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Abstract	At present, functional foods are seen as a good alternative to maintain or even improve human health, mainly for the well-known correlation between diet and health. This fact has brought about a great interest for seeking new bioactive products of natural origin to be used as functional ingredients, being, nowadays,

one of the main areas of research in Food Science and Technology. Among the different sources that can be used to extract bioactives, algae have become one of the most promising. Algae have an enormous biodiversity and can be seen as natural factories for producing bioactive compounds since either by growing techniques or by genetic engineering approaches, they can improve their natural content of certain valuable compounds. In this book chapter, a revision about the different types of bioactives that have been described in algae is presented including compounds, such as lipids, carotenoids, proteins, phenolics, vitamins, polysaccharides, etc. Also, the modern green techniques used to achieve the selective extraction of such bioactives are presented and the methods for fast screening of bioactivity described.

Screening for Bioactive Compounds from Algae

Miguel Herrero, Jose A. Mendiola, Merichel Plaza, and Elena Ibañez

Abstract At present, functional foods are seen as a good alternative to maintain or even improve human health, mainly for the well-known correlation between diet and health. This fact has brought about a great interest for seeking new bioactive products of natural origin to be used as functional ingredients, being, nowadays, one of the main areas of research in Food Science and Technology. Among the different sources that can be used to extract bioactives, algae have become one of the most promising. Algae have an enormous biodiversity and can be seen as natural factories for producing bioactive compounds since either by growing techniques or by genetic engineering approaches, they can improve their natural content of certain valuable compounds. In this book chapter, a revision about the different types of bioactives that have been described in algae is presented including compounds, such as lipids, carotenoids, proteins, phenolics, vitamins, polysaccharides, etc. Also, the modern green techniques used to achieve the selective extraction of such bioactives are presented and the methods for fast screening of bioactivity described.

1 Bioactive Compounds and Functional Foods

The important economic, cultural, and scientific development of our society has strongly contributed to changes in life-style and food habits. For instance, highly caloric and unbalanced diets are commonly consumed in developing countries; this fact, together with a decrease in physical activity has raised the incidence of cardiovascular diseases, diabetes, obesity, etc. [41]. If we also consider the increasing life expectancies, it is easy to realize that different solutions should be found to reduce the expected health costs in a near future.

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One of the possible solutions are the so called functional foods. The concept of functional food as a mean to protect consumer's health was developed at the beginning of the 1980s in Japan, based on several scientific studies demonstrating the correlation between diet and a lower incidence of chronic diseases [3]. In 1993, the Ministry of Health and Welfare established a policy for "Foods for Specified Health Uses" (FOSHU) by which health claims of some selected functional foods were legally permitted and regulated [4]. In Europe, in the second half of the 1990s, a working group coordinated by the European Section of the International Life Science Institute (ILSI) and supported by the European Commission, was created to promote the action FUFOSE (Functional Food Science in Europe, IV Framework Program) to encourage the scientific study on functional foods. A definition of functional food as "the food that besides its nutritious effects, has a demonstrated benefit for one or more functions of the human organism, improving the state of health or well-being or reducing the risk of disease" [26] was established. In this definition, it is necessary to emphasize some new aspects: (a) the functional effect is different from the nutritious one; (b) the functional effect must be demonstrated satisfactorily; and (c) the benefit can consist in an improvement of a physiological function or in a reduction of risk of developing a pathological process. Besides, the functional foods need to be effective at the normal consumed doses and should have a presentation typical of a food product. At present, functional foods are regulated in the European Union by the guideline approved in December 2006 (Regulation (CE) 1924/2006 of the European Parliament and of the Council, December 20, 2006: nutrition and health claims made on foods). In this directive, the nutritional allegations and/or healthy properties of the new products are regulated, including their presentation, labeling, and promotion.

Considering this background, it is easy to understand the interest that functional foods have raised not only for consumers, but also for the food industry. Thus, we can consider that a new, enormous market for the food industry has been opened; as Sloan in 1999 already suggested: "foods for the not-so-healthy" [180].

But, how it is possible to convert a traditional food into a functional food? Again, there is not a single answer since many approaches can be used in order to improve the beneficial action of a certain food, ranging from more or less sophisticated biotechnological processes to several other processes to remove or increase the content of a specific compound. Many times, a functional food is obtained through the addition of a component or a series of ingredients that either are not present in the analogous conventional food or are present at lower concentrations. These ingredients are called functional ingredients and are mainly micronutrients, such as ω3 fatty acids, linoleic acids, phytosterols, soluble fiber (inulin and fructooligosaccharides, called prebiotics), probiotics (microorganisms able to improve the activity in the intestinal tract and the immune system), carotenoids, polyphenols, vitamins, etc., able to exert a specific healthy action into the organism [45, 179].

Algae can be found in nearly any aquatic and terrestrial habitat, showing a huge biodiversity and various morphologies ranging from phytoplankton species to large kelp [129]. Algae are photosynthetic organisms that possess reproductive simple structures; the number of algal species remains unknown although has been estimated

at between one and ten million [112] and, as mentioned, can exist from unicellular microscopic organisms (microalgae) to multicellular of great size (macroalgae). For instance, microalgae use light energy and carbon dioxide with higher photosynthetic efficiency than plants for the production of biomass [7, 113] and have been suggested as a source of biofuel production, to purify wastewater [123, 134], to extract high added value foods and pharmaceutical products, or as food for aquaculture [182].

In fact, algae are organisms that live in complex habitats sometimes submitted to extreme conditions (changes of salinity, temperature, nutrients, UV–Vis irradiation), thus, they have to adapt rapidly to the new environmental conditions to survive, producing a great variety of secondary (biologically active) metabolites, which cannot be found in other organisms [18]. Moreover, most of them are easy to cultivate, they grow rapidly (for many of the species) and there exists the possibility of controlling the production of some bioactive compounds either by manipulating the cultivation conditions or by using more sophisticated genetic engineering approaches. Therefore, algae and microalgae can be considered as genuine natural reactors being, in some cases, a good alternative to chemical synthesis for certain compounds. Therefore, considering the enormous biodiversity of algae and the recent developments in genetic engineering, investigations related to the search of new biologically active compounds from algae can be seen as an almost unlimited field, being this group of organisms one of the most promising sources for new products. In this sense, previous reports have suggested both, micro- and macroalgae as a very interesting natural source of new compounds with biological activity that could be used as functional ingredients [141, 142].

Moreover, another important aspect to be considered is the development of appropriate, fast, cost-effective, and environmental-friendly extraction procedures able to isolate the compounds of interest from these natural sources. In this chapter, green extraction techniques, such as supercritical fluid extraction (SFE) and pressurized liquid extraction (PLE) together with ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) are presented, and applications to algae bioactive's extraction are discussed. A revision about the different types of bioactives that have been described in algae is presented, including compounds such as lipids, carotenoids, proteins, phenolics, vitamins, polysaccharides, etc. In this chapter, a short description of methods for fast screening of bioactivity (mainly antioxidant activity) is included, considering chemical and biological methods. Finally, future research trends and research needs for the attainment of bioactives from algae are critically commented.

2 Green Extraction Techniques for Bioactive Compounds

Today, there is a wide range of classical or conventional extraction techniques that have been traditionally employed for the extraction of interesting compounds from natural matrices, such as algae. In this group, techniques such as Soxhlet, liquid–liquid

extraction (LLE), solid–liquid extraction (SLE), and other techniques based on the use of organic solvents are included. Although these techniques are routinely used, they have several well-known drawbacks; they are time consuming, laborious, they lack of automation and therefore are more prone to present low reproducibility, have low selectivity and/or provide low extraction yields. These shortcomings can be partially or completely overcome by using the newly developed advanced extraction techniques. This new kind of extraction techniques are characterized by being faster, more selective towards the compounds to be extracted, and also very important nowadays, these techniques are more environmentally friendly. In fact, by using the considered advanced extraction techniques, the use of toxic solvents is highly limited. In the next sections, the most important advanced extraction techniques that have been employed to extract bioactive compounds from algae are briefly described and commented.

2.1 Supercritical Fluid Extraction

SFE is based on the use of solvents at temperatures and pressures above their critical points. This technique has been already employed to extract a wide variety of interesting compounds from very different food-related materials [108], and algae are no exception [54]. One of the most valuable characteristics of SFE is the highly reduced (often to zero) employment of toxic organic solvents. In this sense, carbon dioxide is the most used supercritical solvent employed to extract bioactives from natural samples. In fact, CO_2 has a series of interesting properties for bioactives extraction; is cheap, its critical conditions are easily attainable (30.9°C and 73.8 bar), is an environmentally friendly solvent that, besides, is considered generally recognized as safe (GRAS) for its use in the food industry. When submitted to supercritical conditions, CO_2 presents a high diffusivity whereas its solvent strength and density can be highly modified by tuning the temperature and pressure applied. Another important characteristic of this technique, when using supercritical CO_2, is the possibility of attaining solvent-free extracts. Once the extraction procedure is finished, the depressurization of the system allows the gasification of the CO_2, remaining in the collector the compounds that were extracted from the matrix and solubilized in the CO_2 at high pressures. These properties are responsible for the great use of supercritical CO_2 for extraction of bioactive compounds.

Nevertheless, in spite of the potential of this technique, its usefulness will be related to the type of compounds to be extracted from the algae. Considering the low polarity of supercritical CO_2, SFE will be more suitable for the extraction of compounds with low polarity. In this regard, SFE using CO_2 has proven useful for the extraction of fatty acids [159], carotenoids from *Dunaliella salina* [66] and other microalgae [97], pigments from *Chlorella vulgaris* [82], or even interesting volatile compounds from the brown alga *Dictyopteris membranacea* [31], among other interesting applications. Supercritical CO_2 has also the advantage of obtaining a

quite "clean" extract when compared to other conventional extraction techniques. In fact, the selectivity obtained through the use of supercritical CO_2 will also allow the attainment of more purified extracts reducing to a great extent the amount of interfering compounds extracted from the complex algae matrix. However, if the extraction of more polar compounds is aimed, other strategies have to be devised. The main alternative in this case is the use of a given percentage of a modifier together with the supercritical fluid. This modifier (entrainer or cosolvent) typically is a polar organic solvent. When added to the supercritical fluid, this modifier will produce a change on the properties of the extracting mixture, allowing the collection of more polar compounds, increasing the polarity of the solvent used for the extraction and also the range of applications for SFE.

Several parameters are involved in the extraction of bioactives from algae by SFE. Among them, it is necessary to precisely control the effect of the extraction temperature, pressure, addition and, in that case, proportion and type of modifier, amount of sample to be extracted as well as its particle size and use of dispersing agents. The first parameters are more related to the solubility of the interesting compounds in the supercritical fluid, since changes on the extraction temperature and pressure will have a strong influence on the solvent properties, such as density. The type and proportion of modifier are also key factors in determining the solubility of the compound of interest in the supercritical fluid; in this sense, the most commonly employed organic solvent to extract bioactives from algae is ethanol in a range of 5–10% [127, 136]. Other modifiers, such as methanol [85] or acetone [82], have been also employed in some SFE algae applications, although the latter was shown to be less effective for pigments extraction from algae than ethanol [82]. Vegetable oils, notably olive oil, also demonstrated to be effective when added to supercritical CO_2 as modifiers or cosolvents in a proportion of 10%, for the extraction of the carotenoid astaxanthin from *Haematococcus pluvialis* [88]. In fact, in this application, the addition of 10% olive oil provided comparable results to those obtained using ethanol as cosolvent [88]. In contrast, the rest of parameters are more related to the efficiency of the extraction procedure. It is well known that the influence of the physical state of the sample on the outcome of the extraction, as well as its particle size. The crushing degree was a very significant factor in the extraction of carotenoids from *H. pluvialis* microalga [127]. It was demonstrated how an increase in the crushing procedure produced an enhancement in the carotenoid extraction yield. This effect could respond to an increase of the mass transfer rates as a consequence of the lower particle size as well as to the increase of carotenoids in the medium as a result of the disruption of cells in the heavier crushing procedure [127].

Although supercritical solvents have a diffusivity in the matrix higher than liquids, a decrease in the sample particle size generally produces an increase in the extraction yield obtained, mainly due to the increment in the contact surface between sample and solvent, thus increasing the mass transfer. Nevertheless, in some applications the use of dispersing agents (e.g., diatomaceous earth) as well as the employment of Hydromatrix in order to absorb the liquid portion from the sample can be useful.

2.2 Pressurized Liquid Extraction

PLE is another technique that, nowadays, is regarded as an advanced extraction technique, due to the advantages that presents over other traditional extraction mechanism. PLE is based on the use of high temperatures and pressures so that the solvent is maintained in the liquid state during the whole extraction procedure. As a result of the application of these particular conditions, faster extraction processes are obtained in which generally the extraction yield is significantly higher than that obtained using traditional extraction techniques, besides, using lower amounts of organic solvents. Moreover, most of the instruments used for PLE are automated, allowing the development of less labor intensive methods and improving reproducibility.

The principles governing this kind of extraction and providing the above mentioned characteristics are: (a) the mass transfer rate is improved as a result of the increment on the solubility of the compounds as a consequence of the increase of the extraction temperature; (b) under the PLE experimental conditions, the surface tension of the solvent is reduced, allowing a better penetration of the solvent into the sample matrix, increasing likewise the mass transfer; (c) the effect of the pressure theoretically could help to matrix disruption, increasing again the mass transfer rate.

Method development in PLE is by far easier than in SFE, since less parameters influencing the extraction should be considered. Once the solvent has been selected according to the nature of the compounds to be extracted, only two parameters are of significant importance: extraction time and extraction temperature. Although the extraction pressure could help to disrupt the matrix enhancing the mass transfer of the analytes contained on it, as it has been already mentioned, in practice, several reports have shown that the influence of this parameter is not significant once the pressure is high enough to maintain the solvent in the liquid state. The extraction temperature has to be optimized always keeping in mind the possible thermal degradation effects that might occur over the interesting extracted compounds. Although generally an increase in the temperature produces the subsequent increase in the extraction yield, for bioactive compounds, too high temperatures might lead to the degradation of these compounds. Therefore, this value should be carefully maximized just to the level in which the interesting compounds start to get degraded. On the other hand, the extraction time has to be minimum enough to have an adequate mass transfer. Longer extraction times would result on slower extraction procedures and could also favor the thermal degradation, once the solvent solution is saturated with analytes from the food matrix. Therefore, quite simple experimental designs, such as full factorial designs with two factors and three levels can be useful to optimize the bioactives PLE extraction conditions.

Compared to SFE, the possibility of choosing among a high number of solvents causes PLE to be more versatile in terms of polarity of the bioactive compounds to be extracted and thus, the solvent will be selected depending on their nature. However, this technique is considered by far less selective than SFE. Therefore, it is

important to keep in mind, that even if the extraction of the bioactives is attained, it would be possible to find other interfering compounds in the obtained extract. To avoid this problem, other steps can be included. For instance, an extraction step using hexane/acetone as solvent was performed before the PLE of phenolic compounds from several algae species using 80% methanol in water at 130°C for 20 min (two 10 min cycles) [132]. Ethanol has been selected to extract antioxidants from different species, such as *Synechocystis* sp. and *Himanthalia elongata* [143] or antimicrobial compounds from *H. pluvialis* [165]. Generally, the best extraction conditions in these applications were obtained at mild temperatures, around 100°C.

Moreover, PLE can be applied using a wide variety of extraction solvents, although GRAS extraction solvents, like ethanol, are most commonly used. When the extraction solvent is water, this technique is commonly called subcritical water extraction (SWE). The principles of extraction are the same, but in this case, another parameter has critical importance, the dielectric constant of water. This property of water is greatly modified with the increasing temperature when water is maintained in the liquid state. In fact, the value of dielectric constant of water (ε) can vary from 80 at room temperature to values around 25 when is submitted to temperatures of ca. 250°C. This value is similar to the one presented by some organic solvents at room temperature, such as ethanol or methanol, and thus, the use of SWE could be an alternative to the use of this type of solvents in some applications. This technique has been already used to explore the possibility of obtaining antioxidants from different microalgae species [52, 55]. However, the wide development of novel applications for the extraction of bioactives from algae by using SWE has not been fully explored so far.

2.3 Others

Ultrasound-assisted extraction (UAE) is also widely considered as an advanced extraction technique. This technique uses high-frequency sounds, usually higher than 16 kHz and a limited amount of solvent in order to produce an effective extraction of the compounds of interest in the solvent employed, increasing their mass transfer and solubility, by disrupting the food matrix being extracted. As in PLE, the selection of the suitable solvent for extraction by UAE will be made depending on the compounds of interest. For instance, a mixture of dichloromethane/methanol (2:1) was employed to extract lipids from microalgae using UAE [140]. For more polar compounds, such as chlorophylls, methanol was demonstrated as a more effective solvent [175]. This technique has the advantage of providing faster extraction processes compared to conventional techniques. UAE was compared to other solvent-based extraction of pigments and fatty acids from several algae samples. It was demonstrated that UAE was simple, allowed extraction of interesting compounds and did not produce alteration or breakdown products [197]. However, when this technique was directly compared to SFE for the extraction of carotenoids from *D. salina*, it was shown that SFE was more effective for the extraction of these low

polarity compounds, above all in terms of selectivity [98]. At certain conditions, in which a complex sample is being extracted containing the interesting compounds as well as other polar compounds, SFE was demonstrated to be more selective than UAE [98]. UAE has been also employed to extract polysaccharides derived from *Chlorella pyrenoidosa* [171].

When sonicating the samples for a given period of time, an increase in the temperature of the sample can be observed as a result of the vibration of the molecules. For this reason, considering that most of bioactives are thermally labile compounds, it is common to proceed in a temperature controlled environment. For instance, pigments and fatty acids were obtained from algae at −4°C using 35 kHz and 80 W for 90 min [197]. The use of temperatures below 4–5°C allows a better preservation of the extracted compounds, that otherwise, could be degraded.

The last advanced extraction technique also used for bioactives extraction from algae is MAE. In MAE, the sample is heated by using microwaves, at typical powers of 700 W for a short time. Compared to traditional extraction techniques, the use of microwaves allows the decreasing of extraction times significantly limiting also the amount of solvent needed. Again, the temperature will be an important parameter to be controlled. Once selected the extraction solvent for the extraction of bioactives from algae, the microwaves power as well as the extraction time has to be defined. Experimental designs can be useful in determining the best extraction conditions. For instance, response surface methodology was employed to optimize the MAE of astaxanthin from *H. pluvialis* [203]. By using this statistical approach, the microwave power (141 W), extraction time (83 s), solvent volume (9.8 mL), and number of extracting cycles (4 cycles) were optimized. At present, MAE has not been extensively applied to extraction of bioactives from algae, although given its success in the extraction of plant materials, it can be easily inferred the great possibilities for its application to algae samples.

3 Fast Screening for Bioactivity

In general terms, the bioactivity of algal and microalgal extracts can be tested using two big groups of techniques: chemical and biological methods. Since no universal method to test bioactivity exists, marine extracts are commonly evaluated by using several methods.

As will be seen in Sect. 4, most of the bioactive compounds that can be found in algae and microalgae have been described to possess antioxidant activity; thus, most of the chemical methods that will be explained in this section are directed to measure different parameters related to the antioxidant activity.

On the other hand, marine compounds have been associated with a high number of bioactivities (mainly pharmacological activities) that can be tested by biological or biochemical methods. In this sense, several reviews covering both general and specific subject areas of marine pharmacology have been published. This kind of review articles has been grouped by Mayer et al. [105] as: (a) general marine pharmacology;

(b) antimicrobial marine pharmacology; (c) cardiovascular pharmacology; (d) antituberculosis, antimalarial, and antifungal marine pharmacology; (e) antiviral marine pharmacology; (f) anti-inflammatory marine pharmacology; (g) nervous system marine pharmacology; and (h) miscellaneous molecular targets.

3.1 Chemical Methods

3.1.1 Antioxidant Activity

Interest in natural antioxidants for both health and improved food stabilization has intensified dramatically since the last decade of the twentieth century. Health applications have been stimulated by observations that free radicals and oxidation are involved in many physiological functions and cause pathological conditions. Natural antioxidants offer food, pharmaceutical, nutraceutical, and cosmetic manufacturers a "green" label, minimal regulatory interference with use, and the possibility of multiple actions that improve and extend food and pharmaceutical stabilization [168]. Determining antioxidant capacity has become a very active research topic, and a plethora of antioxidant assay methods are currently in use. Despite of it, there are no standard methods due to the sheer volume of claims and the frequent contradictory results of "antioxidant activities" of several products.

Reactive oxygen species which include superoxide anion ($O_2^{-\bullet}$, a free radical), the hydroxyl radical ($^{\bullet}OH$) and hydrogen peroxide (H_2O_2) are produced by ultraviolet light, ionizing radiation, chemical reaction, and metabolic processes. These reactive species may contribute to cytotoxicity and metabolic changes, such as chromosome aberrations, protein oxidation, muscle injury, and morphologic and central nervous system changes in animals and humans [34]. Effective antioxidants must be able to react with all these radicals in addition to lipids, so, consideration of multiple radical reactivity, in antioxidant testing, is critical.

In general terms, three big groups can be distinguished: chain reaction methods, direct competition methods, and indirect methods [154].

1. Among the *chain reaction methods* two approaches have been used: measuring the lipid peroxidation reactions or the kinetics of substrate oxidation.

   There are two modes of lipid peroxidation that may be used for testing. The first one is autoxidation, in which the process is progressing spontaneously, with self-acceleration due to accumulation of lipid hydroperoxide (LOOH). The kinetics of autoxidation is highly sensitive to admixtures of transition metals and to the initial concentration of LOOH. As a result, the repeatability of experiments based on the autoxidation is still a problem. The second, much more promising approach, is based on the use of the kinetic model of the controlled chain reaction. This mode offers to obtain reliable, easily interpretable, and repeatable data. This approach has been applied, among others, to test natural water-soluble antioxidants, microheterogeneous systems, micelles, liposomes, lipoproteins (basically low-density lipoprotein [LDL]), biological membranes, and blood plasma [154].

When choosing a substrate of oxidation, preference should be given to individual compounds. Among individual lipids, methyl linoleate, and linoleic acid seem to be the most convenient. These compounds are relatively cheap and their oxidation is quite representative of the most essential features of biologically relevant lipid peroxidation. The main disadvantage, when using them in biological materials, is that the extract must be free of the elected compound, as it is impossible to provide the identity of substrate. Besides, biologically originated substrates usually contain endogenous chain-breaking antioxidants (vitamin E, etc.), which can intervene in the testing procedure.

2. The *direct competition methods* are kinetic models, where natural antioxidants compete for the peroxyl radical with a reference-free radical scavenger:

   - β-Carotene bleaching: competitive bleaching β-carotene during the autoxidation of linoleic acid in aqueous emulsion monitored as decay of absorbance in the visible region. The addition of an antioxidant results in retarding β-carotene decay [114].
   - Free-radical induced decay of fluorescence of R-phycoerythrin: The intensity of fluorescence of phycoerythrin decreases with time under the flux of the peroxyl radical formed at the thermolysis of APPH (2,2′-azobis-2-methyl-propanimidamide) in aqueous buffer. In the presence of a tested sample containing chain-breaking antioxidants, the decay of PE fluorescence is retarded [147].
   - Crocin bleaching test: Crocin (strongly absorbent in the visible range) undergoes bleaching under attack of the peroxyl radical. The addition of a sample containing chain-breaking antioxidants results in the decrease in the rate of crocin decay [12].
   - Potassium iodide test: KI reacts with the AAPH-derived peroxyl radical with the formation of molecular iodine. The latter is determined using an automatic potentiometric titrator with sodium thiosulfate. In the presence of antioxidant-containing samples, the rate of iodine release decreases [154].

3. When the *indirect approach method* is applied, the ability of an antioxidant to scavenge some free radicals is tested, which is not associated to the real oxidative degradation, or effects of transient metals. For instance, some stable colored free radicals are popular due to their intensive absorbance in the visible region [154]. There are two ways for presenting results, as equivalents of a known antioxidant compound (i.e., Trolox Equivalent Antioxidant Capacity, TEAC) or as the concentration needed to reduce concentration of free radicals by 50% (EC_{50}).

   - DPPH$^{\bullet}$ test: It is based on the capability of stable-free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) to react with H-donors. As DPPH$^{\bullet}$ shows a very intensive absorption in the visible region (514–518 nm), it can be easily determined by the UV–Vis spectroscopy [13]. This method has been applied online with TLC [65] and HPLC [5] to determine antioxidant activity in different algae extracts.
   - ABTS test: The decay of the radical cation ABTS$^{+\bullet}$ (2,2′-azinobis(3-ethylbenzothiaziline-6-sulfonate) radical cation) produced by the oxidation of ABTS$^{+\bullet}$

caused by the addition of an antioxidant-containing sample is measured. $ABTS^{+\bullet}$ has a strong absorption in the range of 600–750 nm and can be easily determined spectrophotometrically. In the absence of antioxidants, $ABTS^{+\bullet}$ is rather stable, but it reacts energetically with an H-atom donor, such as phenolics, being converted into a noncolored form of ABTS [115].

- Ferric reducing antioxidant power (FRAP): The FRAP assay is based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} (Benzie and Strain 1996); if the reaction is coupled to the presence of some colored Fe^{2+} chelating compound like 2,4,6-trypyridyl-*s*-triazine, it can be measured spectrophotometrically.
- Cyclic voltammetry: The general principle of this method is as follows: the electrochemical oxidation of a certain compound on an inert carbon glassy electrode is accompanied by the appearance of the current at a certain potential; while the potential at which a cyclic voltammetry peak appears is determined by the redox properties of the tested compound, the value of the current is proportional to the quantity of this compound, in the presence of an antioxidant compound the signal will be lower [155].

3.2 *Biological Methods*

3.2.1 Antihelmintic, Antifungal, and Antibacterial Activity

In terms of antibacterial and antifungal activity, several compounds have been described in extracts from algal origin. Compounds like phenols, indoles, peptides, steroidal glycosides, terpenes, fatty acid, and so on. Basically, the method consists on letting the organism grow in the presence of the extract or compound. For example, Mendiola et al. [109] used a broth microdilution method to test the minimum inhibitory concentration (MIC) of *Spirulina* extracts on the growing of several bacteria and fungi. Tests were done in microwell plates, prepared by dispensing into each well culture broth plus inocula and 30 μL of the different extract dilutions. After incubation, the MIC of each extract was determined by visual inspection of the well bottoms, since bacterial growth was indicated by the presence of a white "pellet" on the well bottom. The lowest concentration of the extract that inhibited growth of the microorganism, as detected as lack of the white "pellet," was designated the MIC. The minimum bactericidal and fungicidal concentration was determined by making subcultures from the clear wells which did not show any growth.

Among antihelmintic compounds derived from algae, sesquiterpenes, like β-bisabolene, are the most actives. The most common method to measure its activity is to grow the helminths (worms, i.e., *Nocardia brasiliensis*) in the presence of the alga extract. For example, Davyt et al. [22] used tissue-culture 24-well plates. They prepared dilutions in DMSO for each compound, in order to obtain the desired concentration after the addition of 10 μL into each well. The percentage of dead worms was determined on day 5 and corrected by controls and compared with synthetic drugs.

3.2.2 Anticoagulant Activity

Polysaccharides, especially sulfated polysaccharides, are the main anticoagulant compounds isolated from algae and microalgae. Its activity is commonly measured providing the compound in vivo and measuring in vitro how coagulant factors are varied. For example, Drozd et al. [27] administered fucoidans (5 or 10 mg/kg) into the jugular vein of male Wistar rats, collected the blood and measure the inhibition of Xa factor (anti-Xa- or aHa-activity) and thrombin (anti-IIa or aIIa-activity). Specific activity was calculated in U/mg by comparison of optical density of the test and standard solutions during hydrolysis of chromogenic substrates.

3.2.3 Antiviral Activity

The antiviral family is one of the widest families of bioactive compounds isolated from marine sources, or at least one of the most studied. In this group, there are compounds like polysaccharides, terpenoids, proteins, sulfated flavones, and fatty acids. When measuring the antiviral activity, the general trend is to treat well-known mammal cells with the extract and then monitor the viral infection with the microscope. Huheihel et al. [63] used green monkey kidney cells (vero cells) treated with polysaccharides extracted from *Porphyridium* sp., the cell culture was treated with herpes simplex viruses. Each day, the cultures were examined for evidence of the cytopathic effect, defined as areas of complete destruction of cells or of morphologically modified cells and expressed as the percentage of damaged cells in the inspected fields.

But similar test can be done in vivo, Huheihel et al. [63] applying locally (eyes and mouth) *Porphyridium* extracts in rabbits and rats; later, the animals were exposed to the virus. Inflammatory effects, illness, and weight changes were recorded over a period of 4 weeks posttreatment.

3.2.4 Anti-inflammatory Activity

Inflammatory processes are related with several cardiovascular diseases and oxidative stress, therefore its study is of high interest. Among anti-inflammatory compounds from algal sources astaxanthine, terpenes, sterols, indols, and shikimate-derivatives have been described [105]. There is a huge amount of enzymes and secondary metabolites involved in inflammatory processes, but the general trend is to measure the expression of some of those metabolites and/or enzymes when cells involved in the inflammatory response are "activated." Leukocytes are among the most studied models; leukocyte migration has been shown to be one of the first steps in the initiation of an inflammatory/immune response and is essential for accumulation of active immune cells at sites of inflammation. The chemotaxis assay used to analyze the test material is designed to assess the ability of a test material to inhibit the migration of polymorphonuclear leukocytes (PMNs) toward a known chemotactic agent.

For example, polysaccharides from red microalga primarily inhibited the migration of PMNs toward a standard chemoattractant molecule and also partially blocked adhesion of PMNs to endothelial cells [101].

3.2.5 Toxicological Tests

It is well known that despite the bioactive (beneficial) compounds, several toxic compounds can be accumulated in algae and microalgae. Compounds like alkaloids, domoic acid, azaspiracid, brevetoxin, okadaic acid, pectenotoxin, or microcystins have been described.

Therefore, sometimes it is required to perform some toxicological tests mainly based in the mouse bioassay. Article 5 of a European Commission Decision dated 15 March 2002, laying down rules related to maximum permitted levels of certain biotoxins and methods of analysis for marine bivalve molluscs and other seafood states: "When the results of the analyses performed demonstrate discrepancies between the different methods, the mouse bioassay should be considered as the reference method." The basic procedure involves i.p. injection of an extract of the sample containing the toxin and observing the symptoms. A deeper review on toxicological analysis can be read in the book edited by Gilbert and Şenyuva "Bioactive compounds in Foods" [42].

4 Bioactive Compounds from Algae and Microalgae

Algae are important sources of various bioactive compounds with different physiological effects (toxic or curative) on human health. Many of them possess antioxidant, antimicrobial, and antiviral activities that are important for the protection of algal cells against stress conditions. The discovery of new analytical methods and techniques is important for the study of metabolites in algae and similar organisms with respect to their applications in pharmacology and the food industry [132].

4.1 Carotenoids

Carotenoids are prominent for their distribution, structural diversity, and various functions. More than 600 different naturally occurring carotenoids are now known, not including *cis* and *trans* isomers, all derived from the same basic C_{40} isoprenoid skeleton by modifications, such as cyclization, substitution, elimination, addition, and rearrangement. The different carotenoids have been isolated and characterized from natural sources as plants [43, 187], algae [142, 143], bacteria [183, 191], yeast [119], and fungi [70].

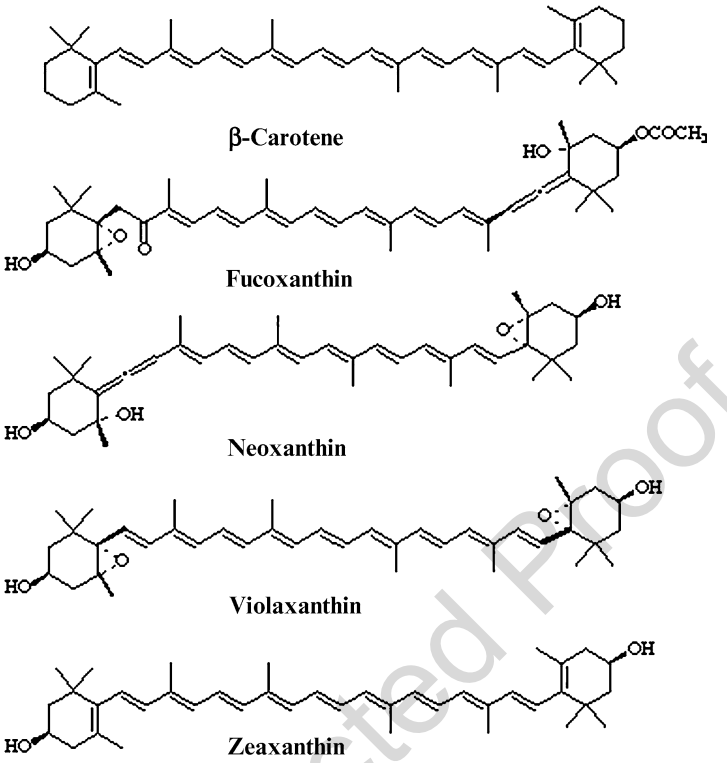

Fig. 1 Structures of the principal carotenoids in algae

Carotenoids play a key role in oxygenic photosynthesis, as accessory pigments for harvesting light or as structural molecules that stabilize protein folding in the photosynthetic apparatus. Carotenoids are powerful antioxidants. The beneficial effects of carotenoids have been well documented from the numerous clinical and epidemiological studies in various populations. Due to its high antioxidant activity, carotenoids have been proposed as cancer prevention agents [173], potential life extenders [88], and inhibitors of ulcer [74], heart attack, and coronary artery disease [151, 194].

All photosynthetic eukaryotes are able to synthesize lycopene, a C_{40} polyene, which is the precursor of two different carotenoid synthesis pathways, the β,ε-carotene and the β,β-carotene pathways [169]. Xanthophylls are oxidation products of carotenes; diversification of xanthophylls increases by the inclusion of allene or acetylene groups. Allenic and acetylenic carotenoids are highly represented in algae, and at least 30 different carotenoids have been identified in this group [169]. The distribution of carotenoids having different molecular structures or the presence of specific biosynthesis pathways can be an index for algae classification. For example, the major carotenoids that occur in seaweeds (Fig. 1) include β-carotene, lutein,

violaxanthin, neoxanthin, and zeaxanthin in green algae (Chlorophytes); α- and β-carotene, lutein, and zeaxanthin in red seaweeds (Rhodophytes) and β-carotene, violaxanthin, and fucoxanthin in brown algae (Phaeophytes).

Carotenoid composition of algae can present great variations mainly related to environmental factors, such as water temperature, salinity, light, and nutrients available. Most of the environmental parameters vary according to season, and the changes in ecological conditions can stimulate or inhibit the biosynthesis of several nutrients, such as carotenoids. For example, *D. salina* is a green microalga, well known for being one of the main natural sources of β-carotene. Under particular conditions, this microalga is able to produce β-carotene up to 14% of its dry weight. Moreover, the particular growing conditions able to maximize the production of β-carotene at industrial scale have been investigated [48–50, 84, 198, 206] (Mojaat 2008). Because β-carotene may play important roles in preventing degenerative diseases due to its associated antioxidant activity, different procedures have been studied, not only for the production of this compound but also for its extraction and isolation [66, 97, 106, 118]. The most widely employed technique has probably been SFE. The low polarity characteristics of the supercritical CO_2 make this solvent appropriate for the β-carotene extraction from this microalga [66, 97, 106, 118].

Other example is the green microalgae *H. pluvialis* that produces chlorophylls a and b and primary carotenoids, namely, β-carotene, lutein, violaxanthin, neoxanthin, and zeaxanthin, while it has the ability to accumulate, under stress conditions, large quantities of astaxanthin, up to 2–3% on a dry weight basis [150]. Using this carotenogenesis process, it undergoes different changes in cell physiology and morphology, giving as a result large red palmelloid cells [76, 204]. Astaxanthin is present in lipid globules outside the chloroplast, its functions in the cell include protection against light-related damage by reducing the amount of light available to the light-harvesting pigmented protein complexes. These pigments possess powerful biological activities, including antioxidant capacity [19], ulcer prevention [74] as well as immunomodulation and cancer prevention [130]. In fact, the extraction of astaxanthin has been thoroughly investigated. Different methods have been tested, including neat supercritical CO_2 [189] or supercritical CO_2 with different cosolvents [127], PLE [25, 67], MAE [203], direct extraction with vegetable oils (Kang et al. 2008) or solvents [75], or even treating cells with various solvents and organic acids at 70°C before acetone extraction, with the aim to facilitate the astaxanthin extraction from the thick cell wall without affecting the original astaxanthin esters profile [166].

Fucoxanthin is the most characteristic pigment of brown algae, and is also one of the most abundant carotenoids in nature [61], accounting for more than 10% of estimated total natural production of carotenoids [103]. Fucoxanthin is an oxygenated carotenoid that is very effective in inhibiting cell growth and inducing apoptosis in human cancer cells [60, 87]; it also has anti-inflammatory [172], antioxidant [158], antiobesity [99], and antidiabetic [100] properties.

4.2 Lipidic Fraction

The content and composition of algal lipids vary with species, geographical location, season, temperature, salinity, light intensity, or combination of these factors. In general, algae contain up to 1–3% of dry weight of lipids, being glycolipids the major lipid class in all algae, followed by neutral and phospholipids.

The major polar lipids that can be found in microalgae are monogalactosyl diacylglycerols (MGDGs), digalactosyl diacylglycerols (DGDGs), and phosphatidylglycerol (PG) [2]. Although these compounds, primarily MGDGs and DGDGs, have been known for more than 40 years, their importance has been recently raised by the description of their different, mainly anti-inflammatory, functional activities [15]. For example, glycol analogs of ceramides and of PG with antithrombotic and anti-inflammatory activities have been reported in cyanobacteria [2]. MGDGs and DGDGs contain a galactose linked to the sn-3 position of the glycerol backbone. These polar lipids are found in the thylakoid membrane of the cells. For instance, several polar lipids have been identified in *Spirulina platensis*, such as, four MGDGs, three PGs and two sulfoquinovosyl diacylglycerol [57], in *Croococcidiopsis* sp. [2], in *Sargassum thunbergii* [81], and *Phormidium tenue* [124] among others.

On the other hand, most of the alga's lipid content is made of polyunsaturated fatty acids (PUFAs) which accumulation also relies on environmental factors. For example, it is known that algae accumulate PUFAs when there is decrease in the environmental temperature [80]. In this sense, it has been described that tropical species contain less lipid (<1%) than cold water species (1.6%) [125].

PUFAs are essential nutrients for humans, and must be obtained from food. ω-3 and ω-6 long chain PUFAs are structural and functional components of cell membranes. The ω-3 to ω-6 ratio is closely matched, a factor that has been found to be important in balanced diet [176]. Likewise, these fatty acids are precursors of eicosanoids, which exert hormonal and immunological activity. This means ω-3 and ω-6 should be consumed in a balanced proportion, with the ideal ratio ω-6:ω-3 ranging from 3:1 to 5:1 [184].

The properties of the long-chain ω-3 fatty acids eicosapentaenoic acid (EPA) (ω-3 $C_{20:5}$) and docosahexaenoic acid (DHA) (ω-3 $C_{22:6}$) have been followed with considerable interest in the last few years. In particular, the vascular protective effects of long-chain ω-3 fatty acids are well documented [17, 170, 207]. Green algae show interesting levels of alpha linolenic acid (ω-3 $C_{18:3}$). The red and brown algae are particularly rich in fatty acids with 20 carbon atoms: EPA and arachidonic acid (ω-6 $C_{20:4}$).

S. platensis is a microalga belonging to the group of cyanobacteria (or blue-green algae) and is a natural source of DHA, which can account for up to 9.1% of the total fatty acids content [199].

Table 1 [133, 161] presents the typical composition of different fatty acids in algae. As can be seen, in all algae studied except *Undaria pinnatifida* and *Ulva lactuca* the single most abundant fatty acid was palmitic acid (which in *Phorphyra* sp. accounted for 63.19% of all fatty acids) while in *U. pinnatifida* the palmitic acid

t1.1 **Table 1** Fatty acids profile of different algae according to Sánchez-Machado et al. [161] and Ortiz et al. [133]

		Chlorophytes	Phaeophytes			Rhodophytes	
t1.2	Fatty acids	*Ulva lactuca*	*Himanthalia elongata*	*Undaria pinnatifida*	*Laminaria ochroleuca*	*Palmaria* sp.	*Porphyra* sp.
t1.3	$C_{14:0}$	1.14±0.22	5.85±0.35	3.17±0.31	4.97±0.20	13.76±0.61	0.53±0.21
t1.4	$C_{16:0}$	14.00±1.12	32.53±1.61	16.51±1.35	28.51±1.87	45.44±1.84	63.19±1.93
t1.5	$C_{16:1}$ ω7	1.87±0.21	2.79±0.25	3.70±0.88	5.62±0.71	5.26±0.63	6.22±0.70
t1.6	$C_{16:3}$ ω4	–	4.38±1.33	2.31±1.94	0.87±0.10	1.20±0.16	1.56±0.51
t1.7	$C_{18:0}$	8.39±0.12	0.68±0.15	0.69±0.08	0.34±0.14	1.28±0.12	1.23±0.10
t1.8	$C_{18:1}$ ω9	27.43±1.91	19.96±2.01	6.79±0.90	13.62±1.24	3.13±0.47	6.70±1.16
t1.9	$C_{18:1}$ ω7	–	–	–	–	2.08±0.68	1.29±0.68
t1.10	$C_{18:2}$ ω6	8.31±1.21	4.39±0.34	6.23±0.32	6.79±0.61	0.69±013	1.17±013
t1.11	$C_{18:3}$ ω3	4.38±0.31	8.79±0.71	11.97±1.75	5.15±0.71	0.59±0.26	0.23±0.16
t1.12	$C_{18:4}$ ω3	0.41±0.01	3.53±0.56	22.60±2.48	10.77±1.85	0.74±0.47	0.24±0.35
t1.13	$C_{20:1}$ ω9	4.21±0.50	–	–	–	0.20±0.10	4.70±0.26
t1.14	$C_{20:4}$ ω6	0.34±0.01	10.69±1.30	15.87±1.68	14.20±0.66	1.45±0.31	6.80±1.18
t1.15	$C_{20:4}$ ω3	–	0.88±1.80	0.70±0.14	0.54±0.90	0.14±0.03	0.07±0.02
t1.16	$C_{20:5}$ ω3	1.01±0.01	5.50±1.78	9.43±0.69	8.62±0.56	24.05±2.59	6.03±0.95
t1.17	Saturated fatty acid	23.53±1.46	30.06±2.11	20.39±1.73	33.82±2.21	60.48±2.58	64.95±2.24
t1.18	Monounsaturated	33.51±2.62	22.75±2.26	10.50±1.78	19.23±1.99	10.67±1.55	18.91±2.81
t1.19	PUFAs	14.45±1.55	38.16±7.84	69.11±9.01	46.94±4.58	28.86±3.94	16.10±3.31
t1.20	PUFAs ω6	8.65±1.22	15.08±1.64	22.10±2.00	20.99±1.27	2.14±0.45	7.97±1.31
t1.21	PUFAs ω3	5.80±0.33	18.70±4.84	44.70±5.05	25.08±3.21	25.52±3.34	7.20±1.48
t1.22	Ratio ω6/ω3	1.49	0.81	0.49	0.83	0.13	1.21

content (16.51%) was only exceeded by that of octadecatetraenoic acid (ω-3 $C_{18:4}$) (22.6%), and in *U. lactuca* the $C_{16:0}$ content (14.0%) was only exceeded by that of oleic acid (ω-9 $C_{18:1}$) (27.43%). However, all the seaweeds also contained the essential fatty acids linoleic acid (ω-6 $C_{18:2}$) and linolenic acid and the icosanoid precursors, arachidonic acid and EPA. Furthermore, the ω-6:ω-3 ratio, which the WHO currently recommends should be no higher than 10 in the diet as a whole, was at most 1.49 so that these algae may be used for reduction of ω-6:ω-3 ratio. Saturated fatty acid contents were higher in the red algae (*Palmaria* sp. and *Porphyra* sp.) than in the brown and green algae, and vice versa for relative total unsaturated fatty acid contents. Whereas in the red algae, C_{20} PUFAs were as a class 8–12 times more abundant than C_{18} PUFAs, in green algae the opposite occur while in brown algae these two classes of fatty acids were more or less equally abundant. Relative essential fatty acid contents were higher in brown and green algae than in red algae.

Several researchers have reported the fatty acid composition of total lipids of different species of *Sargassum*. Heiba et al. [47] studied the fatty acids present in four different *Sargassum* species in the Phaeophyta class that contained heptadecanoic acid ($C_{17:0}$), eicosanoic acid ($C_{20:0}$), eicosatrienoic acid (ω-3 $C_{20:3}$), and DHA. On the other hand, Khotimchenko [80], working with seven *Sargassum* species from different parts of the world, determined similar fatty acid compositions in all of them. The site of collection only seemed to affect palmitic acid ($C_{16:0}$) and C_{20} PUFA contents and was connected mainly with water temperature.

Aquatic plants possess conjugated fatty acids (CFA) with carbon chain length varying from 16 to 22, as natural constituents in their lipids; both trienes and tetraenes occur in aquatic plant lipids. There is not much information available on the literature, only a few reports on the occurrence of these conjugated polyenes in *Tydemania expeditionis*, *Hydrolithon reinboldii* [69], *Ptilota* [205], *Acanthophora* [8], and *Anadyomene stellata* [6] have been published. Various enzymes in aquatic plants are thought to be responsible for the formation of conjugated trienes/tetraenes endogenously. The enzymes responsible for the formation of CFA can be grouped into three main categories of conjugases, oxidases, and isomerases. Hideki and Yuto [58] studied the selective cytotoxicity of eight species of marine algae extracts to several human leukemic cell lines. It has been reported recently that conjugated PUFA, such as conjugated EPA, conjugated AA, and conjugated DHA, prepared by alkali isomerization had profound cytotoxic effects against human cancer cell lines [102].

Besides fatty acids, unsaponifiable fraction of algae contain carotenoids (see Sect. 4.1), tocopherols (see Sect. 4.5), and sterols. The distribution of major sterol composition in macroalgae has been used for chemotaxonomic classification. Recent biological studies have demonstrated that sterols and sterol derivatives possess biological activities. Currently, phytosterols (C_{28} and C_{29} sterols) are playing a key role in nutraceutic and pharmaceutical industries because they are precursors of some bioactive molecules (e.g., ergosterol is a precursor of vitamin D_2, also used for the production of cortisone and hormone flavone and has some therapeutic applications to treat hypercholesterolemia). Phytosterols have also been shown to lower total and LDL cholesterol levels in human by inhibiting cholesterol absorption from

the intestine [37]. High serum concentrations of total or LDL cholesterol are major risk factors for coronary heart disease, a major cause for morbidity and mortality in developed countries. In addition to their cholesterol lowering properties, phytosterols possess anti-inflammatory and anti-atherogenicity activity and may possess anticancer and antioxidative activities [37].

From a chemotaxonomic point of view, literature data show that major sterols in red algae are C_{27} compounds and cholesterol occur in substantial amount. It is generally the primary sterol. Desmosterol and 22E-dehydrocholesterol are present in high concentrations and may even be the major sterols in any red algae.

Sterol content in green algae is similar to higher plants, and also contains large amounts of cholesterol. But in green algae, the dominant sterol seems to vary within the order and within the family.

In brown algae, the dominant sterol is fucosterol and cholesterol is present only in small amounts.

Fucosterol content in *H. elongata* and *U. pinnatifida* was 1,706 μg/g of dry weight and 1,136 μg/g of dry weight, respectively, as demonstrated by Sánchez-Machado et al. [162]. Mean desmosterol content in the red algae ranged from 187 μg/g for *Palmaria* sp. to 337 μg/g for *Porphyra* sp. Cholesterol, in general, was present at very low quantities, except in *Porphyra* sp. that can contain up to 8.6% of the total content of sterols as cholesterol [162].

Sterol content determined in red alga *Chondrus crispus* showed that the main sterol was cholesterol (>94%), containing smaller amounts of 7-dehydrocholesterol and stigmasterol and minimum amounts of campesterol, sitosterol, and 22-dehydrocholesterol [188].

According to the investigation carried out by Kapetanovic et al. [77], the sterol fractions of the green alga *Codium dichotomum* and the brown alga *Fucus virsoides* contained practically one sterol each, comprising more than 90% of the total sterols (cholesterol in the former and fucosterol in the latter). The main sterols in the green alga *U. lactuca* were cholesterol and isofucosterol, while in the brown algae *Cystoseira adriatica*, the principal sterols were cholesterol and stigmast-5-en-3 beta-ol, while the characteristic sterol of the brown algae, fucosterol, was found only in low concentration [77]. However, fucosterol was the major sterol present in *Cystoseira abies-marina* (96.9%), containing low concentration of 24-methylenecholesterol (1.1%), brassicasterol (1.2%), and cholesterol (0.7%) [120].

4.3 Proteins

The protein content in algae can be as high as 47% of the dry weight [35], but these levels vary according to the season and the species. The protein content of brown algae is generally low (5–15% of the dry weight), whereas higher protein contents are recorded for green and red algae (10–30% of the dry weight). Except for brown algae *U. pinnatifida* which has a protein level between 11 and 24% (dry weight) [35]. Higher protein level were recorded for red algae, such as *Porphyra tenera*

t2.1 **Table 2** Amino acid profile of different algae according to Dawczynski et al. [23] (g/16 g N)

t2.2 Amino acids	*Porphyra* sp.	*Undaria pinnatifida*	*Laminaria* sp.	*Hizikia fusiforme*
t2.3 *Essential amino acids*				
t2.4 Histidine	2.6±0.4	2.5±0.3	2.2±0.4	2.6±0.4
t2.5 Isoleucine	3.1±0.5	4.1±0.3	2.7±0.9	4.0±0.4
t2.6 Leucine	5.5±0.9	7.4±0.6	4.9±1.7	6.7±0.6
t2.7 Lysine	4.9±0.9	5.6±0.4	3.9±1.4	3.1±0.3
t2.8 Methionine	1.8±0.7	1.7±0.5	0.9±0.2	1.6±0.1
t2.9 Phenyl alanine	3.3±0.4	4.7±0.3	3.2±1.0	4.6±0.4
t2.10 Tyrosine	3.4±2.1	2.9±0.5	1.7±0.5	2.8±0.4
t2.11 Threonine	5.3±0.8	4.4±0.6	3.5±0.6	4.1±0.5
t2.12 Tryptophan	0.7±0.1	0.7±0.1	0.5±0.5	0.4±0.0
t2.13 Arginine	5.9±0.4	5.2±0.2	3.3±1.1	4.5±0.3
t2.14 Cysteine	1.2±0.2	0.9±0.2	1.2±0.3	0.9±0.1
t2.15 Valine	5.2±1.0	5.2±0.5	3.8±1.0	4.9±0.5
t2.16 *Nonessential amino acids*				
t2.17 Asparagine/aspartate	8.5±1.0	8.7±1.1	12.5±2.8	9.1±1.0
t2.18 Glutamine/glutamate	10.2±2.6	14.5±3.2	23.8±7.5	18.7±2.4
t2.19 Serine	4.0±0.5	4.0±0.4	3.3±0.6	3.7±0.3
t2.20 Glycine	5.1±1.3	5.1±0.7	4.0±1.1	4.8±0.5
t2.21 Alanine	6.2±2.2	4.7±0.6	5.7±2.8	4.3±0.4
t2.22 Proline	3.5±1.0	3.6±1.6	3.1±1.1	3.8±0.4
t2.23 Taurine	4.3±2.1	0.1±0.1	0.3±0.2	0.6±0.2

709 (33–47% of dry mass) [35] or *Palmaria palmata* (8–35 of dry mass) [121]. These
710 levels are comparable to those found in soybean.

711 There are studies about the variation of protein content of marine algae as a func-
712 tion of the seasonal period [1, 39]. Higher protein levels were observed during the
713 end of the winter period and spring whereas lower amounts were recorded during
714 summer.

715 The in vivo digestibility of algal protein is not well documented, and available
716 studies about their assimilation by humans have not provided conclusive results.
717 However, several researchers have described a high rate of alga protein degradation
718 in vitro by proteolytic enzymes. For instance, the relative digestibility of alkali-
719 soluble proteins from *P. tenera* is higher than 70% [38]. On the other hand, some
720 compounds limiting the digestibility of alga proteins, such as phenolic compounds
721 or polysaccharides, have been described. Studies performed on brown algae show
722 the strong inhibitory action of soluble fiber on in vitro pepsin activity and their
723 negative effects on protein digestibility [59].

724 Typical amino acid composition of different species of algae is outlined in Table 2
725 according to Dawczynski et al. [23]. The quality of food protein depends on its
726 essential amino acids. These algae present high concentration of arginine, valine,
727 leucine, lysine, threonine, isoleucine, glycine, and alanine, although the predomi-
728 nant amino acids are glutamine and asparagine. Glutamine and asparagine exhibit
729 interesting properties in flavor development, and glutamine is the main responsible
730 in the taste sensation of "Umami."

The concentration of essential amino acids, such as, threonine, valine, isoleucine, leucine, phenyl alanine, lysine, and methionine, are higher in *U. pinnatifida* than in *Laminaria* sp. *U. pinnatifida* has higher concentrations of Lysine that has *Hizikia fusiforme* and *Laminaria* sp. has higher concentrations of Cysteine than has *U. pinnatifida.* Interestingly, taurine is not a typical component of traditional European food and taurine content represents a nutrient feature which is characteristic of red algae, such as *Phorphyra* sp. Taurine is detected at low concentrations in brown algae varieties.

In general, algae possess proteins that have a high nutritional value since they contained all the essential amino acids in significant amounts (see Table 2).

The organoleptic characteristic of algae are principally due to their free amino acid profile [126], which in turn depends on environmental factors in its culture grounds [44]. Generally, the free amino acid fraction of algae is mainly composed of alanine, aminobutyric acid, taurine, ornithine, citrulline, and hydroxyproline [89].

Other proteins present only in red and blue-green algae are phycobiliproteins (phycocyanin in blue-green algae, phycoerythrin in red algae), a group of protein involved in photosynthesis. Purified phycobiliproteins can have several uses, such as cosmetics, colorants in food, and fluorescent labels, in different analytical techniques [33, 138]. These proteins are characterized by having a tetrapyrrolic pigment, called phycobilin, covalently attached to their structure. Important medical and pharmacological properties, such as hepatoprotective, anti-inflammatory, and antioxidant properties [9, 10, 156], have been described and are thought to be basically related to the presence of phycobilin. Besides, phycobiliproteins might have an important role in different photodynamic therapies of various cancerous tumors and leukemia treatment [157]. Different works have been aimed to the selective extraction and analysis of the phycobiliproteins from algae, such as Herrero et al. [53] and Simó et al. [174], that identified the two subunits of each protein, namely allophycocyanin-α, allophycocyanin-β, c-phycocyanin-α, and c-phycocyanin-β, from *S. platensis*. In the red microalga *Porphyridium* spp., the red-colored pigment phycoerythrin [62, 195] has been described.

4.4 Polysaccharides and Dietary Fibers

Algae contain large amounts of polysaccharides, notably cell wall structural polysaccharides that are extruded by the hydrocolloid industry: alginate from brown algae, carrageenans, and agar from red algae. Edible algae contain 33–50% total fibers, which is higher than the levels found in higher plants. Other minor polysaccharides are found in the cell wall: fucoidans (from brown algae), xylans (from certain red and green algae), ulvans (from green algae), and cellulose (which occur in all genera, but at lower levels than found in higher plants). Algae also contain storage polysaccharides, notably laminarin (β-1,3 glucan) in brown algae and floridean starch (amylopectin-like glucan) in red algae [16]. Most of these polysaccharides are not digested by humans and can be regarded as dietary fibers.

t3.1 **Table 3** Dietary fiber contents of sea vegetables, seaweed by-products, and land
t3.2 plants (according to Mabeau and Fleurence [95])

t3.3 Source	Fiber (% dry weight) Soluble	Insoluble	Total
t3.4 Phaeophytes			
t3.5 *Undaria pinnatifida*	30.0	5.3	35.3
t3.6 *Hizikia fusiforme*	32.9	16.3	49.2
t3.7 *Himanthalia elongate*	25.7	7.0	32.7
t3.8 *Laminaria digitala*	32.6	4.7	37.3
t3.9 Chlorophytes			
t3.10 *Ulva lactuca*	21.3	16.8	38.1
t3.11 *Enteromorpha* spp.	17.2	16.2	33.4
t3.12 Rhodophytes			
t3.13 *Porphyra tenera*	17.9	6.8	34.7
t3.14 *Kappaphycus*	41.5	29.2	70.7
t3.15 High plants			
t3.16 Apple	5.9	8.3	14.2
t3.17 Cabbage	16.8	17.5	34.3

772 Water soluble and water insoluble fibers have different physiological effects associated.
773 Insoluble fiber primarily promotes the movement of material through the digestive
774 system, thereby improving laxation. Therefore, insoluble fiber can increase feelings
775 of satiety [178]. The majority of insoluble fiber is fermented in the large intestine,
776 supporting the growth of intestinal microflora, including probiotic species. Soluble
777 fiber can help to lower blood cholesterol and regulate blood glucose levels [190].
778 The insoluble fibers include cellulose, hemicellulose, and lignin; the soluble fibers
779 include the oligosaccharides, pectins, β-glucans, and galactomanan gums.
780 Table 3 shows, for comparison, the dietary fiber content in some sea vegetables,
781 seaweed by-products and plants [95]. As can be seen, algae contain slightly more
782 fiber than cabbage, although the amounts consumed in the diet would be lower. The
783 red alga *Kappaphycus* shows the highest levels of total fiber (70.7% dry weight).
784 Algae contain sulfated polysaccharides which possesses important functional
785 properties. For instance, fucoidans (soluble fiber), polysaccharides containing sub-
786 stantial percentages of L-fucose and sulfate ester groups, are constituents of brown
787 algae. For the past decade, fucoidans isolated from different brown algae have been
788 extensively studied due to their varied biological activities, including anticoagulant
789 and antithrombotic, antiviral, antitumoral and immunomodulatory, anti-inflammatory,
790 blood lipids reducing, antioxidant and anticomplementary properties, activity against
791 hepatopathy, uropathy, and renalpathy, gastric protective effects, and therapeutic
792 potential in surgery [94]. Compared to other sulfated polysaccharides, fucoidans are
793 widely available from various kinds of cheap sources, so more and more fucoidans
794 have been investigated in recent years as natural sources of drugs or functional ingre-
795 dients. Fucoidans had been isolated of different brown algae, such as, *U. pinnatifida*

[92], *Laminaria angustata* [83], *Sargassum stenophyllum* [29], *H. fusiforme* [93], *Adenocytis utricularis* [146], and *Cystoseira canariensis* [149].

Red algae contain water soluble sulfated polysaccharide galactan, agar, and carrageenans. One of the most studied marine-sulfated homopolysaccharides class, together with fucoidans, are the sulfated galactans. In general, the sulfated galactans are polymers of α-L- and α-D- or β-D-galactopyranosyl units. Unrelated to their natural biological roles as components of the biological wall, the sulfated galactans show important and potent pharmacological actions. These include antiviral, antitumoral, immunomodulation, antiangiogenic, anti-inflammatory, anticoagulant, and antithrombotic properties [144]. Their beneficial effects on the cardiovascular system are the most studied and exploited clinical actions, especially due to the serious need for new antithrombotic drugs as a consequence of the continuously increasing incidence of thromboembolic diseases [145]. Sulfated galactans have been identified in several red algae, among others, *Grateloupia elliptica*, *Sinkoraena lancifolia*, *Halymenia dilatata*, *Grateloupia lanceolata*, *Lomentaria catenata*, *Martensia denticulata*, *Schizymenia dubyi*, and *C. crispus* [91].

Agar extracted from species, such as *Gracilaria* and *Gelidium*, is composed of a mixture of the sulfated galactans D-galactose and 3,6-anhydro-α-L-lactose. The term agarose and agaropectin represent an oversimplification of the agar structure.

Carrageenan is a generic name for a group of linear-sulfated galactans, obtained by extraction from numerous species of marine red algae. These carbohydrates consist of a linear structure of alternating disaccharide repeat units containing 3-linked β-D-galactopyranose and 5-linked α-D-galactopyranose.

Porphyrans, the sulfated polysaccharides making up the hot-water soluble portion of the cell wall, are the main components of *Porphyra*. Structurally, they have a linear backbone of alternating 3-linked β-D-galactosyl units and 4-linked α-L-galactosyl 6-sulfate or 3,6-anhydro-α-L-galactosyl units. In a former study, the content of ester sulfate in porphyran extracted from *Porphyra haitanensis* was measured ranging from 16 to 19% and showing generic antioxidant activity [202]. Several investigations of the structure and function of porphyrans isolated from different species have been undertaken [122, 201]. Although the chemical components and structures show great variation, porphyrans have also been shown to have immunoregulatory and antitumor activities [128, 135].

On the other hand, green algae, such as those of the genera *Ulva* and *Enteromorpha*, contain sulfated heteropolysaccharides in their mucilaginous matrix [72]. Sulfated polysaccharides extracted from the green algae belonging to Ulvales (*Ulva* and *Enteromorpha*) are ulvan. Ulvan is a heteropolysaccharide, mainly composed of rhamnose, xylose, glucose, glucuronic acid, iduronic acid, and sulfate, with smaller amounts of mannose, arabinose, and galactose. The mainly repeating disaccharide units are (β-D-Glcp A-(1→4)-α-L-Rhap 3S) and (α-L-ldop A-(1→4)-α-L-Rhap 3S) [139]. Most of the recent work on Ulvales cell wall polysaccharides focused on ulvan as it display several physicochemical and biological features of potential interest for food, pharmaceutical, agricultural, and chemical applications. Ulvans have been shown to have antioxidant [148], antitumor [104], and antihyperlipidemic [139] activities.

4.5 Vitamins

As marine algae can carry on photosynthesis, they are able to synthesize all vitamins that high plants produce. The vitamin profile of algae can vary according to algal species, season, alga growth stage, and environmental parameters. The edible algae (especially of *Porphyra* spp.) contain large amounts of water-soluble vitamin C and B complex, and the fat-soluble vitamin A and E [71]. Algae are a good source of pro-vitamin A (see Sect. 4.1).

Algae provide a worthwhile source of vitamin C. The levels of vitamin C average 500–3,000 mg/kg of dry matter for the green and brown algae, which are comparable to concentration in parsley, blackcurrant, and peppers; whereas the red algae contain vitamin C levels of around 100–800 mg/kg [16]. Vitamin C is of interest for many reasons: it strengthens the immune defense system, activates the intestinal absorption of iron, controls the formation of conjunctive tissue and the protidic matrix of bony tissue, and also acts in trapping free radicals and regenerating vitamin E [16].

Brown algae contain higher levels of vitamin E (23–412 mg/kg of dry matter) than green and red algae (8 mg/kg of dry matter). *H. elongata* presents high levels of α-tocopherol as demonstrated by Sánchez-Machado et al. [160]; for example, the content of α-tocopherol in *H. elongata* dehydrated (33 μg/g dry weight) was considerably higher than *H. elongata* canned (12.0 μg/g dry weight), which clearly indicates the important effect of the processing on this compound. The highest levels of vitamin E in brown algae are observed in Fucaceae (e.g., *Ascophyllum* and *Fucus* sp.), which contain between 200 and 600 mg of tocopherols/kg of dry matter [96]. The red microalga *Porphyridium cruentum* also presents high levels of tocopherols as demonstrated by Durmaz et al. [30]; for example, the contents of α- and γ-tocopherols were 55.2 and 51.3 μg/g dry weight, respectively. These tocopherols (vitamin E) are lipid-soluble antioxidants that are considered essential nutrients because of their ability to protect membrane lipids from oxidative damage [193]. Vitamin E has effect in the prevention of many diseases, such as atherosclerosis, heart disease, and also neurodegenerative diseases, such as multiple sclerosis [68, 73], thus also making it a very interesting functional compound. Generally, brown algae contain α-, β-, and γ-tocopherols, while green and red algae contain only α-tocopherol [16]. Mendiola et al. studied the possible use of SFE to obtain fractions enriched with vitamin E from *S. platensis* [110].

Algae are also an important source of B vitamins; for instance, algae contain vitamin B_{12}, which is particularly recommended in the treatment of the effects of aging, of chronic fatigue syndrome and anemia. Algae are also one of the few vegetables sources of vitamin B_{12}. *U. lactuca* can provide this vitamin, in excess of the recommended dietary allowances for Ireland fixed at 1.4 μg/day, with 5 μg in 8 g of dry foodstuff [36]. *Spirulina* is the richest source of B_{12} and the daily ingestion of 1 g of *Spirulina* would be enough to meet its daily requirement [196]. This may provide an alternate source of vitamin B_{12} for vegetarians or vegans.

On a dry matter basis, thiamine (vitamin B_1) content ranges from 0.14 μg/g in dried *H. elongata* to 2.02 μg/g in dried *Porphyra*, and riboflavin (vitamin B_2)

content varies between 0.31 μg/g in canned *H. elongata* to 6.15 μg/g in dried *Porphyra* [163]. The amount of folate (as folic acid or vitamin B_9) in the algae studied by Rodríguez-Bernaldo de Quirós et al. [153] (*H. elongata*, *Laminaria ochroleuca*, *Palmaria* spp., *U. pinnatifida*, and *Porphyra* spp.) ranged from 61.4 to 161.6 μg/100 g of dry matter.

In conclusion, the algae have an original vitamin profile, which might complement the vitamin profiles of land vegetables.

4.6 Phenolic Compounds

Phenols are an important group of natural products with antioxidant and other biological activities. These compounds play an important role in algal cell defense against abiotic and biotic stress. Several authors have recently published results regarding the total phenol content and antioxidant activity of algae [40]. Cinnamic acid esters (*n*-butyl 3,5-dimethoxy-4-hydroxycinnamate and isopropyl 3,5-dimethoxy-4-hydroxycinnamate) and methyl 3,4,5-trihydroxybenzoate were studied using 1H and 13C NMR in brown algae *Spatoglossum variabile* [46]. Some of the first polyphenols found in algae (*Fucus* and *Ascophyllum* spp.) were phlorotannins. They are formed from the oligomeric structures of phloroglucinol (1,3,5-trihydroxybenzene) [137]. Also, some flavanone glycosides have been found even in fresh water algae [86].

The main bioactivity associated to phenolic compounds is antioxidant activity, which is also the main bioactivity of algal and microalgal phenolics ~~(Kumar 2008)~~. Duan et al. [28] have demonstrated that antioxidant potency of crude extract from red algae (*Polysiphoma urceoiata*) correlated well with the total phenolic content. Strong correlation also existed between the polyphenol content and DPPH radical scavenging activity of a seaweed (*H. fusiformis*) extract [177]. Using electron spin resonance spectrometry and comet assay, Heo et al. [51] found that phenolic content in seaweeds could raise up to 1,352 μg/g on dry weight basis. The content and profile of phenolic substances in marine algae vary with the species. In marine brown algae, a group of polymers called phlorotannins comprises the major phenolic compounds [20], such as fucols, phlorethols, fucophlorethols, fuhalols, and halogenated and sulfited phlorotannins. Takamatsu et al. [186] showed that bromophenols isolated from several red marine algae exhibited antioxidant activities. These findings suggest that phlorotannins, the natural antioxidant compounds found in edible brown algae, can protect food products against oxidative degradation as well as prevent and/or treat free radical-related diseases ~~(Kumar 2008)~~.

Some algal phenolic compounds have been associated with anti-inflammatory activity, such as rutin, hesperidin, morin, caffeic acid, catechol, catechin, and epigallocatechin gallate, whose have been identified in *Porphyra* genus. Kazłowska et al. [79] have studied recently the phenolic compounds in *Porphyra dentata*, they identified catechol, rutin, and hesperidin in crude extract using HPLC-DAD. They demonstrated that the crude extract and the phenolic compounds inhibited the

production of nitric oxide in LPS-stimulated RAW 264.7 cells. Their results indicate that catechol and rutin, but not hesperidin, are primary bioactive phenolic compounds in the crude extract to suppress NO production in LPS-stimulated macrophages via NF-κB-dependent iNOS gene transcription. Data also explained the anti-inflammatory use and possible mechanism of *P. dentata* in iNOS-implicated diseases.

4.7 Bioactive Volatiles

In another chapter of the present book, volatile compounds from algae and microalgae are studied as an energy production source. Biogeneous hydrocarbons of the marine system, alkenes (mono, di, and cyclic) were originated from algae. One characteristic of crude oils that distinguishes them from biogeneous hydrocarbons is their content in cyclo alkenes and aromatic compounds [32]. But hydrocarbons are not the only volatile compounds that can be found in algae and microalgae. In fact, there is a huge number of secondary metabolites with proved antimicrobial and therapeutic activities while some of these volatile compounds have been also related to climate modifications.

When attacked by herbivores, land plants can produce a variety of volatile compounds that attract carnivorous mutualists. Plants and carnivores can benefit from this symbiotic relationship, because the induced defensive interaction increases foraging success of the carnivores, while reducing the grazing pressure exerted by the herbivores on the plants. Steinke et al. [185] reviewed whether aquatic plant use volatile chemical cues in analogous tritrophic interactions.

In general, naturally produced volatile and semivolatile compounds play an essential role in the survival of organisms for chemical defense and food gathering, but high amounts of volatile compounds could produce tremendous environmental actions. Marine algae produce several classes of biogenic gases, such as nonmethane hydrocarbons, organohalogens, ammonia and methylamines, and dimethylsulfide. These gases can transfer to the air, affect atmospheric chemistry, and are climatically important. Grazing increases dimethylsulfide and ammonia concentrations, and it is possible that other environmentally relevant volatiles are also produced during this process.

Other compounds produced by seaweeds with high importance for environment are halogenated hydrocarbons. Stratospheric ozone depletion and volatile-halogenated compounds are strongly connected with each other since the discovery that a massive loss of ozone in the polar stratosphere is catalyzed by halogen radicals derived from chlorocarbons and chlorofluorocarbons. Furthermore, so far unknown natural sources of volatile organohalogens may also contribute to a further destruction of the ozone layer. Marine macroalgae species from the polar regions were investigated [90] for their importance as natural sources of volatile halogenated compounds released into the biosphere. Several different halogenated C_1 to C_4 hydrocarbons were identified and their release rates determined. Although, at present,

marine macroalgae are apparently not the major source on a global scale, they may become more important in the future due to the influence of changing abiotic factors, such as photon fluence rate, nutrient concentration, temperature, and salinity on the formation of volatile organohalogens.

The release of volatile compounds with defensive functions has been studied in many algae, for example the brown alga *Dictyota menstrualis* [21]. Although the amphipod *Ashinaga longimana* preferentially consumes the alga *D. menstrualis*, its feeding rates can be reduced significantly by high concentrations of diterpenoid dictyols (dictyol E, pachydictyol A, and dictyodial) produced by the alga. The pattern of variation in the chemical defenses of some seaweed species suggests herbivore-induced increases of chemical defenses may be responsible for intraspecific variation in chemical defenses. For example, seaweeds from areas of coral reefs where herbivory is intense often produce more potent and higher concentrations of chemical defenses than plants from habitats where herbivory is less intense. Their findings suggested that seaweeds are not passive participants in seaweed-herbivore interactions, but can actively alter their susceptibility to herbivores in ecological time. Induced responses to herbivory help explain both spatial (i.e., within-thallus, within-site, and among-site) and temporal variation in the chemical defenses of the algae.

As seen above, macroalgae produce volatiles with defensive functions against herbivores, but microalgae also produce defensive volatile compounds. In this sense, it is common in many microalgae to share the ecological niche with bacteria and other microorganism. Therefore, the defensive compounds secreted by microalgae possess antibacterial, antifungal or antiprotozoal activity. The nature of these compounds is highly varied. Microalgae have been screened for potential antimicrobial activity, which have been attributed to different compounds belonging to a range of chemical classes, including indoles, terpenes, acetogenins, phenols, fatty acids, and volatile-halogenated hydrocarbons [105]. For example, pressurized ethanol and supercritical CO_2 extracts of microalgae *D. salina* were studied for their antibacterial activity against *Escherichia coli* and *S. aureus* and for their antifungal activity against *Candida albicans* and *Aspergillus niger* [56, 111]. In the broth microdilution assay, a high antimicrobial activity against *C. albicans*, *E. coli*, and *S. aureus* was observed but not against *A. niger*. In this work, a GC-MS analysis was performed to associate the antimicrobial activity found, it was concluded that antimicrobial activity of *D. salina* extracts could be linked to the presence of terpenic (β-cyclocitral and α and β-ionone) and indolic (methyl-1H-indole derivative) compounds, Fig. 2.

Terpenoids from algae have also been associated with antiviral activity, for example the above mentioned *D. menstrualis* produces a terpenoid able to inhibit HIV-1 reverse transcriptase as demonstrated by Souza et al. [181]; or terpenoid derived from plastoquinone that produces *Sargassum* sp., which acts in the lipid oxidation chain and inhibit cytomegalovirus growing [64].

Short chain fatty acids from microalgae are also volatile compounds associated with antibacterial activity. Santoyo et al. [165] tested, using the broth microdilution assay, extracts obtained from the red hematocysts without flagella (red phase) of

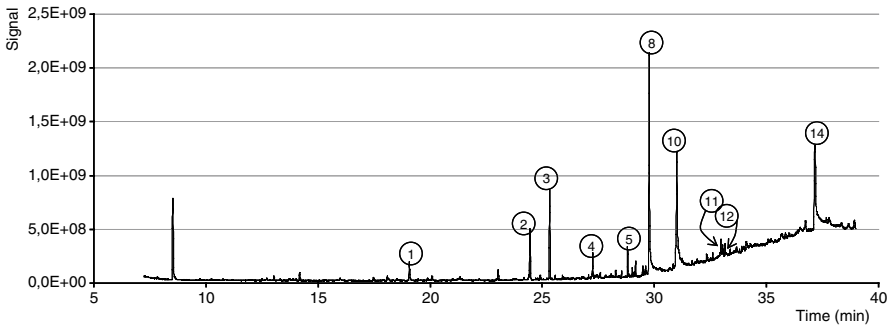

Fig. 2 GC-MS chromatogram of the volatile fraction of *Dunaliella salina* extract [111]. (1) 3,3-Dimethyl-2,7-octanedione; (2) β-ionone; (3) 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone; (4) 4-oxo-β-ionone; (5) neophytadiene; (6) nerolidol; (7) 9-hexadecanoic ethyl ester; (8) hexadecanoic acid; (9) phytol; (10) 9,12,15-octadecatrienoic acid methyl ester; (11) 1H-indole derivative; (12) hexadecanoic acid monoglyceride; (13) neophytadiene derivative; (14) vitamin E. Reprinted with permission from the *Journal of Food Protection*. Copyright held by the International Association for Food Protection, Des Moines, IA, USA

H. pluvialis microalga. In this work, it was concluded that the presence of short chain fatty acid (butanoic, hexanoic) highly inhibited the growing of gram positive and negative bacteria.

4.8 Other Bioactive Compounds

Seaweeds are known to be high in mineral content. More than 30% of the dry weight of marine algae is ash which contains various kinds of minerals, as they are bathed in the rich seawater. Some of the minerals are necessary for our health while some are toxic in varying degrees. Most of the macroalgae have high Ca, Mg, P, K, Na, and Fe contents [116], as can be seen in Table 4.

In comparison with higher plants, their outstanding feature is their high iodine content (Kumar 2008). Seaweeds are the best natural sources of biomolecular dietary I. Some seaweeds contain 1,000 times as much iodine as found in a marine fish like cod. Seaweeds provide di-iodotyrosin (I_2T) which is precursor to essential thyroid hormones thyroxine (T4) and triiodothyronine (T3) [14].

The mineral content in general is highly dependant on the environmental growing conditions (season, temperature, physiological state, geographic variations…). For example, in a recent study of *Porphyra* and *Laminaria* from France, Spain, Korea, and Japan [152] it was found, by using ICP-MS, that seaweeds from Korea and Japan tended to display the highest concentrations of Pb and Cd. In contrast, Spanish and French samples showed the highest levels of some microelements essential to human nutrition. Moreover, *Porphyra* presented

Table 4 Mineral content of some edible seaweeds [116]

Seaweed	Na[a]	K[a]	Mg[a]	Ca[a]	P[a]	Fe[b]	Zn[b]	Cu[b]	Mn[b]	Cr[b]	B[b]
Chlorella	10.4	11.0	3.53	2.30	19.2	1,185	24.7	6.21	77.8	1.38	27.5
Spirulina	10.1	14.9	4.76	2.96	12.6	1,480	59.2	7.26	240	1.08	33.0
Arame	12.0	14.5	6.55	6.79	0.78	63.4	27.2	4.30	3.94	0.77	37.0
Hijiki	16.2	54.5	6.85	6.49	1.02	56.4	16.2	2.02	6.20	0.55	117
Kombu	27.1	90.9	6.72	5.74	4.76	73.8	18.2	1.64	4.67	0.71	89.5
Kombu-Kelp	21.2	48.7	5.61	4.52	2.35	76.4	19.3	1.95	3.90	0.43	87.5
Wakame	62.6	64.8	12.0	4.94	6.04	70.9	22.5	3.41	6.94	0.40	69.0
Wakame-instant	74.9	1.49	9.43	5.31	3.52	304	50.7	3.07	11.4	0.93	33.0
Dulse	22.8	105	3.46	2.08	4.97	717	37.0	4.60	27.5	0.98	52.0
Korzický čaj	20.8	20.4	11.4	52.8	0.60	283	16.4	4.70	20.0	8.01	107
Nori	8.55	26.0	40.6	5.72	2.02	1,833	19.4	15.8	360	4.90	69.5

[a]Results expressed in mg/kg dry weight
[b]Results expressed in μg/kg dry weight

higher concentrations of most elements (Cd, Co, Cr, Mo, Ni, Pb, Sb, Se, and V), except for As, than *Laminaria*.

However, the linkage of certain minerals with anionic polysaccharides (alginate, agar, or carrageenan) might limit the absorption and extraction of these minerals. In such cases, mineral availability is a function of the type of linkage between the polysaccharide and the mineral. For instance, the weakness of the linkages between polysaccharides and iodine allows rapid release of this element. In contrast, the strong affinity of divalent cations (particularly Ca^{2+}) for carboxylic polysaccharides (alginates) probably limits the availability of associated minerals. From a nutritional standpoint, this high affinity might be compensated by the high mineral contents of seaweeds [95].

Other compounds with proven bioactivity are those related with photosynthesis, mainly pigments such as chlorophylls, carotenoids or proteins like opsins. Among them chlorophylls are the most wide spread compounds. Chlorophylls and their intermediate metabolites have proved its contribution to antioxidant and antimicrobial activities. For example, in supercritical CO_2 extracts of *S. platensis*, chlorophyll-*a*, pheophytin-a, pheophytin-a O-allomer, and pyropheophytin-a were detected by LC-MS/MS among the contributors to antioxidant activity measured by DPPH radical scavenging method [107]. On the other hand, phytol was detected by GC-MS among the bactericidal compounds present in *D. salina* extracts [111], as can be observed in Fig. 2, being all of them secondary metabolites of chlorophylls.

Certain alkaloids have been isolated from seaweeds. Among the many chemical classes present in plant species, alkaloids stand out as one of major importance in the development of new drugs, because they possess a wide variety of chemical structures and have been identified as responsible for many of the pharmacological properties of medicinal plants. Caulerpin, a bisindole alkaloid, was isolated from the green alga *Caulerpa racemosa* in 2009 [24]. This alkaloid showed low toxicity and a variety of important biological activities already described in the literature,

among which it is important to mention the antitumor, growth regulator and the plant root growth stimulant properties. De Souza et al. isolated caulerpin from lipoid extract of *C. racemosa* and its structure was identified by spectroscopic methods, including IR and NMR techniques and demonstrated in vivo and in vitro its anti-nociceptive and anti-inflammatory activities [24].

Microalgae have been also studied in the search of alkaloids, in this sense most of this research has been conducted to identify toxins [78]. The non-sulfated alkaloid toxins of freshwater cyanobacteria (anatoxins and saxitoxin) are all neurotoxins. The sulfated polysaccharides, C-toxins and gonyautoxins are also neurotoxins, but the sulfated alkaloid cylindrospermopsin blocks protein synthesis with a major impact on liver cells. Some marine cyanobacteria also contain alkaloids (lyngbyatoxins, aplysiatoxins) which are dermatoxins (skin irritants), but have also been associated with gastroenteritis and more general symptoms such as fever [78]. Several freshwater bloom forming cyanobacterial genera, including Anabaena, Aphanizomenon, Oscillatoria, and Cylindrospermum, produce the neurotoxin, anatoxin-a, an alkaloid with a high toxicity to animals [117].

5 Conclusions and Future Outlooks

In this book chapter, we presented some of the bioactive compounds that can be obtained from algae (macro- and microalgae) with potential use as functional food ingredients. The description did not attempt to be exhaustive since, considering the huge biodiversity of algae and the strong influence of growing conditions on bioactive formation, the list of compounds and combination could be countless. On the other hand, we try to give an overview of the enormous possibilities of algae as natural reactors able to synthesize a myriad of compounds of different polarities and with different physiological effects on human health. Many of these compounds can be major components, such as proteins, lipids, and carbohydrates and other minor components (metabolites) generated to protect algal cells against stress conditions. Most of them are useful for the food industry as macronutrients (fiber, proteins, etc.) while others have an enormous future as functional ingredients to prevent or even improve the health status of a human being.

In this chapter, we also presented new technologies to extract valuable compounds from algae, these processes have in common their "green" label, the possibility of improving the efficiency through process optimization, the removal of toxic solvents, the improved cost efficiency and the enhancement of selectivity and isolation steps. Several examples are described in the text demonstrating the usefulness and the advantages of such processes compared to conventional extraction ones. But, this step cannot be considered isolated but integrated in a more holistic concept of what should be a sustainable process considering algae as raw materials.

In this sense, we can think about algae (mainly microalgae) as (1) a sustainable source of mass and energy, since their processing meets the requirements for energy efficiency (transformation, growing biomass [164]; (2) a supply of clean energy for

the future if overproduction of oil is obtained that can be lately used for large-scale biodiesel production [112, 192]; (3) an efficient CO_2 sequestrant for greenhouse gas emissions control (Kyoto Protocol) [167, 200]; and (4) a valuable source of bioactives [11, 131].

If we are able to think about a whole process involving the optimization of all these steps: efficient production of biomass using CO_2 formed by combustion of fossil fuels in thermoelectric power plants, extraction of valuable bioactives using environmentally friendly processes to obtain high added value products that, on the other hand, leave intact residues, and process of oily fraction of biomass to produce biofuels, we will be able to work toward a sustainable, efficient, and economically viable process with many important positive implications for the economy, the environment and the human health. But, to reach this goal, it is mandatory to work with multidisciplinary teams involving scientists with expertise from phycology, molecular biology, agronomy, chemical engineering, food science and technology, environmental chemistry, economics, and so on.

Other nondirect benefits from this sustainable process are: the recovery of lands unsuitable for agricultural purposes, since the requirements for algae are less demanding, the advancement of genetic engineering basic studies, since more knowledge is needed to select and manipulate the most convenient strains and genes to overproduce the substances of interest, and a more efficient use of energy and sunlight. Working on sustainable processes is one of the best ways of investing in our future and in our planet's future.

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Author Queries

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Queries	Details Required	Author's Response
AU1	Please provide complete details for the reference Benzie and Strain 1996; Mojaat 2008; Kang et al. 2008; Kumar 2008 to be added to the reference list.	
AU2	Please provide complete details for reference [41]	

The reference Benzie & Strain has been deleted since has no relation with the text, the others have been changed by their corresponding numbers

More details about ref [41] has been included:
Geslain-Lanéelle C (2006) Summary report: EFSA Conference on Nutrition and Health Claims, 8-10 November 2006, Bologna, Italy ISBN: 978-92-9199-063-4