# Extraction of value-added compounds from microalgae

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## 19.1 Introduction

Microalgae are known worldwide by their application as bioremediation players due to their outstanding capacity to sequester carbon dioxide ($CO_2$) from the atmosphere, and as raw materials considering the large variety of bioactive compounds in their constitution. Microalgae (mainly *Dunaliella salina*, *Chlorella* sp., and *Spirulina platensis*) were firstly used as food additives or functional food, recently reaching the status of "superfood of the future" (http://www.businessinsider.com/algae-is-the-superfood-of-the-future-2014-6). Their production is recognized as much more interesting from the industrial point of view due to their rich biochemical composition. Actually, microalgae are recognized as rich raw materials since they are composed of a large plethora of bioactive compounds, namely pigments (like carotenoids and chlorophylls), proteins, polysaccharides, and long fatty acids, all of which have been applied over the years in different industries, including cosmetics, animal feed, human food, and energy. Behind the fact that the microalgae production requires simple conditions (of temperature, nutrients, and light) to produce large amounts of different value-added bioactive compounds, the microalgae production can be carried out during the entire year, allowing for high yields of biomass production and bioactive compounds accumulation.

Despite the high economical value of some of the ingredients accumulated in microalgae cells, their commercialization has still not reached its maximum. This fact is normally attributed to the high costs of the extraction and purification processes being applied to date. These high costs are normally related to the complexity of the processes that use large amounts of organic solvents, with a high environmental footprint or that use more sophisticated equipment and specialized human resources, making the compounds' profitable commercialization impossible. In this context, this chapter aims to present the oldest and newest processes of extraction and purification being developed to extract and purify different classes of compounds composing microalgae. Moreover, some of the most recent extractive approaches as well as

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the commercial impact of the main classes of bioactive compounds considering their main sectors of application are evaluated.

## 19.2 Extraction of carotenoids

Carotenoids are organic pigments belonging to the tetraterpenes class consisting of eight isoprene units occurring in chloroplasts and chromoplasts. Like chlorophylls, carotenoids (the most common structures being fucoxanthin, astaxanthin, lutein, zeaxanthin, and β-carotene) are also part of the process of photosynthesis (Anon, 2014), absorbing in the regions of blue, green, and violet light in the visible spectrum of light and reflecting the wavelengths of yellow, red, and orange (Anon, 2014). This class of compounds has been focus of attention in recent years; however, their industrial application is still deficient, mainly due to the high costs of the extraction and purification processes being applied. The normal process to purify carotenoids is described by their extraction from the solid biomass followed by the purification step, in which carotenoids are isolated from other contaminant compounds. The cell wall disruption from the microalgae is one of the most important tasks included in the extraction of carotenoids from biomass. The set of techniques/technologies/processes is extensive (Günerken et al., 2015; Kim et al., 2015) and can be classified as (1) mechanical or (2) non-mechanical techniques, and these can be used individually or combined. Since the cell wall of microalgae is a multilayered structure acting as a physical barrier to solvent input, it is the main item responsible for the isolation of the intracellular core of the cell, in which carotenoids are concentrated. Thus, the understanding of the cell wall disruption mechanisms is important and has been well reviewed in recent years (Günerken et al., 2015; Kim et al., 2015). After the adequate cellular disruption, the extracts rich in carotenoids need to be refined or purified. The number of techniques being applied with the purification purpose is somewhat high: examples are the solid-liquid extraction (SLE) (Hosokawa et al., 1999) with or without mechanical processes associated, like the Soxhlet extraction (Kim et al., 2012); the subcritical and supercritical fluid extraction (SFE) (Herrero et al., 2006; Guedes et al., 2013); the microwave-assisted extraction (MAE) (Pasquet et al., 2011); the ultrasound-assisted extraction (UAE) (Fan et al., 2015); the pulsed electric field-assisted extraction; the enzyme-assisted extraction (Wijesinghe and Jeon, 2012); and more recently, the extraction with surfactants, switchable solvents, and ionic liquids (for more details, see Section 19.7).

One of the methodologies most reported in the literature about the extraction of carotenoids is the SLE, normally using solvents as the extractive media, which are generally put in direct contact with the biomass (Xia et al., 2013). This methodology can use organic solvents (Rodrigues et al., 2015; Günerken et al., 2015). The most common organic solvents investigated are methanol (Foo et al., 2015; Pereira et al., 2015), ethanol and acetone (Amaro et al., 2015), ethyl acetate, n-hexane (Kim et al., 2012), and their mixtures (Rodrigues et al., 2015; Singh et al., 2015) separately or in sequential steps (Hosokawa et al., 1999). Despite the production of extracts rich in carotenoids, some purification may be needed, depending on their final application. Chromatography is the purification method most used when organic solvents are applied as solvents.

Other methods such as SFE, subcritical dimethyl ether, and pressurized liquid extraction (PLE) were already indicated as promising extraction techniques, namely

those based in compressed fluids (Goto et al., 2015; Guedes et al., 2013). The authors claim that it is possible to increase the amount of carotenoids being extracted to 450 mg/g of astaxanthin recovered using ethanol as a cosolvent, considerably reducing the extraction times (from 24–48 h to 2 h, respectively) (Herrero and Ibáñez, 2015). Another example (Fan et al., 2015) reflected the combination of ultrasound techniques with subcritical fluid extraction in which the lutein yield of extraction was practically doubled (124.01 mg/100 g crude extract when compared with the Soxhlet extraction 54.64 mg/100 g crude extract).

## 19.3 Extraction of chlorophylls

About 1.2 billion tons of chlorophyll (*a* and *b*) are produced annually on the planet (Humphrey, 2004). Chlorophyll is commonly preferred as a natural coloring agent due to its alluring green color although it is very susceptible to weak acids, oxygen, and light (Cubas et al., 2008). As a source of chlorophyll, microalgae are one of the most relevant alternatives found in terrestrial plants. Most algae cultured under optimum conditions were reported containing about 4% (dry weight) of chlorophyll (Chen and Jiang, 1999). Its extraction can be performed using solvent extraction, namely UAE, SFE, and MAE, in the same way as described for carotenoids (Hosikian et al., 2010). Considering these extraction techniques, some conditions, such as solvent type, pressure, temperature, efficiency of the contact biomass-solvent, and time of extraction (Simpson and Wells, 2000) have been evaluated (Table 19.1).

The solvent extraction techniques involve a SLE (with or without the Soxhlet assistance) to remove the pigments from the biomass followed by a liquid-liquid extraction, in which the different compounds removed from the microalgae cells are isolated and further purified. For the first step of SLE, the most common solvents being used are methanol, ethanol, acetone, and/or dimethylformamide as pure compounds or as aqueous solutions. Also in the chlorophylls extraction, some authors studied different cell disruption methods aiming at the increase of the extraction yields. The most common techniques of cell disruption being evaluated are the grinding, homogenization, ultrasound, and sonication of the cells that considerably improve the effectiveness of chlorophyll extraction in the presence of organic solvents (Jeffrey et al., 1997; Macías-Sánchez et al., 2009; Schumann et al., 2005). As an example, Simon and Helliwell (1998) demonstrated that with sonication, methanol has removed three times more chlorophyll *a* when compared with the use of 90% of acetone. Recently, some other studies have been developed on the use of UAE, SFE, and MAE to improve the process sustainability, namely by maintaining the extraction yields but at the same time trying to decrease the solvent consumption and/or the time of extraction (Hosikian et al., 2010) as described in more detail in Section 19.7.

## 19.4 Extraction of polysaccharides

Extracellular polysaccharides from microalgae are considered as structurally diverse polysaccharides, which justifies their large plethora of applications (Mishra and Jha, 2009). In general, the extracellular polysaccharides from microalgae have called the attention of the scientific community for their therapeutic application mainly based on

**Table 19.1** **Studies on chlorophyll extraction from microalgae**

| Algea species | Extraction method and conditions | Key results | Reference |
|---|---|---|---|
| Marine diatoms<br>• *Skeletonema costatum* strain 1 to 5<br>• *Rhizosolenia setigera*<br>• *Chaetoceros affinis*<br>• *Chaetoceros didymus*<br>• *Chaetoceros socialis*<br>• *Melosira nummuloides*<br>• *Nitzschia closterium*<br>• *Asterionella japonica*<br>• *Phaeodactylum tricornutum*<br>Raphidophycean<br>• *Heterosigma akashiwo* | Solvent extraction<br>• N-dimethyl formamide<br>• 90% acetone (v/v) | N-dimethyl formamide was found more efficient than acetone | Suzuki and Ishimaru (1990) |

| | | | |
|---|---|---|---|
| Green alga<br>• *Dunaliella primolecta* | | | |
| Green microalga<br>• *Chlorella* sp. | Solvent extraction<br>• Pure methanol<br>• 95% ethanol (v/v) | Chlorophyll extraction with methanol was carried out up to 10 times faster than with ethanol | Miazek and Ledakowicz (2013) |
| Green microalgae<br>• *Stichococcus* sp.<br>• *Chlorella* sp. | Solvent extraction<br>• 90% aqueous acetone (v/v)<br>• Pure dimethyl formamide | The most efficient extraction solvent was dimethyl formamide<br>Mechanical homogenization improved extraction by up to 20% | Schumann et al. (2005) |
| *Dunaliella salina* | Ultrasound-assisted extraction<br>• Dimethyl formamide<br>• Methanol<br>Supercritical fluid extraction<br>• Carbon dioxide | Dimethyl formamide was found to outstrip methanol and ultrasound-assisted extraction overwhelmed supercritical fluid extraction in terms of chlorophyll extraction yield | Macías-Sánchez et al. (2009) |
| *Selenastrum obliquus* | Probe sonication<br>Bath sonication<br>Tissue grinding<br>Maceration by mortar and pestle<br>• Methanol<br>• Acetone | Using methanol, a probe sonicator was more efficient in chlorophyll a extraction than the other extraction methods | Simon and Helliwell (1998) |

*Continued*

**Table 19.1 Continued**

| Algea species | Extraction method and conditions | Key results | Reference |
|---|---|---|---|
| *Chlorella vulgaris* | Ultrasound-assisted extraction<br>• Methanol (100% and 80%)<br>• Ethanol (100% and 80%)<br>• Acetone (100% and 80%) | The optimum extraction conditions were defined as extraction temperature, 61.4°C; extraction time, 78.7 min; and ethanol volume, 79.4% in case of ultrasonic power of 200 W | Kong et al. (2014) |
| *Synechococcus* sp. | Supercritical fluid extraction<br>• Carbon dioxide | The most appropriate operating conditions to obtain the best yield in the extraction of chlorophyll a were 500 bar and 60°C | Macías-Sánchez et al. (2007) |
| *Nannochloropsis gaditana* | Supercritical fluid extraction<br>• Carbon dioxide<br>Solvent extraction<br>• Methanol | The maximum chlorophyll extraction yield was found that at a temperature of 60°C is 400 bar<br>The solvent extraction with methanol was superior to supercritical fluid extraction | Macías-Sánchez et al. (2005) |
| *Scenedesmus obliquus* | Supercritical fluid extraction<br>• Carbon dioxide<br>• Ethanol (co-solvent) | The yields of chlorophylls increased slightly with pressure but decreased with temperature and $CO_2$ flow rate<br>The chlorophyll extraction yield increased in presence of ethanol | Guedes et al. (2013) |

| | | | |
|---|---|---|---|
| Phytoplankton | Solvent extraction<br>• Methanol (90%)<br>• Ethanol (90% and 100%)<br>• Dimethyl formamide | DMF is superior to all the other solvents used, and cell lysis improves extraction in all cases | Jeffrey et al. (1997) |
| *Dunaliella tertiolecta*<br>*Cylindrotheca closterium* | Cold soaking<br>Hot soaking<br>Ultrasound assisted extraction<br>Microwave-assisted extraction<br>Vacuum microwave-assisted extraction<br>• Acetone | Chlorophyll extraction yields highly depend on temperature that was demonstrated by cold and hot soaking<br>Microwave-assisted extraction did not damage chlorophyll a when a short time and weak irradiation were applied<br>Chlorophyll extraction yield obtained vacuum microwave–assisted extraction was lower than that obtained by microwave-assisted extraction | Pasquet et al. (2011) |

their antiviral and antibacterial bioactivities, but also for their antioxidant, antiinflammatory and immunomodulatory, antitumor, antilipidemic, antiglycemic, anticoagulant, antithrombotic, biolubricant, and antiadhesive properties (Raposo et al., 2014a). Other uses have been tested for the extracellular polysaccharides produced by cyanobacteria, which include the improvement of the soil capacity to retain water, the bioremediation of heavy metals/radionuclides, and the elimination of solid matter from contaminated waters (Bender and Phillips, 2004). Due to the importance of polysaccharides for certain sectors of industry, their recovery from the microalgae is actually relevant. In this sense, several authors have been working on the extracellular polysaccharide extraction and purification as described in Table 19.2.

**Table 19.2 Extraction methods of extracellular polysaccharides from different microalgae species with the purification methods used recorded**

| **Extracellular polysaccharides from microalgae** | | | |
|---|---|---|---|
| ***Microalgae* sp.** | **Extraction method** | **Purification** | **References** |
| *Gyrodinium impudicum* KG03 | Centrifugation followed by ethanol precipitation and cetyltrimethylammonium bromide reprecipitation. The pellet collected was resuspended in NaCl precipitated with ethanol and dialyzed | Gel permeation chromatography | Bae et al. (2006) |
| *Porphyridium* sp. | Centrifugation followed by precipitation of the supernatant with NaOH and then different solvents (HCl, NaCl, ethanol, cetyltrimethylammonium bromide, and acetone) | | Geresh et al. (2009) |
| *Porphyridium cruentum* | Diafiltration (tangential flow filtration with 300 kDa membrane) | High-pressure size exclusion chromatography | Patel et al. (2013) |
| *Porphyridium cruentum* | 2-Step concentration diafiltration with different molecular weight cutoff membranes | – | Marcati et al. (2014) |
| *Trebouxia* sp. from lichen *Ramalina farinacea* | Centrifugation followed by ethanol precipitation two times that of the supernatant | SDS PAGE | Casano et al. (2015) |

In general, most of the studies available in the recent literature include one or two steps of centrifugation and/or filtration to remove the microalgae biomass or the remaining cells from the extracts rich in the compounds of interest. The wide majority of researchers applied the traditional solvent precipitation methods, in which the soluble polymers could undergo concentration steps combined with low temperature heating (Raposo et al., 2014b) followed by one or several precipitation steps with a wide variety of solvents. These solvents could be cationic surfactants (Geresh et al., 2009) or organic solvents (ethanol, methanol, acetone, ether) or a combination of some of these solvents in different proportions. More recently, novel technologies like the membrane filtrations have been adapted to extract and purify these extracellular polysaccharides from a wide variety of microalgae species such as *Amphora* sp., *Ankistrodesmus angustus*, *Phaeodactylum tricornutum*, *Graesiella emersonii*, *Graesiella vacuolata*, and *Porphyridium cruentum* (Patel et al., 2013; Marcati et al., 2014).

## 19.5 Extraction of essential lipids/long-chain fatty acids

Lipids in microalgae are classified primarily as polar and nonpolar. From the lipids class, the long-chain polyunsaturated fatty acids (PUFAs) are among the most interesting and studied due to their healthful effects on humans. These fatty acids have been linked to the potential prevention and risk reduction of coronary heart diseases, arthritis and other autoimmune disorders, inflammation, hypertension, psoriasis, and cancer (Lands, 2014). PUFAs are long-chain unsaturated fatty acids that cannot be synthesized by humans. Omega-3 and omega-6 are well-known derivative products from PUFA. Although fish are known as one of the main sources of PUFAs basically, their PUFAs are obtained by the ingestion of microalgae. As a result, microalgae attracted the attention as one of the most important and sustainable producers of PUFAs (Ward and Singh, 2005). As well as in the extraction of pigments, the extraction of lipids and, particularly, fatty acids from microalgae also starts with a first step of cell disruption aided or followed by the use of organic solvents to recover the lipid fraction from the microalgae. In this sense, for both tasks of cell disruption and extraction, an appropriate solvent is necessary to efficiently recover the fatty acids from the biomass (Natarajan et al., 2015). Furthermore, the solvent should be inexpensive, nontoxic, volatile, and highly selective to lipid components of the cell. For this purpose, chloroform, methanol, n-hexane, propan-2-ol, are some of the solvents being evaluated in lipid extraction (Bermúdez Menéndez et al., 2014). Kusdiana and Saka (2004) claimed that dewatering the microalgae was necessary before lipid extraction. Associated with the use of different solvents, different cell disruption techniques have been tested in the same purpose, namely osmotic shock, microwave treatment, autoclave, bead-beating, and ultrasonication (Kim et al., 2013; Bermúdez Menéndez et al. 2014; Lee et al., 2010). The method used for extraction should be fast, easily scalable, effective, and biocompatible with the compounds being extracted, which means that the method or methods should be harmless for the maintenance of the biological activities and chemical structures of the lipids extracted. According to the main results found in this field, it seems that the use of ultrasound and microwave-assisted methods allows the use of low amounts of solvents, thus decreasing the time required for the

extraction (Bermúdez Menéndez et al. 2014; Lee et al., 2010); however, significant energy costs and sometimes the compounds' degradation when long times of extraction are applied, are associated (Bermúdez Menéndez et al., 2014). Meanwhile, ultrasonication has recently gained attention because of its possible use in industry (Natarajan et al., 2014; Han et al., 2016). It was found that the efficiency of this process technique of cell disruption correlates well with ultrasonication energy consumption despite the main ultrasonication conditions.

One alternative to traditional extraction using organic solvents is the use of supercritical fluids, namely $CO_2$, to perform the extraction (Crampon et al., 2011). Actually, the use of SFEs is being recognized as a sustainable alternative considering the lipids' extraction not only due to the high efficiency and selectivity in the recovery of these compounds from microalgae, but also the use of nonflammable and volatile solvents, which allow an easy separation of the target compounds from the solvents of extraction (Crampon et al., 2011). The authors concluded that the high selectivity of this process is devoted to the use of variations of pressure and temperature. The extraction results found for this method are in fact substantially better than those obtained for other techniques like simple extractions using different organic solvents, not only in terms of extraction efficiency, but also because the use of supercritical fluids allows a significant decrease in the time of extraction (Crampon et al., 2011). Finally, the conjugation of supercritical fluids with some other physical methods has been performed, mainly with microwaves (Dejoye et al., 2011). In this work, higher extraction yields (4.73%) were obtained by the use of microwave as a pretreatment when compared with just the application of supercritical $CO_2$ extraction (1.81%).

The research on this field is quite significant, mainly due to the fact that the microalgae lipids have been considered in recent decades as valuable and alternative compounds to be used in the production of biodiesel. So in this context, the number of processes evaluated so far is quite large, and thus other techniques have been investigated to increase in the extraction efficiency (González et al., 1998; Crampon et al., 2011; Catchpole et al., 2009; Mercer and Armenta, 2011).

## 19.6 Proteins from microalgae

Today microalgae are considered a promising option for the provision of food, feed, and biofuel-based ingredients and compounds based on their productivity and ability to be cultured on nonarable land with either fresh, brackish, or sea water. Microalgal species commonly used and processed for foods and cosmetics include *Arthrospira* (traditional name, *Spirulina*), *Chlorella* spp., *Dunaliella* spp., and *Haematococcus* spp. (Buono et al., 2014). Microalgae and cyanobacteria are rich sources of several bioactive components including proteins, PUFAs, sterols, enzymes, vitamins, and pigments (de Jesus Raposo et al., 2013). These bioactive compounds have potential for use in human and animal food and feedstock (Buono et al., 2014) as well as in aquaculture (Hemaiswarya et al., 2011).

By 2050 the world population will require 70% more food than is currently consumed. In 1960 global consumption of protein per person per day was 25 g. Today global consumption of protein per person has risen to 36 g driven by the consumption

of alternative proteins to dairy and meat, which has increased by 15% within this period (Godfray et al., 2010). Aquaculture will increasingly contribute to future food supply requirements through the production of marine-derived ingredients. Microalgae are considered an important source of protein. The value of microalgae can increase by utilizing as many potentially useful algae biomass components as possible. Protein, especially water-soluble protein, is a promising microalgal ingredient. Protein accounts for about half of the total algae biomass of many species and can be applied in human and animal food products as well as the generation of functional foods through the production of bioactive peptides. Consequently, algae protein may replace alternatives to meat and milk such as soy in the future. Several microalgal species are rich in protein. For example, *Arthrospira platensis* contains 50%–70% protein, *Chlorella vulgaris* 38%–58%, *Nannochloropsis oculata* 22%–37%, *P. cruentum* 8%–56%, and *Haematococcus pluvialis* 45%–50% on a dry weight basis (Safi et al., 2013a,b). Recently, Safi et al. examined the release of protein into water following the application of various cell disruption techniques (Safi et al., 2013a,b). This study found that high-pressure cell disruption was the most efficient technique for protein extraction into aqueous medium but that this method was not sufficient for the green microalgae and that less than 50% of the proteins from green microalgae were recovered, indicating that more energy and therefore more costs would be required for green algae (Safi et al., 2013a,b). Ursu et al. (2014) previously examined the potential of *C. vulgaris* as a protein source. This study found that this species was especially rich in protein and furthermore that extracted proteins showed excellent emulsifying properties when extracted using any extraction method: mechanical, alkaline, or isoelectric precipitation (Ursu et al., 2014). These proteins have potential for use in cosmetic and food formulations. The extraction and application of microalgal-derived proteins and peptides are further explored in another chapter (Hayes et al., in press).

## 19.7 Green extraction and purification techniques

The solution for greener extraction techniques passes through the development of more sustainable technologies that consider the minimization of the use of toxic solvents, the reduction of the waste production, and the decrease of the processes energy consumption. Among the several techniques complying with these requirements, those more suitable for the extraction of bioactive compounds from microalgae are the SFE, PLE, UAE, MAE, pulse electric field (PEF), and alternative solvents like ionic liquids (ILs), surfactants, and alcohols in aqueous solution. Moreover, and included in the use of these alternative solvents, new purification technologies are discussed, namely the use of aqueous biphasic systems and cloud point extractions (see Section 19.7.6).

### *19.7.1 Supercritical fluid extraction*

The main advantages of SFE for the recovery of bioproducts, namely lipids, from microalgae rely on the fact that very high yields of extraction can be achieved together with considerable improved selectivity and good fractionation capability (Nobre et al., 2013). In addition, the remaining biomass after the lipidic extraction, consisting

mainly of protein and carbohydrates, can be used for additional applications since there is no denaturation of the proteins. Finally, the lower environmental impact and economic feasibility of SFE, as well as the potential to scale-up the process, makes SFE a very attractive technique (Herrero et al., 2015).

### 19.7.2 Pressurized liquid extraction

Using PLE, it is possible to achieve high yields with a fast extraction and a very low solvent consumption. Also, the applied high pressure inhibits native enzymes while avoiding the degradation of some compounds (Seabra et al., 2010). Additionally, in the analysis of chlorophylls extracted by PLE (Cha et al., 2010), it was verified that *Pheoporbidea* (a derivative of chlorophyll with toxicity) was not present in the extracts. Moreover, PLE is carried out in an oxygen-free and dark-free environment, which is advantageous for bioproducts (Herrero et al., 2015).

### 19.7.3 Ultrasound-assisted extraction

UAE has been found to increase the rate of extraction and in some cases reach a better selectivity (Toma et al., 2001). Moreover, UAE leads at the same time to a significant reduction in extraction time, temperature, and solvent consumption. Also, ultrasound is a well-known technology with low equipment investment and easy implementation; hence it can enhance existing extraction processes.

The research works detailed in the previous sections have shown that UAE can be a suitable alternative for the extraction of bioproducts from microalgae (Gerde et al., 2012). Compared to conventional extraction, UAE is a more efficient and rapid extraction method, due to the strong disruption of the cell wall that is achieved. Also, UAE has no effect on the chemical structure and biological properties of the bioproducts. Moreover, it is suggested UAE can be the most economical method for the extraction of compounds like carotenoids from microalgae.

### 19.7.4 Microwave-assisted extraction

MAE presents a heating mechanism that significantly reduces the extraction time (usually from a few seconds to half an hour) compared to other techniques (Tatke and Jayswal, 2011). This is an advantage for avoiding thermal degradation and oxidation. In addition, MAE consumes less solvents (easy recycled), presents higher extraction yield and enhanced efficiency, is nontoxic, and can be used for larger volumes. MAE is one of the simplest methods and most effective among the several existing techniques. Nevertheless, the maintenance costs associated are still high.

### 19.7.5 Pulse electric field

PEF is considered an interesting alternative extraction technique for bioactive compounds since it has a short extraction time (usually bellow 1 s), works at low temperatures, has minimal energy losses, and allows the successful breakdown of the cell walls. Moreover, this technique has been considered as a potential and promising

green extraction method of bioproducts from microalgae due to the significant energy savings, high potential of scale-up, and considerable reduction of the operation costs (Joannes et al., 2015).

### 19.7.6 Alternative solvents: ILs, surfactants, and alcohols used as main-phase formers

ILs represent a unique class of molten salts with melting temperatures up to 100°C (Choi et al., 2014) and are recognized by their good solubility in both organic and inorganic materials/solvents and ability to form multiple-phase systems (Fauzi and Amin, 2012). Additionally, ILs present widely tunable properties, mainly their high solvency power, which makes them "designer solvents" adaptable to a plethora of techniques and technologies. ILs can efficiently replace organic solvents disrupting the cellular structure as was recently reviewed by Orr et al. (2016) and promoting the formation of effective extraction/purification methodologies (Desai et al., 2016) because of their ability to dissolve cellulose. In fact, Orr et al. (2016) show not only the potential of different ILs to disrupt distinct microalgae cells, thus promoting the release of some of their specific components, but also the industrial potential of their process. In this sense, IL recycling is addressed for inferring the economic viability of the process being developed. Moreover and behind their use as cell disruptors, ILs can be used to fractionate the microalgae biomass in which studies reported the separation of three distinct fractions composed of lipids, proteins, and carbohydrates.

Recoveries of 60% of β-carotene with ABS based in Tween 20 and sodium citrate (Ulloa et al., 2012) were reported. Another approach using surfactants as phase formers is being developed by Smirnova and collaborators (Glembin et al., 2013, 2014). This group uses surfactants as solvents to extract fatty acids in situ from the biomass in lab and pilot scales (Glembin et al., 2013, 2014), which means that the biomass is continuously being produced, and the fatty acids being extracted.

Alcohol-salt-based ABS were applied in the purification of fucoxanthin (Gómez-Loredo et al., 2014) with recoveries from 89.18% (purity of 78.74%) to 63% (conjugation of ABS and ultrafiltration) (Gómez-Loredo et al., 2014). Summing up, it seems that the complete (or partial) substitution of the organic solvents by aqueous solutions is advantageous because they decrease the economic impact, time consumption, and environmental footprint of the whole process while maintain the process efficiency and the compounds' main properties.

## 19.8 Market and commercialization of high-value products from microalgae

Microalgae (mainly *Spirulina* sp. and *Chlorella* sp.) are traditionally commercialized for human food or animal feed and have a market that represents approximately $80 million of annual turnover (Vigani et al., 2015). There are also specific high-value products from microalgae in the market with important applications in different industries belonging to the nutritional (Plaza et al., 2008), pharmaceutical (Guedes et al., 2011), cosmetic, and energetic sectors (Spolaore et al., 2006) as described in Table 19.3.

**Table 19.3 High valuable compounds produced by microalgae: Commercial products, potential competitors, uses, future microalgae producers, and market prospects**

| Compound class | Main compounds | Products from algae on the market | Alternate source(s) | Main uses | Potential producers of the compounds | Market prospects (US$)[a] | Reference |
|---|---|---|---|---|---|---|---|
| Carotenoids | β-Carotene, astaxanthin, canthaxantin, zeaxanthin, lutein, fucoxanthin | β-Carotene (*Dunaliella* sp.), astaxanthin (*Haematococcus* sp.) | Synthetic alternative, fungus *Blakeslea trispora*, green algae (canthaxantin and lutein), and brown macroalgae (fucoxanthin) | Nutraceutical and feed additive | *Chlorella* sp. (asthaxanthin, canthaxantin, lutein and zeaxanthin), *Scenedesmus* sp., *Muriellopsis* sp., and other green microalgae (lutein), *Phaeodactylum tricornatum*, *Odontella aurita*, *Haslea ostrearia* (fucoxanthin) | Carotenoids market of $1428.12 million by 2019 | Borowitzka (2013) |
| PUFA | Omega 3 (γ-linolenic acid, arachidonic acid (ARA), EPA, docosahexaenoic acid (DHA) | DHA (*Crypthecodinium cohnii*), DHA and EPA (*Schizochytrium* sp.), EPA (*Nannochloropsis* sp.) | Fish oil, various plants, fungi (i.e., *Mortierella* sp.) | Nutraceuticals, pharmaceuticals, and feed | ARA (*Parietochloris incisa*), EPA (*Trachydis minutus*, *Nanochloropsis* sp., *Phaeodactylum tricornatum*, *Monodus subterraneus*, *Pavlova lutheri*), DHA (*Isochrysis galbana*, *Crypthecodinium cohnii*, *Schizochytrium* sp., *Ulkenia* sp.) | PUFA market of $18.95 billion by 2020 | Borowitzka (2013); Leu and Boussiba (2014) |
| Polhydroxyalkonates (PHA) | | – | Bacteria (i.e., *Pseudomonas* sp., *Halobacterium marismortui*, *Halomonas boliviensis* and *Azospirillum brasilense*…) | Biodegradable plastics | *Nostoc* sp., *Synechocystis* sp., *Spirulina* sp. | Biodegradable plastics market of $3.4 billion by 2020 | Borowitzka (2013); Singh Saharan et al. (2014) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Polysaccharides | Extracellular polysaccharides and sulfated polysaccharides | Alguronic acid from *Porphyridium*, *Chlorella* and *Spirulina* sp. | Bacteria and fungi | Cosmeceutical, nutraceutical, and pharmaceutical | *Porphyridium* sp., *Chlamydomonas* sp., *Rhodella* sp., and cyanobacteria | – | Borowitzka (2013); Raposo et al. (2013); Laurienzo (2010) |
| Proteins | Phycobilins (phycocyanin, phycoerythrin, allophycocyanin) | *Spirulina* sp. | — | Nutraceutical, pharmaceutical, and cosmeceutical | Cyanobacteria, *Rhodophyta*, *Cryptophyta*, *Glaucophyta* | Protein labeling market of $1894.5 million by 2020 | Borowitzka (2013) |
| | Recombinant proteins (human antibodies, enzymes, and hormones) | – | Mammalian cell cultures | Pharmaceutical | *Chlamydomonas reinhardtii* (erythropoietin), *Dunaliella tertiolecta* (industrial enzymes), *Phaeodactylum tricornutum* (erythropoietin, functional antibodies, and human growth hormone) | Biosimilar market of $6.22 billion by 2020 | Leu and Boussiba (2014) |
| Other compounds | Mycosporine-like amino acids (MAA) | – | – | Cosmeceutical | Cyanobacteria, *Dinophyta*, and other algae | – | Borowitzka (2013) |
| | Marennine | – | – | Food (oyster pigmentation). Possible pharmaceutical, nutraceutical, and cosmeceutical | *Haslea ostearia* | – | Leu and Boussiba (2014) |

[a]Market prospects selected from (http://www.marketsandmarkets.com/, 2016).

Carotenoids with industrial interest currently produced by microalgae are β-carotene (mainly from *D. salina*) and asthaxanthin (mainly from *H. pluvialis*) and the less explored lutein, zeaxanthin, and lycopene. These belong to one of the most competitive classes of bioactive compounds found in microalgae since they could compete with other chemically synthesized carotenoids (Spolaore et al., 2006).

The PUFA DHA was recommended for inclusion in infant formula, which has an estimated world market of $10 billion per year (Ward and Singh, 2005). The main company producing DHA from microalgae is Martek (Columbia, SC) with its products DHASCO and DHA Gold (Spolaore et al., 2006). In the most recent years, new companies have begun producing DHA-enriched products from different microalgae species such as *Schizochytrium* sp. (DSM-NP life's DHA plus EPA and Source-Omega Source Oil), *Crypthecodinium cohnii* (DSM-NP life's DHA and GCI Nutrients DHA Algae 35% Oil), and *Ulkenia* sp. (Lonza DHAid) (Martins et al., 2013). There are several alternative sources (natural and/or chemically synthesized compounds) to most of the existing and potential high-value products from microalgae. The successful establishment of microalgae products will depend not only on the aptitude of the different microalgae species to produce high value compounds, but in reducing the cost in production and downstream, improving the efficiency of the processes and designing new products and creating new markets (Jara et al., 2016). Actually, the success of new bioproducts derived from microalgae will depend on both the production and downstream processes being implemented. If it is necessary to develop simple and effective processes of production of microalgae (Günerken et al., 2015; Chen et al., 2016) able to maximize the accumulation of the most added value biocompounds, efficient and low-cost processes of extraction and purification are required (Koller et al., 2014) to design new products and create new markets (Jara et al., 2016).

## 19.9 Conclusions

Despite the significant number of works dealing with the use of microalgae as feedstocks, chemicals, or natural products, the extraction and mainly the purification processes being applied, need more investigation. If some of these processes/tools are poorly efficient, there are others whose sustainability and economic viability are far from being a reality. In this chapter, different biocompounds were checked (from the simplest to the most complex, ranging from the most hydrophobic to the most hydrophilic ones) and different extraction processes evaluated to identify the best solvents and/or technologies; however, more studies seem to be required to improve the results known up to now. Actually, new systems that have appeared recently using alternative solvents (e.g., ionic liquids and surfactants) with higher solvency power should be deeply evaluated. Researchers need to carefully treat the solvent formulations being used to avoid the application of organic volatile and toxic solvents but should instead treat the biomass with aqueous solutions. Moreover, the correlation between the chemical structures of the compounds extracted from the microalgae biomass and the solvents and technologies being applied should be properly investigated. For example, the use of techniques involving temperature as the driving force to extract thermolabile chemicals should be avoided. Actually,

the application of certain solvents to interact precisely with a specific bioactive compound should be carefully evaluated. In this sense, the creation of heuristic rules appropriately developed considering the correlation between the target compounds' main properties and structures, the solvents, and the extraction techniques being established should improve the appropriate development and implementation of more efficient, environmentally friendly, and mainly economically viable processes, opening the possibility for them to be scaled-up in the near future. The creation of new products and new markets needs to be focused not only by researchers but principally by industries in collaboration with academia.

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## Further reading

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