were low in both species. In *U. lactuca*, small amounts of DHA were observed as well. The ratio of n6/n3 fatty acids was low in both species, with values ranging from 0.2 to 0.6 [122].

Recently, Masatoshi et al. [123] studied the seasonal variations of total lipids and fatty acids composition of two macroalgae, *Sargassum horneri* (Turner) and *Cystoseira hakodatensis* (Yendo), from the northern seashore of Japan. It was observed that the maximum total lipid (TL) contents of *S. horneri* originated from Nesaki and Matsushima areas were 101.9 mg g^{-1} DW (January 2009) and 142.5 mg g^{-1} DW (January 2009), respectively, being palmitic (16:0), oleic (18:1n-9), SDA (18:4n-3), ARA (20:4n-6), and EPA (20:5n-3) the major fatty acids. Regarding *C. hakodatensis*, this macroalga showed the highest total lipids in May 2009 (122.9 mg g^{-1} DW) and in January 2010 (155.9 mg g^{-1} DW). On the other hand, the relative percentages of total n-3 PUFAs were generally larger in winter time, with the highest level of n-3 PUFAs corresponding to those on the total lipid fraction of *C. hakodatensis.*

Another study assessing the seasonal changes versus fatty acid contents was carried out with *U. pinnatifida,* from the Marlborough Sounds, New Zealand [124]. Significant differences in PUFA contents among winter, spring, and summer were observed. PUFA amounts significantly increased during the winter and decreased in spring. *Undaria* also produced more n-3 PUFAs in winter compared with n-6 PUFAs that were found to be the main PUFAs in summer, in which the temperature of water goes from 6 to 8 °C in July to 15–16 °C in December. Among the n-3 PUFAs, the major ones were ALA (18:3n-3), SDA (18:4n-3), and EPA (20:5n-3). On the other hand, the most abundant n-6 PUFAs were LA (18:2n-6), GLA (18:3n-6), DHGLA (20:3n-6), and ARA (20:4n-6) in December.

Similarly, the fatty acids profile of the Rhodophyta *Grateloupia turuturu* collected in Brittany, France, was investigated over four seasons [125]. The two important fatty acids ARA and EPA were produced at higher amount in summer, reaching ARA 11 % and EPA 16 % of total fatty acids. This season apparently was the best to produce large amounts of these two highly valuable PUFAs. Moreover, the ratio n6/n3 was lower than 1.0 in all seasons, which is considered a good nutritional ratio indicating higher amounts of omega-3 than omega-6 fatty acids.

In *Corallina pilulifera,* the maintenance and culture conditions were optimized. It was reported that the optimal temperature for incubation was 16 °C, the optimal light intensity was 40 μE m^{-2} s^{-1} with white fluorescent light, and the optimal light period was a 16 h light:8 h dark cycle. Under these conditions, it was found that from the fatty acids, 45.4 % were PUFAs, with EPA (20:5n-3) comprising 31.4 %, and also recording a very attractive n6/n3 ratio of 0.45 [126].

The influence of heavy metals and the changes produced in the lipid and fatty acid composition of algae has been researched [127]. The modes of action of heavy metals in these processes are not clear, but it is thought that they produce an oxidative stress and also an oxidative damage increasing the concentration of reactive oxygen species (ROS) in the cells [128]. In another recent research with cultures of *Gracilaria tenuistipitata*, the effect of heavy metals in the biosynthesis of fatty acids was evaluated, mimicking two different polluted environmental situations, adding low amounts of Cd^{2+} and Cu^{2+} to the cultures [129]. In a second scenario, and in order to establish how the light intensity variation affects fatty acid profiles, cultures were grown under 100 or 1000 μmol photons m^{-2} s^{-1} [129]. It was shown that the levels of the most important PUFAs, ARA, EPA, and DHA diminished, more significantly after treatment with Cd^{2+}, while high light intensity did not affect the amount of PUFAs in this species.

In *G. dura,* the effects of cadmium (Cd) in fatty acid profile, as well as the protective role of selenium (Se) and

Table 9.3 Summary of some seaweed species analyzed as producers of the nutritionally important omega-3 fatty acids, i.e., DHA and EPA

Seaweed	Fatty acid						
	EPA	DHA	ARA	GLA	SDA	LA	ALA
Ahnfeltiaplicata	++	+−	++	+−	−	+−	+−
Caulerpa racemosa	++	+−	+−	+−	−	++	+−
Caulerpa veravalnensis	+−	+−	++	+−	−	++	+−
Cystoseira indica	+−	+−	++	+−	−	+−	+−
Dictyota spiralis	−	−	++	−	−	+−	−
Georgiella confluens	++	+−	−	−	+−	+−	+−
Grateloupia indica	++	−	++	+−	−	+−	+−
Halopteris scoparia	++	+−	++	−	−	++	−
Myriogrammesmithii	++	−	−	−	−	+−	−
Peyssonnelia sp.	++	+−	++	−	−	+−	−
Sarconema filiforme	+−	−	++	+−	−	+−	+−
Spatoglossum asperum	++	+−	++	+−	−	+−	+−
Stoechospermum marginatum	+−	+−	++	+−	−	+−	+−
Ulva lactuca	+−	+−	+−	+−	−	++	+−
Ulva linza	+−	+−	+−	+−	−	++	+-
Ulva rigida	+−	++	+−	+−	−	++	+−

The appearance of other fatty acids for the same listed species is also displayed

polyamines, such as putrescine (Put) and spermine (Spm), were studied [130]. Treatments with Cd negatively affected the amounts of n-6 PUFAs, with ARA dropping from 51 % in control cultures to 21 % of total fatty acids in Cd-treated cultures. Surprisingly, the addition of Se or Spm had a positive re-establishing effect recovering the amounts of ARA even at the same initial levels. This fact shows the preventive role of both Se and Spm in controlling the Cd negative effect in *G. dura*.

Recently, a very curious experiment has been carried out in which the red algae *Gracilaria vermiculophylla*, a good producer of PUFAs, were transferred from Peter the Great Bay (Sea of Japan) and cultivated in the lagoon of Katba Island (Tonkin Bay in the South China Sea) [131]. It was shown that the amounts of ARA, with a 45 %, and EPA, with 4 % of total lipid in the original culture from Japan, that also showed a n6/n3 ratio of 10, were able to change the PUFA profile once cultivated in other conditions (South China Sea) and produced less amount of ARA (5.9 %), and 2.6 % of EPA adjusting the n6/n3 ratio to 2.3. This strategy appears as a valuable tool to change the fatty acid profiles and n6/n3 ratios far from 1.0.

In Table 9.3, a short summary of some macroalgae species analyzed as producers of the nutritionally important omega-3 fatty acids, i.e., DHA and EPA, is presented. The appearance of other fatty acids for the same species listed is also displayed [108–110].

Biotechnology of Fatty Acids from Marine Algae

The fast growing interests in the use of transgenic microalgae for industrial applications is powered by the rapid developments in microalgal biotechnology, particularly the advances made for biofuel research employing these organisms which can be extrapolated to many of these species. Nowadays, there are many different available genomes that have been successfully sequenced, for instance from the red alga *Cyanidioschyzon merolae* [132], the diatoms *Thalassiosira pseudonana* [133] and *P. tricornutum* [134], and the unicellular green alga *Ostreococcus tauri* [135]. Available tools for genetic modification and in silico predictive capacity have allowed the characterization of algal genomes and manipulation of different desirable pathways in an effort to develop and establish algal biofuel, and desirable fatty acids and PUFA production strains [136].

Microalgae are the natural initial EPA and DHA producers in the marine food chain and can grow rapidly under a variety of autotrophic, mixotrophic, and heterotrophic culture conditions with high PUFA production potential. Although large-scale cultivation facilities for microalgae exist, often their productivity is not fully optimized because of the lack of ideal strains that can be selectively manipulated for high biomass productivity, high TAG (triacylglycerol), or PUFA content [137]. Genetic engineering has

been used to altered fatty acid profiles in plants [26, 52, 138–140], so it could be easily considered as a promising tool to engineer fatty acids biosynthesis as a powerful strategy to create alternative algal strains with modified lipid yields and improved oil quality [141].

Genetically modified microalgae are emerging as alternative sources of TAG and PUFA [142] and represent progress toward the use of fermentation approaches to commercially exploit microalgae at large scale, thereby reducing the limitations often associated with photobioreactors, such as light dependency and lower growth [143]. However, the lack of efficient genetic modification systems so far has delayed the genetic engineering of microalgae, and not many prosperous attempts of genetically modified, for instance diatoms, have been reported. Genes encoding key enzymes participating in the fatty acid biosynthesis have been identified in *T. pseudonana* [144–146], *P. tricornutum* [147, 148], and in particular in the model organism *Chlamydomonas reinhardtii* [149]. At present, the mechanisms involved in the fatty acid biosynthetic pathways in microalgae have not been extensively studied, and most information has been gathered from studies on plant metabolism [150], and in not all the cases, the biosynthetic route and all the enzymes involved have been fully established yet. Nonetheless, clear advances have been attained which has permitted to draw the likely biosynthetic pathway taking place in these organisms [137, 151–154] (Fig. 9.2).

Numerous studies have been carried out applying the metabolic engineering strategy to boost the lipid accumulation in different species. These can be classified into five different approaches: (1) overexpressing enzymes of the fatty acid biosynthesis pathway; (2) overexpressing enzymes that enhance the TAG biosynthesis pathway; (3) regulation of related TAG biosynthesis bypass approaches; (4) partially blocking competing pathways; and (5) the multigene transgenic approach [155].

Some attractive examples of how PUFA biosynthesis could be improved through the regulation of genes involved in lipid metabolism have been published. For instance, one isoform of the enzyme diacylglycerol acyltransferase type 2 from the marine diatom *P. tricornutum,* which participates in TAG assembly, was overexpressed obtaining a higher productivity of oil bodies, as well as an increase of 35 % on neutral lipid content. Monounsaturated fatty acids showed an increase of 35.6 %, and PUFAs showed an increase of 34.8 %. It should be highlighted the increase of C20:5 (eicosapentaenoic acid, EPA) at 76.2 %, with the proportion increased from 4.21 to 7.42 %. Moreover, the growth rate of transgenic microalgae remained similar, thereby maintaining a high biomass. All these results clearly suggest that increased DGAT2 expression could alter fatty acid profile in the diatom, and these results represent a valuable strategy for PUFA production by genetic manipulation [156].

Another example of boosting EPA, which has been clearly demonstrated to play important roles in a number of aspects of human health and traditionally obtained from marine fish oils [151], was reported in *N. oculata* [157] which was subjected to *N*-methyl-*N*-nitrosourea-induced mutagenesis under the selection pressure of quizalofop, a well-known inhibitor of acetyl-CoA carboxylase (ACCase) activity, with the clear objective of generating genetically tractable mutants with modified fatty acid metabolism.

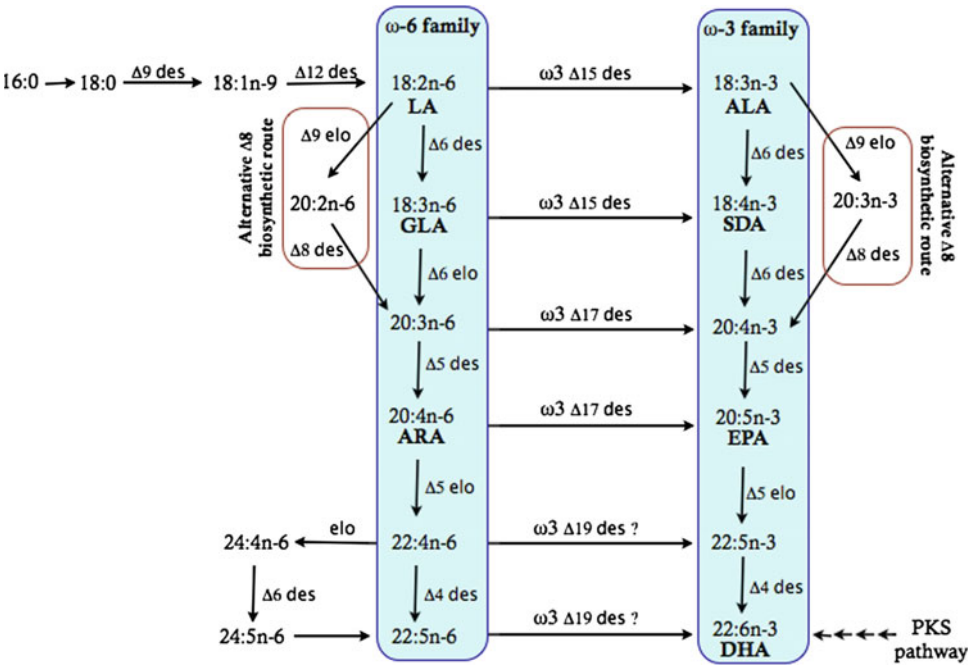

Fig. 9.2 Tentative biosynthetic pathway for the formation of long-chain polyunsaturated fatty acids (PUFAs) n-3 and n-6 in microalgae and microorganisms (*des* = desaturase, *elo* = elongase, *PKS* = polyketide synthase)

Two mutants, QUIZ1 and QUIZ2, showing resistance to quizalofop were isolated and partially characterized. The growth properties and morphology of the mutants appeared identical compared with the wild-type strain. Gas chromatographic analysis of fatty acids revealed how eicosapentaenoic acid (20:5n-3) (EPA) accumulated in higher amounts. The content of mutant strains QUIZ1 (28.29 mg g^{-1} dry matter) and QUIZ2 (25.2 mg g^{-1} dry matter) was higher than that of the parental strain (23.90 mg g^{-1} dry matter) under standard conditions (25 °C, a salinity of 31 %, pH 8.0, and a light intensity of 90 µmol photon m^{-2} s^{-1}, 12 h photoperiod). Mutant strains were rich in PUFAs (n-3 PUFAs), as well as total fatty acid contents; this was accompanied by a parallel increase in triacylglycerol (TAG), followed by linoleic acid (18:2) and arachidonic acid (20:4n-6). Random mutagenesis was shown to be a good tool to manipulate PUFAs and EPA in *Nannochloropsis*. The development of genetic manipulation as well as biochemical tools of this strain could be useful in understanding fatty acid metabolism and also for their direct use in mariculture.

An alternative approach consists of the elevation, and redirection of the endogenous accumulation of omega-3 long chain-PUFAs, and their incorporation into neutral lipids, such as triacylglycerol (TAG). Biolistic transformation has been used to engineer the diatom *P. tricornutum*, introducing and expressing, both combined or individually, the foreign Δ6-desaturase and Δ5-elongase from the picoalga *Ostreococcus tauri*. Some manipulated strains were able to accumulate the high-value omega-3 docosahexaenoic acid (DHA). Transgenic strains expressing the Δ6-desaturase showed no significant alteration of EPA and/or DHA, while the expression of the Δ5-elongase dramatically increased the amounts of DHA 8-fold compared with controls, representing a marked and valuable change in the fatty acid profile of this microalga. Importantly, DHA was shown to accumulate in triacylglycerols, with several novel triacylglycerol species being detected in the transgenic strains. In addition, the coexpression of both transgenes was carried out for the first time in a transgenic diatom, demonstrating further increases in DHA levels through simultaneous expression. Together, this demonstrates the high potential of using iterative metabolic engineering to optimize and overproduce omega-3 content in algae [153].

There are many other instances of how to manipulate microalgae to overproduce other desirable fatty acids apart from PUFAs. Overexpression of the endogenous PtTE (thioesterase) in the diatom *P. tricornutum,* using microparticle bombardment, did not lead to significant changes on fatty acid composition; nonetheless, the total fatty acid content increased by 72 % (from 78 to 133 mg g^{-1} dry cell weight) compared to control, especially both C16:0 and C16:1. This novel thioesterase gene shows its potential in metabolic engineering for enhancing lipid yield of microalgae [158]. In another attractive approach, the overexpression of the endogenous *C. reinhardtii* thioesterase (CrTE) increased the levels of short-chain fatty acids, although engineering the *Umbellularia californica* thioesterase (plant UcTE) into the *C. reinhardtii* chloroplast did not alter the fatty acid profile. These findings show how important and critical the interaction between proteins is in manipulating fatty acid biosynthesis in algae [159].

These methods are paving the way for a more thorough characterization of the algal lipid biosynthetic enzymes, which appear to be distinct from those found in terrestrial plants. In a similar fashion, transgenically overexpressing two different shorter chain length fatty acid acyl-ACP thioesterases, one from *Cinnamomum camphora* (myristic acid biased thioesterase) and another from *U. californica* (lauric acid biased thioesterase) in the eukaryotic microalga *P. tricornutum*, resulted in higher levels of lauric and myristic acids, which are more desirable as a fungible biofuel feedstock. This study shows the feasibility of using such an approach to modify algal oil composition, and how valid this strategy is for improving the fuel producing properties of algae [160].

Synechocystis sp. PCC6803 mutant strains where generated to study the effect of fatty acids activation, displaying either overexpression or deletion of the slr1609 gene, which encodes an acyl-ACP synthetase, which converts and activates free fatty acid (FFA) molecules into fatty acyl-ACP [161]. Similar growth rate was displayed by the wild-type and slr1609 deletion mutant strains, but the content of FFA showed substantial differences between both strains. In the slr1609 deletion mutant, the concentration of total FFA was 6.7 µg mL^{-1}/OD, while that of the wild type was 3.5 µg mL^{-1}/OD. FFA accumulation increased close to 2-fold after knocking out *slr1609*. This indicates that the dysfunction of FA activation caused by the deletion of *slr1609* results in an increase of FFA accumulation. Regarding unsaturated fatty acids with carbon chain length of C16 and C18, as in the case of FFA, the amount of unsaturated fatty acids was significantly higher in the slr1609 knockout mutant compared to the wild type. On the other hand, mutant strain GQ3, overexpressing *slr1609*, showed no significant changes on the production of FFA compared to the wild type. This approach showed how blocking FA activation by deleting *slr1609* gene in wild-type *Synechocystis* sp. PCC6803 led to doubling the amount of FFA. Similarly, other model cyanobacterium, *Synechococcus elongatus* PCC 7942, may be an advantageous host for biofuel production [162]. Unlike *Synechocystis* 6803, *S. elongatus* 7942 does not contain the pathway for polyhydroxybutyrate (PHB) synthesis [163]. As PHB synthesis is a competitive pathway of FFA synthesis for the available carbon flux, the PHB pathway is undesirable from a fuel production perspective, making *S. elongatus* 7942 an attractive host

candidate. In order to allow for FFA accumulation, the acyl-ACP synthetase gene was suppressed, and a truncated thioesterase, which allows removal of feedback inhibition of fatty acids biosynthesis, was expressed. The resulting engineered strain SE02 expresses a thioesterase, therefore reduces potential inhibition of fatty acids biosynthesis, and lacks the ability to metabolize FFA as a result of the *aas* knockout. As expected, FFA excretion by wild-type *S. elongatus* 7942 was negligible, whereas removing feedback inhibition of fatty acids biosynthesis through thioesterase expression in SE02 showed FFA production and excretion being further improved [162].

Another interesting approach consists of blocking competitive pathways. For instance, highly energetic compounds such as starch, which shares common carbon precursors with lipid synthesis, could be an interesting strategy for modifying TAG and fatty acid profiles in microalgae [164]. Several studies have been published indicating the interaction between the two pathways, although the regulation of carbon partitioning into starch and lipid synthesis pathways is not well understood. It could be hypothesized that controlling carbon precursors from the starch synthesis pathway might result in overproduction of fatty acids and TAG. Inactivation of ADP-glucose pyrophosphorylase in a *Chlamydomonas* starchless mutant led to a 10-fold increase in TAG, suggesting that shunting of photosynthetic carbon partitioning from starch to TAG synthesis may represent a more effective strategy than direct manipulation of the lipid synthesis pathway to overproduce TAG [165].

Likewise, it was shown that BAFJ5, one of the *C. reinhardtii* mutants defective in the small subunit of ADP-glucose pyrophosphorylase, accumulated neutral and total lipids up to 32.6 and 46.4 % of dry weight corresponding to 8- and 3.5-fold higher than the wild type, respectively [166]. Another example of this simple approach was studied in a novel starchless mutant of the unicellular green alga *Chlorella pyrenoidosa* [167] designated as STL-PI, which was isolated from this parental wild type after application of ultraviolet to induce mutagenesis [168, 169]. This starchless mutant had a 22 % higher growth rate and 24.5 % more protein than the parental strain, 82T. Furthermore, a very important fact was that the STL-PI mutant accumulated 20.4 % more PUFAs and 18 % less saturated fatty acids than the parental 82T. These results confirmed how promising these approaches are in order to increase lipid production through redirecting photosynthetically assimilated carbon away from starch synthesis into neutral lipid synthesis.

Another pathway that competes with lipids synthesis and should be taken into account is the conversion of phosphoenolpyruvate to oxaloacetate, catalyzed by the phosphoenolpyruvate carboxylase (PEPC) enzyme. TAG biosynthesis also requires phosphoenolpyruvate which converts successively to pyruvate, acetyl-CoA, malonyl-CoA, and then FA [170]. Some preliminary results also indicate that PEPC plays a role in the regulation of fatty acid accumulation and reduced PEPC activity by antisense expression which was correlated with an increase on lipid content in the cyanobacterium *Synechococcus* sp. [170].

A very interesting approach to altering fatty acid metabolism is to redirect carbon flux toward desired pathways, as was shown in *Schizochytrium* sp., a microalga that has been established as a candidate for commercial production of PUFAs, and able to produce large amounts of oil with 35 % dehydroacetic acid of total fatty acids [171]. During the aerobic fermentation of *Schizochytrium*, large amounts of acetate were produced, which is harmful to cell growth. Therefore, enhancing the capability of *Schizochytrium* to assimilate acetate would be useful in the fermentation process. The introduction of an *Escherichia coli* acetyl-CoA synthetase (ACS) gene was carried out, which carries an irreversible reaction that converts acetate into acetyl-CoA, in the marine microalga *Schizochytrium* sp. TIO1101. Some resulting mutants, such as ACS1 and ACS3, were able to produce a proportion of fatty acid content of 44.7 and 46.8 %, respectively, which were 1.06- and 1.11-fold larger than that of the wild-type strain (42.0 %). Finally, the fatty acid composition determined by GC-MS showed that both the wild-type strain and ACS transformants accumulated mainly six types of fatty acids. Furthermore, the dehydroacetic acid content of ACS1 and ACS3 was 43.4 and 43.7 %, slightly higher than that of the wild-type strain (43.3 %) [172]. On the contrary, it has been proposed that unlike disrupting carbohydrate pools, which are the primary carbon storage product of many microalgae, knockdown of lipid catabolism would have less impact on the primary carbon pathways associated with growth, increasing lipid accumulation while growth is not negatively affected. Only a few attempts have been made to engineer lipid metabolism in microalgae, and no targeted manipulation to date has significantly increased lipid yields without simultaneously decreasing growth. It has been reported that the targeted knockdown of a multifunctional lipase/phospholipase/acyltransferase version 3 protein ID 264297 (Thaps3_264297) increased lipid yields without affecting growth in the diatom *T. pseudonana*. Antisense-expressing knockdown strains 1A6 and 1B1 exhibited a comparable growth rate with the wild type, as well as an increased lipid content, under both continuous light and alternating light/dark conditions. Strains 1A6 and 1B1 contained 2.4- and 3.3-fold higher lipid content than wild type during exponential growth and 4.1- and 3.2-fold larger lipid content than wild type after 40 h of silicon starvation. These results clearly confirm that targeted metabolic manipulations can be employed to increase lipid accumulation in eukaryotic microalgae without affecting growth rates [173].

Analogously, it has also been demonstrated that multigene manipulation of different genes participating in TAG

metabolism is a very powerful tool to boost lipid yields. In *Chlorella minutissima,* five different genes involved in TGA biosynthesis, G3PDH: glycerol-3-phosphate dehydrogenase, GPAT: glycerol-3-phosphate acyltransferase, LPAAT: lysophosphatidic acid acyltransferase, PAP: phosphatidic acid phosphatase, and DGAT: diacylglycerol acyltransferase, were overexpressed [174]. Expression profiles using real-time analysis of the quintuple gene construct showed that the relative expression levels of G3PDH, GPAT, LPAAT, PAP, and DGAT were 27, 17, 16, 22, and 18 %, respectively. Furthermore, G3PDH showed the highest mRNA expression level, and thus, more glycerol-3-phosphate (G3P) should be introduced into the acyl pool to increase the metabolic flux for GPAT, LPAAT, PAP, and DGAT. After 14 days of cultivation of *C. minutissima* UTEX 2219 with this construct in MES-volvox medium including vitamin solution, the TAG accumulation achieved 44 wt%, 2-fold higher than control.

Another very attractive multigene approach was that in which the primary pathways toward FFA overproduction were manipulated. First by introducing an acyl-acyl carrier protein thioesterase gene (*E. coli* TE gene *tesA*), and then six successive generations of genetic modifications of the cyanobacterium *Synechocystis* sp. *PCC6803* [175], and at the same time, various enzymes belonging to competing pathways that uncouple the carbon flux from FFA overproduction, were successfully suppressed. It is known that long-chain acyl-ACP molecules are important feedback inhibitors of the activity of fatty acid synthesis (FAS) enzymes [176], such as acetyl-CoA carboxylase (ACC), the enzyme that catalyzes the conversion of acetyl-CoA into malonyl-CoA. Overproduction of enzymes like TE would reduce the cellular acyl-ACP concentrations, thus achieving the stimulation of FAS flow by decreasing feedback inhibition [176]. Except for one strain, SD225, most genetic modifications resulted in increased FFA secretion compared to the parent strains, reaching a fatty acid yield of 197 mg L^{-1} of culture in one improved strain at a cell density of 1.0×10^9 cells mL^{-1}. This result suggests that fatty acid secreting cyanobacteria could be considered as a feasible organism for renewable biofuel production [175].

In another study, *Synechocystis* 6803 was engineered for the accumulation of FFA, modifying different strains. One strain (F3), in which TE, a thioesterase catalyzing the liberation of FFAs from acyl-ACP, was overexpressed. Another strain (F16) in which inactivation of the AAS gene, encoding acyl-ACP synthetase, prevented the rethioesterification of FFA generated from membrane lipid recycling and led to elevated levels of intracellular FFA. And finally, strain (F3:16), in which both modifications were introduced and expressed [177]. Contrary to expectations, strain F3 displayed no increase in the amounts of intracellular FFA, while F16 showed a modest but notable increase. On the other hand, strain F3:16 demonstrated a dramatic 30-fold rise in FFA levels, being those FFA mostly stearic acid (octadecanoate; 18:0) and palmitic acid (hexadecanoate; 16:0), and the rest being monounsaturated octadecanoates, primarily oleic acid (18:1-Ω9), vaccenic acid (18:1-n11), and euric acid (22:1-n9). FFA secreted by the F3:16 cells differed significantly from the intracellular pool, with the majority being unsaturated fatty acids, chiefly linoleic acid (18:2n6), palmitoleic acid (16:1n9), vaccenic acid (18:1n11), and α-linolenic acid (18:3n3). Again, it has been demonstrated how a multigene modification is much more desirable and effective in order to achieve a higher production of fatty acids.

We need to take into account many other findings related to the metabolic engineering or manipulation of growing conditions, which could facilitate production of PUFAs, fatty acids, and biofuel [178]. Likewise, we could not disregard to positively use all the information available from other organisms, such as the cyanobacterium *Synechocystis* sp. PCC6803 [179], the yeast *Yarrowia lipolytica* [180, 181], and other findings, for instance related with new metabolic pathways [182].

All these results, together with the continuous important contributions, and new results on the field of PUFAs and biofuels, will shed light on how to develop and optimize different alternative sources of production of those long-chain n-3 fatty acids in high demand for human life, health, and food, compounds whose current sources, particularly fish, are being consumed at a high rate and therefore could result in a complete depletion in the near future.

Concluding Remarks

It is well demonstrated the tremendous importance of PUFA consumption for cell and body function, regardless of the organisms (animals, plants, fungi, algae, etc.). In humans, PUFA plays a high role in our well-being, and for attaining a healthy status, as has been presented in depth in different chapters of this book. Gradually, this is being understood and accepted by the health authorities and by the general public more concerned with their nutrition. These compounds cannot be synthesized de novo by the human biochemical machinery, although some of them can be produced through some intermediates but in a very inefficient manner, as encountered particularly with the vegan communities, and therefore must necessarily be taken up through diet.

The main source of PUFA for humans derives from fish oil, and very worrisome, this depends on extractive fishing practices that also contributes to the depletion of fish stocks, which, despite many efforts and policies, has not been managed to be exploited in a sustainable manner in order to provide a continuous and ordered supply of omega-3 fatty acids. The estate of fish stocks depicts an alarming scenario,

with 32 % of fish stocks overexploited, other 50 % highly exploited, 4 % already exhausted, and just 2 % on the process of being recovered [9]. Therefore, the demand for finding and establishing new sources of PUFA in a more sustainable fashion continues to be highly required, and thus, the search began a few decades ago, particularly in the oceans. Currently, many different species are being cultured and exploited for the controlled production of omega-3 fatty acids as presented in this chapter. Some show strong potential and attractive scale-up capabilities and are being exploited accordingly. These successful industrial examples provide clear evidences that one of the ways to accomplish a continuous supply of these metabolites is that offered by the microalgae, particularly autotrophic microalgae that do not demand an organic carbon source for their growth [150]. Furthermore, more research into the genetics of these organisms is required to gain full knowledge and understanding of their biochemical potential. On the other hand, the genetic engineering of the biosynthetic route leading to EPA and DHA accumulation has been attempted, particularly in microalgae, but its full potential has not been accomplished yet. Surely, this will generate fascinating and encouraging outcomes, permitting also a full understanding of this biosynthetic route in these organisms, ending up in establishing more manageable and productive systems.

Other alternatives, such as the ultimate advances made with higher plants, to which the complete biosynthetic route of the omega-3 fatty acids has been introduced and expressed, resulting in high yields of EPA and DHA, should be also considered [51, 52]. Macroalgae, although presenting interesting profiles and yields of fatty acids, seems to be less exploited for omega-3 fatty acid production than the microalgae, although they also accumulate an extensive array of very important bioactive compounds already under exploitation.

Regarding regulations on the production, use, and consumption of omega-3 fatty acids derived from these sources, this is defined by different governing bodies, but the most general and internationally applied is the Codex Alimentarius, which formulates and harmonizes food standards ensuring their global implementation [54]. Furthermore, it also allows them a role in the development of codes governing hygienic processing practices, and recommendations relating to compliance with those standards, and in general pursues consumer health protection. In Europe, the European Food Safety Authority is responsible to assess the safety of any new food and feed compound before they are authorized for production and commercialization. Similarly, in USA, the agency conducting all these policies and regulations is the Food and Drug Administration (FDA).

Recently, two meetings dedicated to the topic of biomarine advances, novel food from algae, aquaculture, etc., took place in Portugal (Alga biomass Novel Food Workshop; 5th BioMarine International Business Convention, Marine Bio-Resources for a New Blue Economy 2014). How the regulatory process and requirements, which are not always at pace with the fast scientific advances and discoveries, came out and were debated. Despite the need for assuring the safety on the consumption of food and food ingredients, a faster, agile, and clear regulatory framework that would make it possible to bring to the market a wide range of new species and related products and extracts, in a faster way, was demanded. This would be required for an optimal and rapid exploitation of food ingredients, such as, for instance, the omega-3 fatty acids obtained from newly determined novel microalga species with strong industrial potential.

Over the past decades, algae biotechnology has been growing increasingly with many successful examples of many enterprises exploiting different aspects of the biochemical potential of these organisms. Moreover, research continues, although most of the microalga species estimated to exist in our oceans (1–10 million) remains to be discovered and evaluated for their omega-3 production potential, as only 10,000–20,000 have been taxonomically described, and just a minor percentage of them has been screened for determining their omega-3 fatty acid profiles. Thus, the quest must continue, with many interesting and exciting results waiting to be accomplished.

In order to establish and offer an adequate continuous supply of these highly valuable metabolites needed for maintaining a healthy human population, we ought to consider all possible alternatives to thus satisfy the increasing world demand for these nutrients, including obviously algae.

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References

1. Qin S, Lin HZ, Jiang P. Advances in genetic engineering of marine algae. Biotechnol Adv. 2012;30(6):1602–13.
2. Falkowski PG, Barber RT, Smetacek V. Biogeochemical controls and feedbacks on ocean primary production. Science. 1998;281 (5374):200–6.
3. Apt KE, Behrens PW. Commercial developments in microalgal biotechnology. J Phycol. 1999;35(2):215–26.
4. Chisti Y. Biodiesel from microalgae. Biotechnol Adv. 2007;25 (3):294–306.
5. Ahmed F, Fanning K, Netzel M, Turner W, Li Y, Schenk PM. Profiling of carotenoids and antioxidant capacity of microalgae from subtropical coastal and brackish waters. Food Chem. 2014;165:300–6.

6. Skulberg OM. Microalgae as a source of bioactive molecules—experience from cyanophyte research. J Appl Phycol. 2000;12(3–5):341–8.
7. Simopoulos AP. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exp Biol Med. 2008;233(6):674–88.
8. Simopoulos AP. Omega 3-fatty acids in health and disease and in growth and development. Am J Clin Nutr. 1991;54(3):438–63.
9. FAO. The State of World Fisheries and Aquaculture. 2010.
10. Adarme-Vega TC, Thomas-Hall SR, Schenk PM. Towards sustainable sources for omega-3 fatty acids production. Curr Opin Biotechnol. 2014;26:14–8.
11. Spolaore P, Joannis-Cassan C, Duran E, Isambert A. Commercial applications of microalgae. J Biosci Bioeng. 2006;101(2):87–96.
12. Peter Castro MEH. Marine biology. New York: McGraw-Hill Science/Engineering/Math; 2012.
13. Hedgpeth JW. Classification of marine environments. Geol Soc Am Memoirs. 1957;67:12.
14. Fenchel T. The microbial loop-25 years later. J Exp Mar Biol Ecol. 2008;366(1–2):99–103.
15. Boyce DG, Lewis MR, Worm B. Global phytoplankton decline over the past century. Nature. 2010;466(7306):591–6.
16. Ausubel JH, Trew Crist D, Waggoner PE. First census of marine life 2010. Highlights of a decade of discovery. Washington, USA: Census of Marine Life; 2010.
17. Barsanti LGP. Algae: anatomy, biochemistry, and biotechnology. UK: CRC Press, Taylor & Francis Group, LLC; 2006.
18. Rogers K. Fungi, algae, and protists. 1st ed. USA: Britannica Educational Publishing; 2011.
19. Yool A, Tyrrell T. Role of diatoms in regulating the ocean's silicon cycle. Glob Biogeochem Cycle. 2003;17(4):21.
20. Berner RA. Atmospheric oxygen over phanerozoic time. Proc Natl Acad Sci USA. 1999;96(20):10955–7.
21. Kenrick P, Crane PR. The origin and early evolution of plants on land. Nature. 1997;389(6646):33–9.
22. Wodniok S, Brinkmann H, Glockner G, Heidel AJ, Philippe H, Melkonian M, et al. Origin of land plants: do conjugating green algae hold the key? BMC Evol Biol. 2011;11:10.
23. Harwood JL. Recent advances in the biochemistry of plant fatty acids. Biochim Biophys Acta. 1996;1301:7–56.
24. Alonso DL, Maroto FG. Plants as 'chemical factories' for the production of polyunsaturated fatty acids. Biotechnol Adv. 2000;18(6):481–97.
25. Guil-Guerrero JL. Stearidonic acid (18: 4n–3): metabolism, nutritional importance, medical uses and natural sources. Eur J Lipid Sci Technol. 2007;109(12):1226–36.
26. Haslam RP, Ruiz-Lopez N, Eastmond P, Moloney M, Sayanova O, Napier JA. The modification of plant oil composition via metabolic engineering-better nutrition by design. Plant Biotechnol J. 2013;11(2):157–68.
27. Bhardwaj HL, Hamama AA. Oil, erucic acid, and glucosinolate contents in winter hardy rapeseed germplasms. Ind Crop Prod. 2000;12(1):33–8.
28. Federica Zanetti TV. Stefano Bona, Giuliano Mosca Can we "cultivate" erucic acid in southern Europe? Ital J Agron. 2006;1(1):3–10.
29. Cequier-Sanchez E, Rodriguez C, Dorta-Guerra R, Ravelo AG, Zarate R. Echium acanthocarpum hairy root cultures, a suitable system for polyunsaturated fatty acid studies and production. BMC Biotechnol. 2011;11:14.
30. Guil-Guerrero JL, Garcia-Maroto F, Campra-Madrid P, Gomez-Mercado F. Occurrence and characterization of oils rich in gamma-linolenic acid part II: fatty acids and squalene from *Macaronesian echium* leaves. Phytochemistry. 2000;54(5):525–9.
31. Guil-Guerrero JL, Garcia-Maroto F, Vilches-Ferron MA, Lopez-Alonso D. Gamma-linolenic acid from fourteen boraginaceae species. Ind Crop Prod. 2003;18(1):85–9.
32. Guil-Guerrero JL, Gomez-Mercado F, Garcia-Maroto F, Campra-Madrid P. Occurrence and characterization of oils rich in gamma-linolenic acid—part I: Echium seeds from Macaronesia. Phytochemistry. 2000;53(4):451–6.
33. Guil-Guerrero JL, Gomez-Mercado F, Rodriguez-Garcia I, Campra-Madrid P, Garcia-Maroto F. Occurrence and characterization of oils rich in gamma-linolenic acid (III): the taxonomical value of the fatty acids in Echium (Boraginaceae). Phytochemistry. 2001;58(1):117–20.
34. Guil-Guerrero JL, Maroto FFG, Gimenez AG. Fatty acid profiles from forty-nine plant species that are potential new sources of gamma-linolenic acid. J Am Oil Chem Soc. 2001;78(7):677–84.
35. Muuse BG, Essers ML, Vansoest LJM. Oenothera species and Borago officinalis—sources of gamma linolenic acid. Neth J Agric Sci. 1988;36(4):357–63.
36. Sayanova O, Napier JA, Shewry PR. Delta(6)-unsaturated fatty acids in species and tissues of the Primulaceae. Phytochemistry. 1999;52(3):419–22.
37. Murphy DJ. Manipulation of lipid metabolism in transgenic plants—biotechnological goals and biochemical realities. Biochem Soc Trans. 1994;22(4):926–31.
38. Sayanova OV, Napier JA. Eicosapentaenoic acid: biosynthetic routes and the potential for synthesis in transgenic plants. Phytochemistry. 2004;65(2):147–58.
39. Somerville C, Browse J. Plants lipids—metabolism, mutants and membranes. Science. 1991;252(5002):80–7.
40. Somerville C, Browse J. Dissecting desaturation: plants prove advantageous. Trends Cell Biol. 1996;6(4):148–53.
41. Voss A, Reinhart M, Sankarappa S, Sprecher H. The metabolism of 7,10,13,16,19-docosapentanoic acid to 4,7,10,13,16,19-docosahexanoic acid in rat liver is independent of a delta4 desaturase. J Biol Chem. 1991;266(30):19995–20000.
42. Sprecher H. An update on the pathways of polyunsaturated fatty acid metabolism. Curr Opin Clinic Nutri Metab Care. 1999;2(2):135–8.
43. Sprecher H, Chen Q, Yin FQ. Regulation of the biosynthesis of 22: 5n–6 and 22: 6n–3: a complex intracellular process. Lipids. 1999;34:S153–6.
44. Buzzi M, Henderson RJ, Sargent JR. The desaturation and elongation of linolenic acid and eicosapentaenoic acid by hepatocytes and liver microsomes from rainbow trout (*Oncorhynchus mykiss*) fed diets containing fish oil or olive oil. Biochim Biophys Acta-Lipids Lipid Metab. 1996;1299(2):235–44.
45. Buzzi M, Henderson RJ, Sargent JR. Biosynthesis of docosahexaenoic acid in trout hepatocytes proceeds via 24-carbon intermediates. Comp Biochem Physiol B-Biochem Mol Biol. 1997;116(2):263–7.
46. Rodriguez C, Henderson RJ, Porter AEA, Dick JR. Modification of odd-chain length unsaturated fatty acids by hepatocytes of rainbow trout (*Oncorhynchus mykiss*) fed diets containing fish oil or olive oil. Lipids. 1997;32(6):611–9.
47. Sayanova O, Haslam R, Guschina I, Lloyd D, Christie WW, Harwood JL, et al. A bifunctional Delta 12, Delta 15-desaturase from *Acanthamoeba castellanii* directs the synthesis of highly unusual n-1 series unsaturated fatty acids. J Biol Chem. 2006;281(48):36533–41.
48. Venegas-Caleron M, Beaudoin F, Sayanova O, Napier JA. Co-transcribed genes for long chain polyunsaturated fatty acid biosynthesis in the protozoon *Perkinsus marinus* include a plant-like FAE1 3-ketoacyl coenzyme A synthase. J Biol Chem. 2007;282(5):2996–3003.

49. Napier JA, Graham IA. Tailoring plant lipid composition: designer oilseeds come of age. Curr Opin Plant Biol. 2010;13(3):330–7.
50. Rogalski M, Carrer H. Engineering plastid fatty acid biosynthesis to improve food quality and biofuel production in higher plants. Plant Biotechnol J. 2011;9(5):554–64.
51. Sayanova O, Napier JA. Transgenic oilseed crops as an alternative to fish oils. Prostaglandins Leukot Essent Fatty Acids. 2011;85(5):253–60.
52. Ruiz-Lopez N, Haslam RP, Napier JA, Sayanova O. Successful high-level accumulation of fish oil omega-3 long-chain polyunsaturated fatty acids in a transgenic oilseed crop. Plant J. 2014;77(2):198–208.
53. Petrie JR, Shrestha P, Belide S, Kennedy Y, Lester G. Metabolic Engineering *Camelina sativa* with fish oil-like levels of DHA. PLoS ONE. 2014;9(4):1 (e85061).
54. Commission CA. Understanding the codex alimentarius. Rome: WHO, FAO; 2006.
55. http://www.fda.gov/AboutFDA/CentersOffices/default.htm.
56. Enzing C, Ploeg M, Barbosa M, Sijtsma L, Vigani M, Parisi C, et al. Microalgae-based products for the food and feed sector: an outlook for Europe. Publications Office; 2014.
57. McHugh DJ. A guide to the seaweed industry:. Food and Agriculture Organization of the United Nations Rome; 2003.
58. Khozin-Goldberg I, Iskandarov U, Cohen Z. LC-PUFA from photosynthetic microalgae: occurrence, biosynthesis, and prospects in biotechnology. Appl Microbiol Biotechnol. 2011;91(4):905–15.
59. Bhatnagar I, Kim SK. Immense essence of excellence: marine microbial bioactive compounds. Mar Drugs. 2010;8(10):2673–701.
60. Milledge JJ. Commercial application of microalgae other than as biofuels: a brief review. Rev Environ Sci Biotechnol. 2011;10(1):31–41.
61. Mimouni V, Ulmann L, Pasquet V, Mathieu M, Picot L, Bougaran G, et al. the potential of microalgae for the production of bioactive molecules of pharmaceutical interest. Curr Pharm Biotechnol. 2012;13(15):2733–50.
62. Doughman SD, Krupanidhi S, Sanjeevi CB. Omega-3 fatty acids for nutrition and medicine: considering microalgae oil as a vegetarian source of EPA and DHA. Curr Diabetes Rev. 2007;3(3):198–203.
63. Hu HH, Gao KS. Response of growth and fatty acid compositions of Nannochloropsis sp to environmental factors under elevated CO_2 concentration. Biotechnol Lett. 2006;28(13):987–92.
64. Gog A, Senila L, Roman M, Luca E, Roman C, Irimie FD. Oil extraction and fatty acid characterization of *Nannochloropsis oculata* microalgae for biodiesel applications. Stud Univ Babes-Bolyai Chem. 2012;57(1):111–8.
65. Martins DA, Custodio L, Barreira L, Pereira H, Ben-Hamadou R, Varela J, et al. Alternative sources of n-3 long-chain polyunsaturated fatty acids in marine microalgae. Mar Drugs. 2013;11(7):2259–81.
66. Krienitz L, Wirth M. The high content of polyunsaturated fatty acids in *Nannochloropsis limnetica* (Eustigmatophyceae) and its implication for food web interactions, freshwater aquaculture and biotechnology. Limnologica. 2006;36(3):204–10.
67. Fajardo AR, Cerdan LE, Medina AR, Fernandez FGA, Moreno PAG, Grima EM. Lipid extraction from the microalga *Phaeodactylum tricornutum*. Eur J Lipid Sci Technol. 2007;109(2):120–6.
68. Sevilla JMF, Garcia MCC, Miron AS, Belarbi E, Camacho FG, Grima EM. Pilot-plant-scale outdoor mixotrophic cultures of *Phaeodactylum tricornutum* using glycerol in vertical bubble column and airlift photobioreactors: Studies in fed-batch mode. Biotechnol Prog. 2004;20(3):728–36.
69. Ceron-Garcia MC, Fernandez-Sevilla JM, Sanchez-Miron A, Garcia-Camacho F, Contreras-Gomez A, Molina-Grima E. Mixotrophic growth of *Phaeodactylum tricornutum* on fructose and glycerol in fed-batch and semi-continuous modes. Bioresour Technol. 2013;147:569–76.
70. Dillschneider R, Posten C. A linear programming approach for modeling and simulation of growth and lipid accumulation of *Phaeodactylum tricornutum*. Energies. 2013;6(10):5333–56.
71. Guihéneuf F, Fouqueray M, Mimouni V, Ulmann L, Jacquette B, Tremblin G. Effect of UV stress on the fatty acid and lipid class composition in two marine microalgae *Pavlova lutheri* (Pavlovophyceae) and *Odontella aurita* (Bacillariophyceae). J Appl Phycol. 2010;22:629–38.
72. Moreau D, Tomasoni C, Jacquot C, Kaas R, Le Guedes R, Cadoret JP, et al. Cultivated microalgae and the carotenoid fucoxanthin from *Odontella aurita* as potent anti-proliferative agents in bronchopulmonary and epithelial cell lines. Environ Toxicol Pharmacol. 2006;22(1):97–103.
73. Haimeur A, Ulmann L, Mimouni V, Gueno F, Pineau-Vincent F, Meskini N, et al. The role of *Odontella aurita*, a marine diatom rich in EPA, as a dietary supplement in dyslipidemia, platelet function and oxidative stress in high-fat fed rats. Lipids Health Dis. 2012;11:13.
74. Xia S, Wan LL, Li AF, Sang M, Zhang CW. Effects of nutrients and light intensity on the growth and biochemical composition of a marine microalga *Odontella aurita*. Chin J Oceanol Limnol. 2013;31(6):1163–73.
75. Rezanka T, Petrankova M, Cepak V, Pribyl P, Sigler K, Cajthaml T. *Trachydiscus minutus*, a new biotechnological source of eicosapentaenoic acid. Folia Microbiol (Praha). 2010;55(3):265–9.
76. Cepak V, Pribyl P, Kohoutkova J, Kastanek P. Optimization of cultivation conditions for fatty acid composition and EPA production in the eustigmatophycean microalga *Trachydiscus minutus*. J Appl Phycol. 2014;26(1):181–90.
77. Jiang Y, Chen F, Liang SZ. Production potential of docosahexaenoic acid by the heterotrophic marine dinoflagellate *Crypthecodinium cohnii*. Proc Biochem. 1999;34(6–7):633–7.
78. Jiang Y, Chen F. Effects of temperature and temperature shift on docosahexaenoic acid production by the marine microalga *Crypthecodinium cohnii*. J Am Oil Chem Soc. 2000;77(6):613–7.
79. Okuda T, Ando A, Sakuradani E, Ogawa J. Isolation and characterization of a docosahexaenoic acid-phospholipids producing microorganism Crypthecodinium sp D31. J Am Oil Chem Soc. 2013;90(12):1837–44.
80. Tatsuzawa H, Takizawa E. Changes in lipid and fatty acid composition of *Pavlova lutheri*. Phytochemistry. 1995;40(2):397–400.
81. Ponis E, Parisi G, Zittelli GC, Lavista F, Robert R, Tredici MR. *Pavlova lutheri*: production, preservation and use as food for *Crassostrea gigas* larvae. Aquaculture. 2008;282(1–4):97–103.
82. Guiheneuf F, Mimouni V, Ulmann L, Tremblin G. Combined effects of irradiance level and carbon source on fatty acid and lipid class composition in the microalga *Pavlova lutheri* commonly used in mariculture. J Exp Mar Biol Ecol. 2009;369(2):136–43.
83. Shah SMU, Radziah CC, Ibrahim S, Latiff F, Othman MF, Abdullah MA. Effects of photoperiod, salinity and pH on cell growth and lipid content of *Pavlova lutheri*. Ann Microbiol. 2014;64(1):157–64.
84. Patil V, Kallqvist T, Olsen E, Vogt G, Gislerod HR. Fatty acid composition of 12 microalgae for possible use in aquaculture feed. Aquac Int. 2007;15(1):1–9.

85. Feng DN, Chen ZA, Xue S, Zhang W. Increased lipid production of the marine oleaginous microalgae *Isochrysis zhangjiangensis* (Chrysophyta) by nitrogen supplement. Bioresour Technol. 2011;102(12):6710–6.
86. Liu J, Sommerfeld M, Hu Q. Screening and characterization of Isochrysis strains and optimization of culture conditions for docosahexaenoic acid production. App Microbiol Biotechnol. 2013;97(11):4785–98.
87. Fradique M, Batista AP, Nunes MC, Gouveia L, Bandarra NM, Raymundo A. *Isochrysis galbana* and *Diacronema vlkianum* biomass incorporation in pasta products as PUFA's source. Lwt-Food Sci Technol. 2013;50(1):312–9.
88. Honda D, Yokochi T, Nakahara T, Erata M, Higashihara T. *Schizochytrium limacinum* sp. nov., a new thraustochytrid from a mangrove area in the west Pacific Ocean. Mycol Res. 1998;102:439–48.
89. Chin HJ, Shen TF, Su HP, Ding ST. *Schizochytrium limacinum* SR-21 as a source of docosahexaenoic acid: optimal growth and use as a dietary supplement for laying hens. Aust J Agric Res. 2006;57(1):13–20.
90. Kamlangdee N, Fan KW. Polyunsaturated fatty acids production by Schizochytrium sp. isolated from mangrove. Songklanakarin J Sci Technol. 2003;25(5):9.
91. Ren L-J, Ji X-J, Huang H, Qu L, Feng Y, Tong Q-Q, et al. Development of a stepwise aeration control strategy for efficient docosahexaenoic acid production by Schizochytrium sp. App Microbiol Biotechnol. 2010;87(5):1649–56.
92. Qu L, Ji XJ, Ren LJ, Nie ZK, Feng Y, Wu WJ, et al. Enhancement of docosahexaenoic acid production by Schizochytrium sp. using a two-stage oxygen supply control strategy based on oxygen transfer coefficient. Lett App Microbiol. 2011;52(1):22–7.
93. Huang JZ, Aki T, Hachida K, Yokochi T, Kawamoto S, Shigeta S, et al. Profile of polyunsaturated fatty acids produced by Thraustochytrium sp KK17-3. J Am Oil Chem Soc. 2001;78 (6):605–10.
94. Shene C, Leyton A, Rubilar M, Pinelo M, Acevedo F, Morales E. Production of lipids and docosahexasaenoic acid (DHA) by a native Thraustochytrium strain. Eur J Lipid Sci Technol. 2013;115(8):890–900.
95. Singh A, Wilson S, Ward OP. Docosahexaenoic acid (DHA) production by Thraustochytrium sp ATCC 20892. World J Microbiol Biotechnol. 1996;12(1):76–81.
96. Akimoto M, Shirai A, Ohtaguchi K, Koide K. Carbon dioxide fixation and polyunsaturated fatty acid production by the red alga *Porphyridium cruentum*. Appl Biochem Biotechnol. 1998;73(2–3):269–78.
97. Colla LM, Bertolin TE, Costa JAV. Fatty acids profile of *Spirulina platensis* grown under different temperatures and nitrogen concentrations. Zeitschrift Fur Naturforschung C— J Biosci. 2004;59(1–2):55–9.
98. Guil-Guerrero JL, Belarbi EH, Rebolloso-Fuentes MM. Eicosapentaenoic and arachidonic acids purification from the red microalga *Porphyridium cruentum*. Bioseparation. 2000;9 (5):299–306.
99. Lang IK, Hodac L, Friedl T, Feussner I. Fatty acid profiles and their distribution patterns in microalgae: a comprehensive analysis of more than 2000 strains from the SAG culture collection. BMC Plant Biol. 2011;11:16.
100. Renaud SM, Thinh LV, Lambrinidis G, Parry DL. Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures. Aquaculture. 2002;211(1–4):195–214.
101. Renaud SM, Zhou HC, Parry DL, Thinh LV, Woo KC. Effect of temperature on the growth, total lipid content and fatty acid composition of recently isolated tropical microalgae *Isochrysis* sp, *Nitzschia closterium*, *Nitzschia paleacea*, and commercial species *Isochrysis* sp (clone T ISO). J Appl Phycol. 1995;7(6):595–602.
102. Wen ZY, Chen F. Production potential of eicosapentaenoic acid by the diatom *Nitzschia laevis*. Biotechnol Lett. 2000;22(9):727–33.
103. Wen ZY, Chen F. Application of statistically-based experimental designs for the optimization of eicosapentaenoic acid production by the diatom *Nitzschia laevis*. Biotechnol Bioeng. 2001;75(2): 159–69.
104. Otero A, Fabregas J. Changes in the nutrient composition of *Tetraselmis suecica* cultured semicontinuously with different nutrient concentrations and renewal rates. Aquaculture. 1997;159(1–2):111–23.
105. Guzman HM, Valido AD, Duarte LC, Presmanes KF. Estimate by means of flow cytometry of variation in composition of fatty acids from *Tetraselmis suecica* in response to culture conditions. Aquac Int. 2010;18(2):189–99.
106. Chang KJL, Dunstan GA, Abell GCJ, Clementson LA, Blackburn SI, Nichols PD, et al. Biodiscovery of new Australian thraustochytrids for production of biodiesel and long-chain omega-3 oils. App Microbiol Biotechnol. 2012;93(5):2215–31.
107. Kumari P, Bijo AJ, Mantri VA, Reddy CRK, Jha B. Fatty acid profiling of tropical marine macroalgae: an analysis from chemotaxonomic and nutritional perspectives. Phytochemistry. 2013;86:44–56.
108. Kumari P, Kumar M, Gupta V, Reddy CRK, Jha B. Tropical marine macroalgae as potential sources of nutritionally important PUFAs. Food Chem. 2010;120(3):749–57.
109. Pereira H, Barreira L, Figueiredo F, Custodio L, Vizetto-Duarte C, Polo C, et al. Polyunsaturated fatty acids of marine macroalgae: potential for nutritional and pharmaceutical applications. Mar Drugs. 2012;10(9):1920–35.
110. Graeve M, Kattner G, Wiencke C, Karsten U. Fatty acid composition of Arctic and Antartic macroalgae: indicator of phylogenetic and trophic relationships. Mar Ecol Prog Ser. 2002;231:67–74.
111. van Ginneken VJT, Helsper J, de Visser W, van Keulen H, Brandenburg WA. Polyunsaturated fatty acids in various macroalgal species from north Atlantic and tropical seas. Lipids Health Dis. 2011;10:8.
112. Sanchez-Machado DI, Lopez-Cervantes J, Lopez-Hernandez J, Paseiro-Losada P. Fatty acids, total lipid, protein and ash contents of processed edible seaweeds. Food Chem. 2004;85(3):439–44.
113. Gressler V, Yokoya NS, Fujii MT, Colepicolo P, Mancini J, Torres RP, et al. Lipid, fatty acid, protein, amino acid and ash contents in four Brazilian red algae species. Food Chem. 2010;120(2):585–90.
114. Nelson MM, Phleger CF, Nichols PD. Seasonal lipid composition in macroalgae of the northeastern pacific ocean. Bot Marina. 2002;45(1):58–65.
115. Ivesa L, Blazina M, Najdek M. Seasonal variations in fatty acid composition of *Caulerpa taxifolia* (M. Vahl.) C. Ag. in the northern Adriatic Sea (Malinska, Croatia). Bot Marina. 2004;47 (3):209–14.
116. Teoh ML, Chu WL, Marchant H, Phang SM. Influence of culture temperature on the growth, biochemical composition and fatty acid profiles of six Antarctic microalgae. J Appl Phycol. 2004;16 (6):421–30.
117. Cyril J, Powell GL, Duncan RR, Baird WV. Changes in membrane polar lipid fatty acids of seashore paspalum in response to low temperature exposure. Crop Sci. 2002;42(6): 2031–7.
118. Winfield MO, Lu CG, Wilson ID, Coghill JA, Edwards KJ. Plant responses to cold: transcriptome analysis of wheat. Plant Biotechnol J. 2010;8(7):749–71.

119. Mendes LF, Stevani C, Zambotti-Villela L, Yokoya N, Colepicolo P. Toxic effect of metal cation binary mixtures to the seaweed *Gracilaria domingensis* (Gracilariales, Rhodophyta). Environ Sci Pollut Res. 2014;21(13):8216–23.
120. Khotimchenko SV, Yakovleva IM. Effect of solar irradiance on lipids of the green alga *Ulva fenestrata* Postels et Ruprecht. Bot Marina. 2004;47(5):395–401.
121. Khotimchenko SV, Yakovleva IM. Lipid composition of the red alga *Tichocarpus crinitus* exposed to different levels of photon irradiance. Phytochemistry. 2005;66(1):73–9.
122. Schmid M, Guiheneuf F, Stengel DB. Fatty acid contents and profiles of 16 macroalgae collected from the Irish Coast at two seasons. J Appl Phycol. 2014;26(1):451–63.
123. Nomura M, Kamogawa H, Susanto E, Kawagoe C, Yasui H, Saga N, et al. Seasonal variations of total lipids, fatty acid composition, and fucoxanthin contents of *Sargassum horneri* (Turner) and *Cystoseira hakodatensis* (Yendo) from the northern seashore of Japan. J Appl Phycol. 2013;25(4):1159–69.
124. Boulom S, Robertson J, Hamida'b N, Ma QL, Lu J. Seasonal changes in lipid, fatty acid, alpha-tocopherol and phytosterol contents of seaweed, *Undaria pinnatifida*, in the Marlborough sounds, New Zealand. Food Chem. 2014;161:261–9.
125. Kendel M, Couzinet-Mossion A, Viau M, Fleurence J, Barnathan G, Wielgosz-Collin G. Seasonal composition of lipids, fatty acids, and sterols in the edible red alga *Grateloupia turuturu*. J Appl Phycol. 2013;25(2):425–32.
126. Kang JY, Benliro IMP, Lee IJ, Choi JY, Joo J, Choi YS, et al. Viability, fatty acid composition and structure of the Corralline alga *Corallina pilulifera*. Bot Sci. 2014;92(1):103–9.
127. Rocchetta I, Mazzuca M, Conforti V, Ruiz L, Balzaretti V, de Molina MDR. Effect of chromium on the fatty acid composition of two strains of *Euglena gracilis*. Environ Pollut. 2006;141(2):353–8.
128. Pinto E, Sigaud-Kutner TCS, Leitao MAS, Okamoto OK, Morse D, Colepicolo P. Heavy metal-induced oxidative stress in algae. J Phycol. 2003;39(6):1008–18.
129. Pinto E, Carvalho AP, Cardozo KHM, Malcata FX, Anjos FMD, Colepicolo P. Effects of heavy metals and light levels on the biosynthesis of carotenoids and fatty acids in the macroalgae *Gracilaria tenuistipitata* (var. liui Zhang & Xia). Braz J Pharmacognosy. 2011;21(2):349–54.
130. Kumar M, Bijo AJ, Baghel RS, Reddy CRK, Jha B. Selenium and spermine alleviate cadmium induced toxicity in the red seaweed *Gracilaria dura* by regulating antioxidants and DNA methylation. Plant Physiol Biochem. 2012;51:129–38.
131. Imbs AB, Latyshev NA, Svetashev VI, Skriptsova AV, Le TT, Pham MQ, et al. Distribution of polyunsaturated fatty acids in red algae of the genus Gracilaria, a promising source of prostaglandins. Russ J Mar Biol. 2012;38(4):339–45.
132. Nozaki H, Takano H, Misumi O, Terasawa K, Matsuzaki M, Maruyama S, et al. A 100 %-complete sequence reveals unusually simple genomic features in the hot-spring red alga *Cyanidioschyzon merolae*. BMC Biol. 2007;5:28.
133. Armbrust EV, Berges JA, Bowler C, Green BR, Martinez D, Putnam NH, et al. The genome of the diatom *Thalassiosira pseudonana*: ecology, evolution, and metabolism. Science (New York, NY). 2004;306(5693):79–86.
134. Bowler C, Allen AE, Badger JH, Grimwood J, Jabbari K, Kuo A, et al. The Phaeodactylum genome reveals the evolutionary history of diatom genomes. Nature. 2008;456(7219):239–44.
135. Derelle E, Ferraz C, Rombauts S, Rouze P, Worden AZ, Robbens S, et al. Genome analysis of the smallest free-living eukaryote *Ostreococcus tauri* unveils many unique features. Proc Nat Acad Sci USA. 2006;103(31):11647–52.
136. Gimpel JA, Specht EA, Georgianna DR, Mayfield SP. Advances in microalgae engineering and synthetic biology applications for biofuel production. Curr Opin Chem Biol. 2013;17(3):489–95.
137. Li HY, Lu Y, Zheng JW, Yang WD, Liu JS. Biochemical and genetic engineering of diatoms for polyunsaturated fatty acid biosynthesis. Mar Drugs. 2014;12(1):153–66.
138. Abbadi A, Domergue F, Bauer J, Napier JA, Welti R, Zahringer U, et al. Biosynthesis of very-long-chain polyunsaturated fatty acids in transgenic oilseeds: Constraints on their accumulation. Plant Cell. 2004;16(10):2734–48.
139. Cheng BF, Wu GH, Vrinten P, Falk K, Bauer J, Qiu X. Towards the production of high levels of eicosapentaenoic acid in transgenic plants: the effects of different host species, genes and promoters. Transgenic Res. 2010;19(2):221–9.
140. Thelen JJ, Ohlrogge JB. Metabolic engineering of fatty acid biosynthesis in plants. Metab Eng. 2002;4(1):12–21.
141. Blatti JL, Michaud J, Burkart MD. Engineering fatty acid biosynthesis in microalgae for sustainable biodiesel. Curr Opin Chem Biol. 2013;17(3):496–505.
142. Ryckebosch E, Bruneel C, Muylaert K, Foubert, I. Microalgae as an alternative source of omega-3 long chain polyunsaturated fatty acids. Lipid Technol. 2012;24:128–30.
143. Zaslavskaia LA, Lippmeier JC, Shih C, Ehrhardt D, Grossman AR, Apt KE. Trophic conversion of an obligate photoautotrophic organism through metabolic engineering. Science (New York, NY). 2001;292(5524):2073–5.
144. Tonon T, Qing R, Harvey D, Li Y, Larson TR, Graham IA. Identification of a long-chain polyunsaturated fatty acid acyl-coenzyme A synthetase from the diatom *Thalassiosira pseudonana*. Plant Physiol. 2005;138(1):402–8.
145. Tonon T, Sayanova O, Michaelson LV, Qing R, Harvey D, Larson TR, et al. Fatty acid desaturases from the microalga *Thalassiosira pseudonana*. FEBS J. 2005;272(13):3401–12.
146. Xu JZZ, Zou J. A membrane-bound glycerol-3-phosphate acyltransferase from *Thalassiosira pseudonana* regulates acyl composition of glycerolipids. Botany. 2009;87(6):544–51.
147. Domergue F, Lerchl J, Zahringer U, Heinz E. Cloning and functional characterization of *Phaeodactylum tricornutum* front-end desaturases involved in eicosapentaenoic acid biosynthesis. Eur Jo Biochem/FEBS. 2002;269(16):4105–13.
148. Domergue F, Spiekermann P, Lerchl J, Beckmann C, Kilian O, Kroth PG, et al. New insight into *Phaeodactylum tricornutum* fatty acid metabolism. Cloning and functional characterization of plastidial and microsomal delta12-fatty acid desaturases. Plant Physiol. 2003;131(4):1648–60.
149. Chi X, Zhang X, Guan X, Ding L, Li Y, Wang M, et al. Fatty acid biosynthesis in eukaryotic photosynthetic microalgae: identification of a microsomal delta 12 desaturase in *Chlamydomonas reinhardtii*. J Microbiol (Seoul, Korea). 2008;46(2):189–201.
150. Adarme-Vega TC, Lim DK, Timmins M, Vernen F, Li Y, Schenk PM. Microalgal biofactories: a promising approach towards sustainable omega-3 fatty acid production. Microb Cell Fact. 2012;11:96.
151. Cao Y, Cao Y, Zhao M. Biotechnological production of eicosapentaenoic acid: from a metabolic engineering point of view. Proc Biochem. 2012;47:1320–6.
152. Guedes AC, Amaro HM, Barbosa CR, Pereira RD, Malcata FX. Fatty acid composition of several wild microalgae and cyanobacteria, with a focus on eicosapentaenoic, docosahexaenoic and alpha-linolenic acids for eventual dietary uses. Food Res Int. 2011;44(9):2721–9.
153. Hamilton ML, Haslam RP, Napier JA, Sayanova O. Metabolic engineering of *Phaeodactylum tricornutum* for the enhanced

accumulation of omega-3 long chain polyunsaturated fatty acids. Metabol Eng. 2014;22:3–9.
154. Harwood JL, Guschina IA. The versatility of algae and their lipid metabolism. Biochimie. 2009;91(6):679–84.
155. Liang MH, Jiang JG. Advancing oleaginous microorganisms to produce lipid via metabolic engineering technology. Prog Lip Res. 2013;52(4):395–408.
156. Niu YF, Zhang MH, Li DW, Yang WD, Liu JS, Bai WB, et al. Improvement of neutral lipid and polyunsaturated fatty acid biosynthesis by overexpressing a type 2 diacylglycerol acyltransferase in marine diatom *Phaeodactylum tricornutum*. Mar Drugs. 2013;11(11):4558–69.
157. Chaturvedi R, Uppalapati SR, Alamsjah MA, Fujita Y. Isolation of quizalofop-resistant mutants of *Nannochloropsis oculata* (Eustigmatophyceae) with high eicosapentaenoic acid following N-methyl-N-nitrosourea-induced random mutagenesis. J App Phycol. 2004;16:135–44.
158. Gong Y, Guo X, Wan X, Liang Z, Jiang M. Characterization of a novel thioesterase (PtTE) from *Phaeodactylum tricornutum*. J Bas Microbiol. 2011;51(6):666–72.
159. Blatti JL, Beld J, Behnke CA, Mendez M, Mayfield SP, Burkart MD. Manipulating fatty acid biosynthesis in microalgae for biofuel through protein-protein interactions. PLoS ONE. 2012;7(9):e42949.
160. Radakovits R, Eduafo PM, Posewitz MC. Genetic engineering of fatty acid chain length in *Phaeodactylum tricornutum*. Metab Eng. 2011;13(1):89–95.
161. Gao Q, Wang W, Zhao H, Lu X. Effects of fatty acid activation on photosynthetic production of fatty acid-based biofuels in *Synechocystis sp*. PCC6803. Biotechnol Biofuels. 2012;5(1):17.
162. Ruffing AM, Jones HD. Physiological effects of free fatty acid production in genetically engineered *Synechococcus elongatus* PCC 7942. Biotechnol Bioeng. 2012;109(9):2190–9.
163. Miyake M, Takase K, Narato M, Khatipov E, Schnackenberg J, Shirai M, et al. Polyhydroxybutyrate production from carbon dioxide by cyanobacteria. App Biochem Biotechnol. 2000;84–86:991–1002.
164. Korkhovoi VI, Blium IB. Biodiesel from microalgae: ways of increasing effectiveness of lipids accumulation by genetic engineering methods. TSitologiia i genetika. 2013;47(6):30–42.
165. Li Y, Han D, Hu G, Dauvillee D, Sommerfeld M, Ball S, et al. Chlamydomonas starchless mutant defective in ADP-glucose pyrophosphorylase hyper-accumulates triacylglycerol. Metabol Eng. 2010;12(4):387–91.
166. Li Y, Han D, Hu G, Sommerfeld M, Hu Q. Inhibition of starch synthesis results in overproduction of lipids in *Chlamydomonas reinhardtii*. Biotechnol Bioeng. 2010;107(2):258–68.
167. Ramazanov A, Ramazanov Z. Isolation and characterization of a starchless mutant of *Chlorella pyrenoidosa* STL-PI with a high growth rate, and high protein and polyunsaturated fatty acid content. Phycol Res. 2006;54:255–9.
168. Ball S, Marianne T, Dirick L, Fresnoy M, Delrue B, Decq A. A *Chlamydomonas reinhardtii* low-starch mutant is defective for 3-phosphoglycerate activation and orthophosphate inhibition of ADP-glucose pyrophosphorylase. Planta. 1991;185(1):17–26.
169. Plumed MP, Villarejo, A., de los Rios, A., García-Reina, G, Ramazanov, Z. The CO_2 concentrating mechanism in a starchless mutant of the green unicellular alga *Chlorella pyrenoidosa*. Planta. 1996;200:28–31.
170. Song D, Fu J, Shi D. Exploitation of oil-bearing microalgae for biodiesel. Chin J Biotechnol. 2008;24(3):341–8.
171. Fan KW, Jiang Y, Faan YW, Chen F. Lipid characterization of mangrove thraustochytrid-*Schizochytrium mangrovei*. J Agric Food Chem. 2007;55(8):2906–10.
172. Yan J, Cheng R, Lin X, You S, Li K, Rong H, et al. Overexpression of acetyl-CoA synthetase increased the biomass and fatty acid proportion in microalga Schizochytrium. App Microbiol Biotechnol. 2013;97(5):1933–9.
173. Trentacoste EM, Shrestha RP, Smith SR, Gle C, Hartmann AC, Hildebrand M, et al. Metabolic engineering of lipid catabolism increases microalgal lipid accumulation without compromising growth. Proc Nat Acad Sci USA. 2013;110(49):19748–53.
174. Hsieh HJ, Su CH, Chien LJ. Accumulation of lipid production in *Chlorella minutissima* by triacylglycerol biosynthesis-related genes cloned from *Saccharomyces cerevisiae* and *Yarrowia lipolytica*. J Microbiol (Seoul, Korea). 2012;50(3):526–34.
175. Liu X, Sheng J, Curtiss R 3rd. Fatty acid production in genetically modified cyanobacteria. Proc Nat Aca Sci USA. 2011;108 (17):6899–904.
176. Magnuson K, Jackowski S, Rock CO, Cronan JE Jr. Regulation of fatty acid biosynthesis in *Escherichia coli*. Microbiol Rev. 1993;57(3):522–42.
177. Hu P, Borglin S, Kamennaya NA, Chen L, Park H, Mahoney L, Kijac A, Shan G, Chavarría KL, Zhang C, Quinn NW, Wemmerc D, Holman H-Y, Jansson C. Metabolic phenotyping of the cyanobacterium *Synechocystis* 6803 engineered for production of alkanes and free fatty acids. App Energy. 2013;102:850–9.
178. Chen CY, Chen YC, Huang HC, Huang CC, Lee WL, Chang JS. Engineering strategies for enhancing the production of eicosapentaenoic acid (EPA) from an isolated microalga *Nannochloropsis oceanica* CY2. Biores Technol. 2013;147:160–7.
179. Chen G, Qu S, Wang Q, Bian F, Peng Z, Zhang Y, et al. Transgenic expression of delta-6 and delta-15 fatty acid desaturases enhances omega-3 polyunsaturated fatty acid accumulation in *Synechocystis* sp. PCC6803. Biotechnol Biofuels. 2014; 7(1):32.
180. Xue Z, Sharpe PL, Hong SP, Yadav NS, Xie D, Short DR, et al. Production of omega-3 eicosapentaenoic acid by metabolic engineering of *Yarrowia lipolytica*. Nat Biotechnol. 2013;31 (8):734–40.
181. Xie D, Jackson EN, Zhu Q. Sustainable source of omega-3 eicosapentaenoic acid from metabolically engineered *Yarrowia lipolytica*: from fundamental research to commercial production. App Microbiol Biotechnol. 2015:1–12.
182. Liang Y, Maeda Y, Sunaga Y, Muto M, Matsumoto M, Yoshino T, et al. Biosynthesis of polyunsaturated fatty acids in the oleaginous marine diatom Fistulifera sp. strain JPCC DA0580. Mar Drugs. 2013;11(12):5008–23.