Downstream green processes for recovery of bioactives from algae

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Abstract Nowadays, macro and microalgae are being increasingly used as promising raw materials for the food, cosmetic and pharmaceutical industries thanks to their biodiversity and its variety on valuable bioactive compounds such as carbohydrates, polyunsaturated lipids, proteins and pigments, among others. Furthermore, more efficient and environmentally friendly processes for bioactives' recovery are requested not only by the industry but also by the society. This book chapter presents an overview on the use of downstream green processes, mainly based on compressed fluids extraction techniques, in order to recover bioactives from algae that can be lately used in several potential applications for the food, pharmaceutical and cosmetic industries, which is the pillar of algae-based biorefinery.

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1. Introduction

The increasing knowledge regarding the positive impact of diet on human health has brought about a great interest for seeking new bioactive products of natural origin to be used as functional ingredients for the development of functional foods. The concept of functional food is defined as food that besides the basic nutritional and energetic value provides additional health benefits thanks to the one or more functional ingredients that contains (Merichel Plaza et al. 2009). This definition implies that a functional food must improve well-being or reduce the risk of illness (Diplock et al. 1999).

Micro- and macroalgae have been suggested as a potential natural source of new compounds with biological activity that could be used as functional ingredients, due to their antioxidant (Lv et al. 2015), anti-inflammatory (Caroprese et al. 2012), antidiabetic (Yu Ran et al. 2015), neuroprotective (Pangestuti and Kim 2011), anticancer (Souza et al. 2018), anti-allergic (Thanh-Sang et al. 2012) and antimicrobial activities (Rodriguez-Meizoso et al. 2010), among others.

The development and production of these functional ingredients has become of great interest for the food industry, although pharmaceutical and cosmetic industries are also aware of the important bioactive compounds that can be obtained from marine natural sources such as algae and microalgae, thus extending its interest and applicability. In this sense, many algae-derived secondary metabolites are known for their skin benefits, which include protection from UV radiations and prevention of aging, rough texture, wrinkles, and skin flaccidity (Ariede et al. 2017), of upmost importance for new cosmetics' development. On the other hand, some important secondary metabolites (such as meroterpenoids) have been isolated from marine organisms presenting interesting pharmacological properties, such as cytotoxic towards several human cell lines, anti-inflammatory, etc. (García et al. 2018).

At present, the world is not only worried about food and human health but also about the global environmental awareness that continues to be on the rise. This is true in many countries, but especially in Europe and the USA. Climate change, global warming, and the realistic threat of a lack of resources in the future for the rapidly growing world population have contributed to push process greenness and sustainability (Herrero and Ibáñez 2018). Sustainability can be understood as a rational way of improving processes to maximize production while minimizing the environmental impact (Herrero and Ibáñez 2015). Considering this framework, the study of the use of solvents that are generally recognized as safe (GRAS) for its use in the food industry, such as water, CO_2 or ethanol, combined with compressed fluids techniques are the most promising engineering approach that offers a fast, cost-effective and environmentally friendly extraction of bioactive compounds from algae. Application of high pressure and moderate-high temperature to the GRAS solvents modifies their properties contributing to a better extraction process, improving the mass transfer rate and preserving the biological potential of

the extracts. In this chapter, green extraction techniques, such as supercritical fluid extraction (SFE), gas expanded liquid (GXL) extraction, pressurized liquid extraction (PLE) and subcritical water extraction (SWE) are presented, and applications to algae bioactives extraction are discussed. Moreover, other important aspects related to upstream processes optimization and biorefinery of algae (achieved through downstream process integration for valorising, in a rational way, all the different algae fractions) are also described.

1.1 Marine Resources

Prokaryotic life was originated in the oceans about 3.6 billion years ago while eukaryotic life was originated between 0.6 and 1 billion years later (Ibáñez and Cifuentes 2013). The long evolution period of marine life compared to terrestrial has generated a huge diversity in terms of number of different species, genes, etc. Furthermore, marine organisms live in hostile environments of light, salinity, and temperature, thus, they must adapt to survive, producing a great variety of secondary (and biologically active) metabolites. This ability, coupled with the immense diversity of species, provides an almost inexhaustible source of natural bioactive compounds from marine resources. Nowadays, the most important source of information for these bioactive compounds is The Dictionary of Marine Natural Products (Blunt and Munro 2008), which lists over 30,000 purified compounds and tends to present a growing number of compounds every year.

Among the marine sources, algae are the most promising due to their easy cultivation and fast growth. Algae are photosynthetic aquatic organisms that possess simple reproductive structures. In general, these can be categorized as unicellular microscopic (microalgae) and multicellular macroscopic organisms (macroalgae). Although the number of different alga species has been estimated to be between one and ten million (Metting 1996), approximately only forty thousand species have been described (Suganya et al. 2016), which involves almost an unlimited field of research.

Macroalgae are classified in groups based on their pigmentation: Chlorophyceae (green algae), Phaeophyceae (brown algae), and Rhodophyceae (red algae) (Oncel 2017). Macroalgae have been extensively utilized as food (or food technological ingredients) for many years, and thus are farmed commercially in several countries (Baghel et al. 2015) (over 30 million tons in 2016) (FAO 2018).

On the other hand, microalgae cultivation is increasing quickly, mainly in large scale, both in outdoor and indoor production. Microalgae could grow in autotrophic conditions, heterotrophic conditions with enough nutrients but no light availability and even in mixotrophic conditions, so that they are able to utilize both inorganic and organic compounds from the medium (Carvalho et al. 2014). Regarding pigment composition, microalgae are classified into nine divisions. Some of the largest groups include Phaeophyceae, Chlorophyceae, Pyrrophyceae

(dinoflagellates), Bacillariophyceae (diatoms), Chrysophyceae (golden-brown) and Rhodophyceae (Oncel 2017).

One of the main applications of micro- and macroalgae biomass is biodiesel production (Mata et al. 2010) because of the high level of triglycerides they contain (Yen et al. 2013). Algae as a potential renewable resource is not only used for biofuels (Suganya et al. 2016) but also for food for aquaculture (Suganya et al. 2016), biofertilizer (Marris 2006), environmental applications such as CO_2 mitigation (Bilanovic et al. 2009) or wastewater treatment (Hodaifa et al. 2008), and to obtain high added value foods (Ibáñez and Cifuentes 2013), cosmetics (Ariede et al. 2017) and pharmaceutical products (Thanh-Sang et al. 2012).

In the following section, algae will be presented as a source of different bioactive compounds of interest for the food, cosmetics and pharmaceutical industry. A revision about the different types of bioactives that have been described in algae is presented, including compounds such as lipids, proteins and peptides, polysaccharides, carotenoids, phenolics, alkaloids, etc. **Table 1** presents a summary of potential functional compounds found in different microalgae and macroalgae, together with their possible health effects.

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2. Algae as source of bioactive or valuable compounds

2.1 Lipids

Algae can produce different kind of lipids such as glycolipids, phospholipids (polar lipids), glycerolipids with neutral storage lipids, and free fatty acids. Lipid percentages vary within the type of algae, containing 7-16% dry weight for macroalgae and from 1.9% up to 40% for microalgae (Suganya et al. 2016).

Among the lipids, polyunsaturated fatty acids (PUFAs) are the most studied compounds in algae. PUFA fraction in algae is often higher than in terrestrial vegetables (Kumari et al. 2010). In fact, several microalgae are able to synthesize ω-3 and ω-6 long chain PUFAs, which are essential natural antioxidants for body health, at levels as high as 10–70% of total fatty acids (Kumari et al. 2013), exceeding 20% of their total lipid content (Bellou et al. 2014). However, the amount of PUFAs and the number or position of double bonds on the carbon chain can vary according to the algal species and growing conditions (Villarruel-Lopez et al. 2017). In general, many microalgae have PUFAs such as EPA (eicosapentaenoic

acid, ω-3 $C_{20:5}$), DHA (docosahexaenoic acid, ω -3 $C_{22:6}$) and ARA (arachidonic acid, ω -6 $C_{20:4}$).

The specific interest in ω-3 essential PUFAs are their beneficial effects such as the reduction of the risks of heart disease (Jinghai Chen et al. 2011), depression (Giles et al. 2013), inflammation (Yates et al. 2014), and cancer (Giros et al. 2009; Pottel et al. 2014).

Since humans have difficulty in synthesizing fatty acids with more than 18 carbons, these fatty acids should be obtained from food (Hamed et al. 2015) and in general, algae have low ω-6:ω-3 ratio, as recommended by the WHO. Although fish and seafood are the major source of long-chain PUFAs, it is important to remark that algae have been suggested as a feed for aquaculture with the idea of obtaining the desired fatty acid profile in fish and seafood for consumers.

Other important microalgae-derived lipids are phytosterols, which have been used as additives in many food products such as spread, dairy products and salad dressing (Luo et al. 2015). Phytosterols have been reported to have many beneficial health effects in humans, including immunomodulatory (Caroprese et al. 2012), anti-inflammatory (Ciliberti et al. 2017), antihypercholesterolemic (Jingnan Chen et al. 2014), antioxidant (Lv et al. 2015) and anticancer (Kazlowska et al. 2013).

2.2 Proteins and peptides

Algae can become a potential protein source. The protein content recorded for green and red algae can reach 47% of the dry weight (Ibáñez and Cifuentes 2013) and ranged between 60-70% in microalgae such as *Arthrospira platensis*, *Chlorella vulgaris* of *Isochrysis galbana* (Matos et al. 2017). These have been used as a supplement in food, animal feed or aquaculture due to their optimal balance of essential amino acids.

Peptides from protein hydrolysis have been studied due to their bioactivities. Some peptides have potential benefits such as antioxidative (Hu et al. 2015), binding or inhibiting specific receptors (Kalpa W. Samarakoon et al. 2014), growth factors, hormones, immunomodulators (de Jesus Raposo et al. 2013), antihypertensive, anticoagulant and antiproliferative (Kalpa Samarakoon and Jeon 2012).

2.3 Polysaccharides

Macroalgae contain large amounts of polysaccharides, mainly cell wall structural polysaccharides such as alginates (brown algae) and carrageenans and agar (red algae) (Ibáñez and Cifuentes 2013), meanwhile microalgae have a low content (approximately 10% of dry matter) of carbohydrates (Villarruel-Lopez et al.

2017). Nevertheless, macro- and microalgal polysaccharides have health promoting properties such as anti-inflammatory, antitumor, anti-adhesive, antiviral, antibacterial, immunomodulatory and infection-prevention activities (Gallego et al. 2018). For example, beta glucans are considered immune stimulators while cellulose and starch can act as dietetic fibres, and sulphated polysaccharides have antioxidant and antitumoral activities (Villarruel-Lopez et al. 2017).

2.4 Phenolic compounds

The main bioactivity associated to algal phenolic compounds is their antioxidant effect through scavenging of reactive oxygen species (ROS) or enhancement of intracellular antioxidant defences. For example, extracts from microalgae *Euglena cantabrica* exhibit high antioxidant activity due to their high concentration of phenolic acids, particularly gallic and protocatechuic acids (Jerez-Martel et al. 2017).

Phlorotannins are the major phenolic compounds in brown macroalgae and the most studied group of phenolic compounds from algae because they constitute an extremely heterogeneous group of molecules, providing a wide range of potential biological activities in addition to antioxidant activity: antiproliferative (Montero et al. 2016), antibiotic (Tanniou et al. 2014), antiallergic (Kim and Himaya 2011), antidiabetic and anti-inflammatory activities (Catarino et al. 2017).

Other phenolic group with interesting bioactive properties are flavonoids. For instance, it has been reported in microalgae that the synergistic effects of chlorogenic and caffeic acids with 13-*cis*-retinoic acid can not only prevent lipid peroxidation, but also regress cancer (de Jesus Raposo and Miranda Bernardo de Morais 2015). The flavonoid content in macroalgae has been also studied (Yoshie-Stark et al. 2003).

2.5 Alkaloids

Alkaloids present special interest because of their pharmacological activities. Structurally, alkaloids isolated from marine algae mostly belong to the phenylethylamine and indole groups.

Some alkaloids detected in marine macroalgae and microalgae have been associated with relieve depression (phenylethylamine), increase heart rate and blood pressure (tyramine), diuretic effects and inhibition of gut movements (hordenine), treat cardiovascular and kidney disorders (dopamine), antitumor, antibacterial and antifungal activity (caulerpin) or antioxidant activity (fragilamide) (Güven et al. 2010).

2.6 Carotenoids

Carotenoids are lipophilic compounds that present significant interest as food colorants, feed supplements, nutraceuticals, and for cosmetic and pharmaceutical purposes. Their C_{40} structure is based on isoprene units which can contain oxygen, so they can be classified in two main groups: carotenes and xanthophylls (Gong and Bassi 2016). More than 600 different naturally occurring carotenoids are now known, not including *cis* and *trans* isomers.

Carotenoids from marine macro- and microalgae have been described as powerful antioxidants and their beneficial physiological functions, such as anti-cancer, anti-obesity, anti-diabetic, anti-inflammatory, and cardioprotective activities have also been reported (Hoang Van and Eun 2017). For instance, some of the most studied carotenoids extracted from algae with beneficial effects on health are fucoxanthin, β-carotene, lutein and zeaxanthin from macro- and microalgae; and astaxanthin, canthaxanthin, capsanthin, α-carotene, crocetin, β-cryptoxanthin, lycopene, neoxanthin and phytoene from microalgae (Christaki et al. 2013; Gallego et al. 2018).

3. How to improve the production of bioactive metabolites

As it was mentioned in the introduction, macro and microalgae have raised an enormous interest thanks to their potential for being a good source of high added value compounds that can be used in cosmetic, food and pharmaceutical industries. Furthermore, it is well established that secondary metabolites production can be strongly increased by many factors. **Figure 1** offers an overview on different ways to increase the production of valuable components from algae: marine biotechnology (through genetic engineering, selection and improvement of strains, metabolic flux modelling…) and optimization of processes including both upstream (strain selection and cultivation conditions), and downstream processes (biomass processing, extraction and purification methods). The main objective would be the integration of these factors in a biorefinery approach which allows a high production of the bioactives of interest.

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3.1 Marine biotechnology

Marine or blue biotechnology can be defined as the application of genetic engineering to marine resources. Thus, by using genetic engineering it is possible to modify genes and improve algae strains obtaining transgenic algae which are able to overexpress genes and overproduce valuable target compounds.

Marine biotechnology involves the study of the metabolic pathways which lead to the synthesis of bioactive compounds. It is important to consider all biochemical reactions, and their stoichiometry, which occur within the metabolic network. This knowledge will lead to modify or model the metabolomic flux, increasing (or decreasing) the production of selective bioactive metabolites (Ibáñez and Cifuentes 2013).

It is true that genetic manipulation in algae has been limited to a few species due to the complexity and large genome size. Microalgal genome sizes range from 12.6 Mbp for the *Ostreococcus tauri* and 168 Mbp for the *Emiliania huxleyi* to an estimated 10,000 Mbp for the *Karenia brevis* (Cadoret et al. 2012). These large genome sizes can be difficult to sequence and transform.

Furthermore, it is very difficult to obtain new microalgal strains since nuclear transformation has a low efficiency and transgenes expression is not stable (Leon and Fernandez 2007).

Recently, some researchers have proposed new methods to ensure stability and a higher expression of transgenes. For instance, Díaz-Santos et al. (Diaz-Santos et al. 2016) proposed an interesting approach to express transgenes in microalgae using co-transformation with two naked promoterless genes, which are randomly inserted into the nuclear genome. They reached a successful co-transformation of *Chlamydomonas reinhardtii*, concluding that this transformation system could be universally applicable to any microalgal species.

In conclusion, more intense research and the study of new genetic engineering techniques are necessary to better understand, both genetically and metabolically, the complex network involved in the synthesis of bioactive compounds of interest; this way, the full potential of macro and microalgae could be reached.

3.2 Optimization of upstream and downstream processes

Upstream and downstream processes involve all stages from the selection of macro and microalgae strains and cultivation to extraction and/or purification of secondary metabolites.

3.2.1 Upstream processes

Of course, depending on the bioactive compound of interest, a specific algae strain must be chosen since metabolite composition is extremely variable among species. Nowadays, there is a huge quantity of compounds obtained from different algae which can be found in many industries. For example, carotenoids such as β-carotene and astaxanthin are obtained from the green microalga *Dunaliella salina* and *Haematococcus pluvialis*, respectively. Another interesting example is the use of *Isochrysis galbana*, which is rich in ω-3, as an ingredient for functional biscuits (Gouveia et al. 2008).

Cultivation conditions are essential in algae biorefinery. The main factors are supply of carbon dioxide (commonly CO_2), nutrient source (i.e. nitrogen and phosphorus), and source and origin of illumination (Vanthoor-Koopmans et al. 2013), but also it is important to take into account other factors such as temperature control, algae concentration, pH, cultivation systems such as ponds or photobioreactors, etc. All these factors are vital for the proper growth of algae. For instance, it is well known that the microalgae *Haematococcus pluvialis* can grow as motile biflagellated green cells when it is subjected to favourable conditions, but under stress conditions (nutrient deficiency, high light intensity or salt stress), the cells lose their motility, their size increases and form red cysts, allowing its survival for long and stressful periods (Hagen et al. 2002). Thus, in green cells, chlorophylls and carotenoids such as lutein and β-carotene can be found while in red cell phase, astaxanthin and its derivatives (esters, mainly) constitute up to 98% of the total carotenoid content (Boussiba et al. 1999). In terms of light, Aravantinou and Manariotis (Aravantinou and Manariotis 2016) observed a greater growth rate of *Chlorococcum sp*. under artificial light conditions instead of direct sunlight, proving the importance of light intensity and light source on biomass production.

The second main step in upstream processing is harvesting, which has to be optimized for each particular algae strain. In this sense, there are many ways to recover biomass but they are mostly focused in centrifugation and filtration.

Biomass processing is related to the proper disruption of the cell, since most of metabolites of interest are located inside the cell. It includes different techniques such as enzymatic treatments, microwave-assisted processes, pulsed electric fields or high pressure homogenization. In this sense, Carullo et al. (Carullo et al. 2018) studied the effect of two different cell disruption techniques in the microalgae *Chlorella vulgaris* and demonstrated that it was possible to selectively recover small-sized cytoplasmic compounds using pulsed electric fields, and high molecular weight intracellular components using high pressure homogenization.

3.2.2 Downstream processes

Downstream processes involve the extraction and purification methods to isolate the valuable compounds of interest from algae. These procedures can be extremely expensive and can consume a huge quantity of organic solvents, so the optimization of these steps is vital for the global economic viability of the algae

biorefinery. As an alternative to conventional processes (such as solid-liquid extraction or Soxhlet extraction), green processes have been proposed as a clean, sustainable and environmentally friendly approach. **Table 2** shows a list of alternative processes that have been recently used to extract compounds from many sources, including macro and microalgae. These processes are microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), enzyme-assisted extraction (EAE), supercritical fluid extraction (SFE), pressurized liquid extraction (PLE) in which subcritical water extraction (SWE) is included, and gas-expanded liquids (GXLs). Even if these techniques are based on different principles, all of them have in common the use of minimal amount of food-grade solvents and its intensification through the employment of microwaves, ultrasound, enzymes or high pressure/temperature (Mendiola et al. 2013) that allows improving the selectivity, and the global efficiency, of the extraction process.

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3.2.2.1 Assisted-extraction techniques

Microwave-assisted extraction is based on the use of microwave radiation that causes heat both inside the matrix and the solvent. In algae, this heat provokes an enormous pressure inside the cells and favours the rupture of the cell wall, thus exposing its constituents to the solvent. Furthermore, the heat helps the solvent to diffuse into the cells, thus improving the transfer of the bioactive compounds between the matrix and the solvent (Tatke and Jaiswal 2011). MAE has been widely used to extract bioactives from algae such as lipids, high value pigments, proteins, vitamins, carbohydrates and others (Kapoore et al. 2018). Although different organic solvents can be employed, those selected for MAE applications should absorb microwave radiation and, therefore, usually polar and protic solvents are used. Some applications of MAE for the extraction of lipids, pigments and proteins from different algae species such as *Chlorella sp*., *Nannochloropsis salina*, *Phaeodactylum tricornutum* and *Porphyridium purpureum* have been developed using GRAS solvents (Gilbert-Lopez et al. 2017a; Juin et al. 2015; Martinez-Guerra et al. 2014; Patil et al. 2013).

For instance, Martínez-Guerra et al. (Martinez-Guerra et al. 2014) studied the extraction of lipids from microalgae using MAE. In this case, algal lipids were extracted from dry *Chlorella* sp. using ethanol as solvent. In comparison to the conventional Bligh and Dyer (BD) method, they obtained an increase in lipid extraction yields (from 13.9 % to 20.1 %) with a higher fatty acids ethyl esters conversion of the algal lipids (from 78.1 % up to 96.2 %) under optimum conditions (algae biomass:ethanol molar ratio of 1:250-500 and 2.0-2.5% sodium hydroxide catalyst with reaction times around 6 min).

Another interesting approach was given by Gilbert-López et al. (Gilbert-Lopez et al. 2017a). They used MAE to obtain high valuable extracts from *Phaeodactylum tricornutum.* Under optimum conditions (30 °C, 100% ethanol and 2 min of extraction), they obtained a higher extraction yield (14.51 %) and recovered a good amount of lipids such as EPA and carotenoids such as fucoxanthin, even higher than those reported from brown algae.

Ultrasound-assisted extraction also relies on the disruption of cell walls, increasing the contact between solvent and matrix. In this case, the driving force that favours the extraction of the bioactives is the acoustic cavitation produced by the use of high-frequency sounds. Some algae such as *Arthrospiraplatensis* and *Chlorella sp.* have been used to extract valuable compounds using UAE, with an important increase in the extraction yield. As an example, Zhao et al. (Zhao et al. 2013) studied different treatments to extract carbohydrates from fresh *Chlorella* sp. UAE treatment showed the best results, reaching the maximum glucose yield (36.94 ± 2.46 g per 100 g dry cell weight) considering the following extraction conditions: ultrasonic power of 800 w, extraction time of 80 min, flow rate of 1.52 L/min and cell concentration of 0.3 g/L. Another interesting example was given by Hadiyanto and Suttrisnorhadi (Hadiyanto and Suttrisnorhadi 2016), who efficiently extracted phycocyanin from *Arthrospiraplatenis* using UAE. Results showed a significant increase of the extraction yield using UAE (up to 15.7 %) in comparison to conventional extraction (11.13 %) under UAE optimal conditions (52.5 ºC, 42 min of extraction time and ultrasound frequency of 42 Hz).

One interesting aspect common to both techniques is that it is possible to extract bioactive compounds directly from wet biomass without using any solvent. For instance, Adam et al. (Adam et al. 2012) performed a solvent-free ultrasound-assisted extraction from fresh *Nannochloropsis oculata* biomass in order to recover lipids. As the water of the wet alga was used as solvent, lipids were effectively separated in two distinct phases, simplifying the oil recovery. Furthermore, using scanning electron microscopy (SEM), they could observe that after UAE, external structure of cells surface had changed, in contrast to non-treated cells, which appear to be intact. This means UAE directly from fresh microalgae cells could be an innovative and sustainable option to extract lipids from microalgae.

Passos et al. (Passos et al. 2015) studied both pretreatment methods (MAE and UAE) directly from microalgal biomass, finding that all pretreated microalgal biomass had a higher content of all soluble organic macromolecules (proteins, carbohydrates and lipids) than non-pretreated biomass. However, these procedures can damage or degrade thermolabile compounds if extraction conditions are carried out under extremely high temperatures.

Another alternative extraction method relies on the use of enzymes, which are capable of degrade or disrupt cell walls and membranes, thus allowing a better release of bioactives (Munish et al. 2012). In vegetable matrices, pectinases, cellulases and hemicellulases are commonly used. Since algae have a similar cell wall, these enzymes have been also employed for degradation of their cell walls, as many authors have confirmed. For instance, Zuorro et al. (Zuorro et al. 2016) used

a multi-enzyme pretreatment based on cellulase and mannanase enzymes for the release of intracellular material, specifically lipids, from the marine microalga *Nannochloropsis* sp, reaching up to 90 % of lipid recovery under optimal conditions. Another interesting example was given by Huo et al. (Huo et al. 2015). They applied a mixture of enzymes (cellulase, pectinase and hemicellulase) to extract oil from wet microalgae *Scenedesmus* sp. G4, obtaining up to 86.1 % of lipids under optimal conditions and proving the great impact of enzymes on the integrity of microalgae cell. The main problem encountered by using this methodology is the low efficiency of the lysis process and the time required to complete the reaction (that can take from hours to days) (Grosso et al. 2015).

3.2.2.2 Compressed fluids' extraction techniques

Compressed fluids' extraction techniques such as SFE, PLE, SWE or GXL are the most innovative methods that have been recently used to obtain high value compounds from many matrices, including macro and microalgae. The main advantage is that all of them can use green solvents such as CO_2, water or ethanol. Furthermore, the possibility of changing the solvent physicochemical properties and solvating power by changes in pressure and/or temperature of the system provides a great selectivity and efficiency for obtaining a huge range of bioactives with different characteristics.

Despite several differences in the basic principles of SFE, GXL and PLE, they all have in common that they must operate under medium-to-high pressures; for this reason, it is possible to use the same equipment for the three extraction techniques. SFE is based on the use of solvents at temperatures and pressures above their critical points, while PLE operates using liquids at temperatures above their normal boiling points and pressures enough to keep the extracting fluid in the liquid state. GXLs extraction is an intermediate technique between PLE and SFE. GXLs are liquids whose volume has been increased when pressurized with a condensable gas (e.g., CO_2). Under these conditions, at least two fluid phases or a single phase above the bubble point curve but below the critical composition exist (Herrero et al. 2013). **Figure 2** shows a general scheme of the equipment that can be used for SFE, GXL and PLE. In the following sections, a more detailed explanation on the different configurations employed for each process is included.

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3.2.2.2.1 Supercritical Fluid Extraction

Briefly, when a fluid is forced to a temperature and pressure above its critical point, it is considered to be a supercritical fluid, and it shares physicochemical characteristics from both liquid and gas states. Some of these properties are low viscosity, high diffusivity and tunable density, which can be easily modified depending on the temperature and pressure applied and, consequently, the solubility of the target compound in the fluid is also modified. Carbon dioxide (CO_2) is the most-employed fluid in SFE, since it has moderate critical temperature and pressure (31.2 °C and 73.8 bar) and it can be recycled, so it can be considered as environmentally friendly. Moreover, a very interesting point is that CO_2 will become a gas at atmospheric conditions, so once the extraction is finished, the CO_2 from the extract is directly evaporated, and the extract is completely solvent-free.

As mentioned, Figure 2 shows the scheme of a pilot plant that can be used for SFE, GXL extraction and PLE. In SFE configuration, the CO_2 is initially cooled to 0-5°C in order to be pumped as a liquid; the system includes the possibility of adding a co-solvent as modifier of the polarity of CO_2. Once the mixture is achieved, the fluid is heated to the selected extraction temperature and pumped at the selected pressure into an extraction vessel (E1 and/or E2), kept at working temperature. Algae are placed inside the extraction cell in a basket. If several extraction vessels are used, it is possible to increase productivity since while one is used for extraction, the other can be simultaneously filled with the material. Once the extraction is finished, the pressure is reduced through a control valve (R1) and the extract precipitates and is recovered in the separator vessels (S1 and/or S2). A series of collection vessels at sequentially lower pressures may be employed to trap and fractionate the extract. Flow rate and extraction pressure is controlled by the pumping rate and by the setting of the control valve for a particular pumping rate, respectively. On a pilot and industrial scale, CO_2 is recycled by condensing it, filtering it and sending it back to the reservoir for being pumped in the following extraction.

There are many reviews which summarize the potential of supercritical fluid extraction to obtain bioactives from different natural sources, including algae. For instance, SFE have been used to extract lipids from *Nannochloropsis oculata*, *Tetraselmis suecica*, *Dunaliela salina* and *Crypthecodinium cohnii*, among others; and carotenoids from *Haematococcus pluvialis*, *Chlorococcum littorale*, *Chlorella vulgaris* or *Scenedesmus almeriensis*, among others (Gallego et al. 2018).

As expected, extraction conditions are different not only depending on the compound of interest but also on the algae species. A clear example of this dependence was given by Bong and Loh (Bong and Loh 2013). In this study, they compared the fatty acid composition and tocopherol content of lipid extracts from *Nannochloropsis oculata* and *Tetraselmis suecica* using supercritical fluid extraction and optimum conditions were totally different in both algae (80 ºC, 20.7 MPa and 40 ºC, 62 MPa, respectively). The same approach occurred for carotenoids. Gilbert-López et al. (Gilbert-Lopez et al. 2017b) reported that lutein was efficiently extracted from *Scenedesmus obliquus* using SFE at 50 ºC, 36 MPa and 120 min as extraction time, whereas Macías-Sánchez et al. (Macías-Sánchez et al. 2010)

reported that the same carotenoid was optimally recovered from *Scenedesmus almeriensis* at 60 ºC, 40 MPa and 300 min as extraction time.

One of the most important drawbacks of using supercritical CO_2 ($scCO_2$) as extracting solvent is its low polarity, so polar bioactive components cannot be extracted. In this case, an alternative is the use of a polar co-solvent or modifier in small percentages (ie. ethanol from 1-15%) that allows increasing the polarity of the resulting supercritical solvent mixture, thus favouring the extraction of more polar compounds.

For instance, Solana et al. (Solana et al. 2014) used a 5 % of ethanol as co-solvent for the extraction of α-linolenic acid (αLnA) from *Scenedesmus obliquus, Chlorella protothecoides* and *Nannochloropsis salina*. The highest amount of αLnA was reached at 45 °C and 15 MPa after 30 min of extraction.

On the other hand, Ota et al. (Ota et al. 2009) extracted β-carotene from *Chlorococcum littorale* comparing SFE with and without ethanol as co-solvent, reaching a high yield (up to 90 %) with 10% of ethanol and optimum conditions of 60 ºC, 30 MPa and 180 min of extraction time; a yield of 40% was obtained with pure CO_2 as extracting solvent.

Selection of co-solvent is also important for the bioactivity of the obtained extract. For example, Saravana et al. (Saravana et al. 2017) compared sunflower oil, soybean oil, canola oil, ethanol, and water as co-solvents to support $scCO_2$ extraction of carotenoids, mainly fucoxanthin, and phlorotannins from brown seaweed *Saccharina japonica*. A 2% sunflower oil as co-solvent showed higher carotenoid content and antioxidant activity than the control ($scCO_2$ only).

Regarding microalgae extraction, in general, a drying step prior to $scCO_2$ extraction is required because they are grown in liquid cultures. Reyes al. (Reyes et al. 2016) studied the direct extraction of carotenoids from *Neochloris oleabundans* paste (containing around 70-80 % water) mixing this paste with adsorbents as supporting media. Results showed that chitosan was the adsorbent with better adsorbent capacities for the recovery of carotenoids. These results are interesting to avoid the drying step, which is energy consuming and could be detrimental for the bioactivity of the extracted compounds.

3.2.2.2.2 Gas Expanded Liquid Extraction

When increasing the amount of polar solvent mixed with CO_2, a different type of solvent is achieved; the so-called “carbon dioxide expanded liquid (CXL)”. CXL is a particular case of gas expanded liquid (GXL) in which carbon dioxide is using as expanding media; CXLs are considered to be half way from pressurized liquids to supercritical fluids (Herrero et al. 2017).

In general terms, GXLs have densities similar to that of organic solvents (without CO_2 added), while their viscosities are between those of supercritical fluids and liquids. GXLs show a wide range of physicochemical properties compared to

supercritical fluids, since more diverse properties can be obtained considering the wide variety of different green organic solvents that can be employed (Cunico and Turner 2017). Several physicochemical properties change by changing the pressure and/or temperature in CXL systems, among them: density, compressibility, viscosity, mass transfer and dielectric properties. For more in depth information about GXLs, readers are referred to Sanchez-Camargo et al. (Sánchez-Camargo et al. 2018).

As shown in **Figure 2**, the equipment needed to work under CXL conditions is the same that the one required for carrying out SFE; the only difference is that under CXLs conditions, a higher amount of solvent is used and, commonly, lower pressures are employed. In general, the instrumentation consists of 2 pumps, one for carbon dioxide and another one for the solvent, a system for heating the extraction cell(s) (medium-high pressure vessel(s)), valves for controlling the fluid flow path and pressure and a collection device. Operation starts by mixing the liquid solvent with CO_2 at medium-high pressures (CO_2 will expand and the volume of the fluid mixture will increase, depending on the pressure conditions); the fluid is then injected in the medium-high pressure vessel where the extraction takes place (at certain temperature conditions controlled by a heating system); after the extraction time, the outlet valve (R1) is open to control flow/pressure and the extract is continuously collected in a separator vessel (S1).

Some interesting applications of CXLs for the extraction of bioactive compounds from algae have been recently published. For instance, Golmakani et al. (Golmakani et al. 2012) described one of the very first uses of GXL to algal biomass. In this case two alternative extraction techniques (GXLs and pressurized ethyl lactate:ethanol) were applied to obtain high-value lipids from *Arthrospira platensis*. Results obtained after chemometric optimization allowed understanding the effects of the different factors involved in the studied processes and provide the optimum conditions to get the maximum γ-linolenic acid (γLnA) recovery and lipids' yield. GXL (40ºC, 300 atm, 50% ethanol, 90 min extraction time) provided γLnA recovery of 24.7% and total yields of 6.7% (w/w), while PLE (180 °C, 20.7 MPa, ethanol:ethyl lactate 1:1 and 15 min extraction time) provided total yields up to 20.7% (w/w) and γLnA recoveries of 68.3%. In this case GXL provided lower yields and recoveries than PLE, but gave higher selectivity and demonstrated its performance as intermediate between PLE and supercritical fluids for the extraction of medium-polar compounds.

Reyes et al. (Reyes et al. 2014) used a Box–Behnken experimental design to examine the effects of mild operating temperature (40–70 °C) and pressure (20–35 MPa), using ethanol in $scCO_2$ (0–13% w/w) on the astaxanthin content, extraction yield, and antioxidant activity of *Haematococcus pluvialis* extract. Since astaxanthin is a carotenoid whose molecular weight and functional groups give low solubility in $scCO_2$, two approaches can be followed to increase its extraction: the first one is to force the extraction by increasing the extraction time and pressure (above 50 MPa), while the other is to employ higher amount of ethanol to increase astaxanthin solubility in $scCO_2$. In the work by Reyes et al. 2014, after demon-

strating the important effect of ethanol content in supercritical CO_2 (more significant than pressure and temperature), authors move to the GXL region using higher ethanol content (50–70%, w/w), mild temperature (30–60 °C) and low pressure (7 MPa). Comparing CXE (Carbon Dioxide Expanded Extraction) with $scCO_2$ at optimum extraction conditions (20 MPa, 13% (w/w), 55 °C for $scCO_2$ and 7 MPa, 50% (w/w) ethanol, 45 °C for CXE), CXE showed better results in terms of extraction yield, astaxanthin content and astaxanthin recovery than $scCO_2$ extraction. In fact, these results were better than any previously published manuscript concerning astaxathin extraction from *H. pluvialis.*

3.2.2.1.3 Pressurized Liquid Extraction

Pressurized liquid extraction (PLE) is based on the use of high temperature (below the critical point) and pressures enough to keep the solvent in liquid state. If water is used as extracting solvent, it is called subcritical water extraction (SWE) or pressurized hot water extraction (PHWE) and can be considered as the greenest alternative involving the use of pressurized liquids. Thanks to the high temperatures and pressures, the solvent possesses increased solubility and decreased viscosity, allowing a better mass transfer rates and penetration into the matrix while improving the efficiency of the extraction process.

When working under PLE conditions, instrumentation needed consists on: a solvent pump, an extraction vessel (E1), pressure valves, heating systems for controlling temperature and a collection vessel (S1). The solvent is introduced inside the extraction cell by the pump (pressures required range between 35 and 200 bar). Pressure is controlled inside the extraction cell by two on/off valves (or one on/off valve and the restrictor, R1) and the extraction cell is placed inside a heating system, which controls the applied temperature (usually, high temperature area employed is above the boiling point of the solvent and below its critical point). A collection vessel is needed to recover the extract. It is important to mention that the solvents employed for the extraction should be oxygen-free in order to avoid oxidation of the bioactives as well as to prevent cavitation in the pump; degassing by ultrasounds or helium purge are two systems that can be employed for this purpose.

Depending on the matrix and on the target compound(s), a proper selection of the extracting solvent is needed. Thus, for the extraction of more polar lipids such as short-chain fatty acids and tocopherol or carbohydrates, water can be chosen, whereas less polar lipids such as PUFAs can be extracted using ethanol (Pieber et al. 2012; Rodriguez-Meizoso et al. 2010). On this sense, Otero et al. (Otero et al. 2018) studied the selectivity of five solvents of different polarities (hexane, ethyl acetate, acetone, ethanol and ethanol:water 50:50) in the lipid composition of *Fucus vesiculosus* by PLE. Results showed that long chain fatty acids including oleic acid, arachidonic acid and EPA are selectively extracted using ethyl acetate, producing extracts that at least double the fatty acids quantity in comparison to the

other solvents. Nevertheless, the lowest ω-6/ω-3 ratio was achieved with ethanol:water 50:50 (the most polar solvent) with a value of 1.92, much lower than those recommended by FAO (ω-6/ω-3 = 10) (FAO 2010). It is well-know that a low ω-6/ω-3 ratio exerts suppressive effects on cardiovascular diseases (Simopoulos 2002).

Several examples can be found in the literature about the use of PLE to extract carotenoids from many different algae species using different solvents such as ethanol, water, acetone and their mixtures. The diversity of solvents that have been employed can be explained by the wide range of polarities of bioactive carotenoids; for example violaxanthin, neoxanthin and lutein could be effectively extracted from *Chlorella vulgaris* using acetone at 50 ºC and 10 MPa (Merichel Plaza et al. 2012); fucoxanthin and zeaxanthin could be extracted from *Himanthalia elongata* using ethanol as solvent at 100ºC and 10.3 MPa (M. Plaza et al. 2010); and also from *Phaeodactylum tricornutum* using ethyl acetate at 100 °C and 10 MPa (Derwenskus et al. 2018); astaxanthin and derivatives could be efficiently extracted from *Haematococcus pluviales* using pressurized ethanol at 50 ºC and 10.3 MPa (Jaime et al. 2010).

3.3 Integrated processes

The integration of processes dealing with the extraction of bioactive compounds from macro- and microalgae is a hot topic. Some interesting approaches have been employed in the literature; for instance, Hernandez et al. (Hernandez et al. 2014) studied the effect of microwave pre-treatment previous to $scCO_2$ extraction in different microalgae. Interestingly, authors reported that the microwave (MW) effect strongly depend on the microalgae tested; whereas in the microalga *Scenedesmus almeriensis* a positive effect on the yield of lipids was shown, *Nannochloropsis gaditana* seemed to be negatively affected by the microwave-assisted pretreatment. The same approach was studied in *Chlorella vulgaris*, in which Dejoye et al. (Dejoye et al. 2011) concluded that the integration of MAE and $scCO_2$ extraction gives a high quality and yield of recovered lipids.

Among the most promising integration of processes are those involving the coupling of extraction and purification, considering that depending on the extraction conditions and the chemical characteristics of the target compound, sometimes it would be difficult to obtain pure extracts. Supercritical antisolvent fractionation (SAF), supercritical antisolvent (SAS) or solution-enhanced dispersion (SEDS) by supercritical fluids are processes that could be coupled on-line to obtain dried encapsulated particles. In general terms, these techniques are based in contacting an organic solution with $scCO_2$. During mixing, the rapid mutual diffusion at the interface of $scCO_2$ and the liquid extract containing the compounds causes the precipitation of solutes, allowing to obtain completely solvent-free products. These processes can also be used to encapsulate or co-precipitate target compounds by super saturation of the polymer/solute, leading to sub-micrometric particles with controlled size. For example, Machado et al. (Machado et al. 2016) coupled an enzymatic lysis assisted by ultrasounds, without biomass freezing, for the cell wall disruption of *Haematococcus pluvialis*, with the subsequent encapsulation of carotenoids in the copolymer poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV) using SEDS technique.

3.4 Biorefinery

The concept of biorefinery relies on the capability of improving the recovery of different products from a unique biomass. In other words, the main idea consists on the integration of multiple and sequential processes that allow the fractionation of a single biomass into different and isolated compounds of high added value (Subhadra and Grinson 2011).

The biorefinery concept not only consists on the integration of multiple processes to obtain different products but also on the optimal exploitation of the available resources. In this sense, a microalgae biorefinery platform was designed in our research group involving the integration of compressed fluids technologies such as SFE, GXLs, and PLE in a holistic approach, in which the residue of each extraction is used as a raw material for the next step.

The compressed fluids' biorefinery platform involves the extraction of target compounds of different polarity through the addition/removal of CO_2 and therefore moving from SFE (with neat CO_2) to conventional organic solvents (working under high pressure and temperature) and considering, as intermediate steps, the use of CO_2 plus modifier and/or CXLs. In this approach, working under medium/high pressures, different physicochemical properties can be conveniently modified through the addition of compressed CO_2 (such as polarity, viscosity and diffusivity) (Herrero et al. 2017).

Figure 3 shows a scheme of the compressed fluids' platform mentioned above. In this kind of biorefinery platform the residue of one extraction is the matrix to be

treated in the next step; taking into account that all the steps are done in the same equipment, different extraction processes (carried out at medium-high pressure) were sequentially used to extract valuable compounds from algae biomass. Biorefinery started using a dry biomass sample and applying a SFE (CO_2 as solvent) as first step to obtain non-polar bioactives, including carotenoids and lipids; the residue of SFE was subsequently treated with a CXL (and/or PLE with ethanol) to obtain the polar lipids, carotenoids and chlorophylls; and finally, by means of SWE, sugars and proteins were obtained. By this approach, the sample is treated with increasing polarity solvents to provide different extracts enriched in valuable compounds. This was the approach followed by Gilbert-López et al. (Gilbert-Lopez et al. 2015) in which different compounds were obtained from *Isochrysis galbana*. Thus, extraction process was partially selective according to the polarity of the solvent/mixture of solvents used. First extracts using $scCO_2$ were rich in triacylglycerides, while extracts obtained using CXL were rich in fucoxanthin, the main carotenoid in *Isochrysis galbana*. Following steps provide with extracts enriched in proteins and carbohydrates.

Similar results were obtained using *Scenedesmus obliquus* as dry biomass. In this case, not fucoxanthin but lutein and β-carotene were extracted in the GXL step (Gilbert-Lopez et al. 2017b).

It is worth mentioning that the same biorefinery approach can be used to extract compounds starting from high polarity to low polarity, by just inverting the order of the processes involved (PLE with water, PLE with ethanol, CXL and SFE with neat CO_2). The viability of this approach has been recently demonstrated considering wet microalgae as starting material (Ibáñez et al. 2017).

It is also important to emphasize that through the integration of green chemistry into biorefineries and the use of low environmental impact technologies such as those based on the use of compressed fluids, future sustainable production chains of biofuels and high value chemicals from biomass can be established, thus improving the economic viability of the whole biorefinery.

4. Conclusions

In this book chapter, we presented and overview of the bioactive compounds that can be obtained from macro and microalgae with potential use in the food, cosmetic and pharmaceutical industries. Although not exhaustive, the information has been selected considering some of the most important compounds that can be synthesized by algae and can provide benefits for human health. Some of them are major components such as proteins, lipids and carbohydrates and other minor components (secondary metabolites) generated to protect algal cells against stress conditions. Emphasis has been put on the different possibilities for promoting the enrichment in high value metabolites, ranging from marine biotechnology to processes (both, upstream and downstream) that can be optimized to obtain highly en-

riched fractions in different components. But, the main focus of the chapter has been the description of new technologies to extract valuable compounds from algae; among them some extraction processes assisted by microwaves, ultrasounds or enzymes and processes based on the use of compressed fluids (SFE, GXL, PLE and SWE). In the framework of this book, these processes have in common that they are greener, more efficient, avoid the use of toxic organic solvents and can be sustainable. Several recent on the application of these technologies to the extraction of valuable compounds from algae are described in the text demonstrating the usefulness and the advantages of such processes compared to conventional ones. Finally, a biorefinery platform based on compressed fluids technology is presented as an example of the possibilities offered by these technologies to completely valorize algae biomass. This platform is intended to be placed in a whole process involving the optimization of the different necessary 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, and processing of the oily fraction to produce biofuels; exhausted material can be also used for other purposes (such as fabrication of furniture, etc.). This way it will be possible to move towards a more sustainable world, in which circular economy will take the lead and sustainable development challenges will start to be met.

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Table 1. Functional compounds found in some algae and possible health effects (based on references Ibañez and Cifuentes 2013; Sathasivam and Ki 2018)

Functional compound	**Possible health benefit**	**Macroalgae**	**Microalgae and cyanobacteria**
PUFAs	Reduce risk of certain heart diseases	*Himanthalia elongate, Undaria pinnatifida, Porphyra* spp., *Chondrus crispus*	*Dunaliella salina, Haematococcus pluvialis, Chlorella* spp., *Arthrospira platensis*
Vitamin E	Antioxidant activity		*Porphyridium* spp.
α-Tocopherol	Antioxidant activity	*Himanthalia elongate*	
Folates	Reduce risk of certain types of cancer	*Undaria pinnatifida*	
Sterols	Reduce total and LDL cholesterol and immunosuppressant effects	*Himanthalia elongate, Undaria pinnatifida, Porphyra* spp., *Chondrus crispus, Cystoseira* spp., *Ulva* spp.	*Dunaliella salina, Haematococcus pluvialis, Chlorella* spp.
Pheophorbide a-, b-like compounds	Inhibition of cytopathic effect of herpes simplex Virus 1		*Dunaliella salina*
Phycobiliproteins	Immunomodulation activity, anticancer activity, and hepatoprotective, anti-inflammatory and antioxidant properties		*Arthrospira platensis*
Allophycocyanin	Inhibition of cytopathic effect, delay in synthesis of viral RNA of enterovirus		*Cryptomonads*
Soluble fiber	Reduce total and LDL cholesterol	*Himanthalia elongate, Undaria pinnatifida, Porphyra* spp., *Chondrus crispus*	
Alginic acid, xylofucans	Antiviral activity	*Sargassum vulgare*	
Sulfated polysaccharides	Regulate the bioactivity of growth factors and cytokines, apoptotic, antiviral, antitumour, antihyperlipidaemia, and anticoagulant activities	*Undaria pinnatifida, Porphyra* spp., *Chondrus crispus, Cystoseira* spp., *Ulva* spp.	*Dunaliella salina, Haematococcus pluvialis, Chlorella* spp.,*Arthrospira platensis, Porphyridium* spp.
Polysaccharides	Inhibition of hyaluronidase of herpes simplex and influenza A virus and antileukaemic activity		*Navicula directa, Gymnodinium* sp., *Gyrodinium impudicum*
Phenolic acids	Antioxidant activity		*Arthrospira platensis*

Terpenes	Valuable curative properties	*Cystoseira* spp.	
Fucoxanthin	Preventive effect on cerebrovascular diseases, increase the metabolism, antioxidant	*Undaria pinnatifida*	*Isochrysis galbana, Phaeodactylum tricornutum*
Diadinochrome A, B, diatoxanthin/cynthiaxanthin	Cytotoxic effect in HeLa cells		*Peridinium bipes*
Carotenoids	Antioxidant, immunomodulation and cancer prevention	*Ulva* spp.	*Haematococcus pluvialis, Chlorella* spp.*, Muriellopsis* spp. *Scenedesmus* sp.*, Porphyridium* sp.
Karatungiols	Antifungal, antiprotozoan		*Amphidinium* spp.

Table 2. Advantages and disadvantages of alternative downstream extraction processes (based on references Grosso et al. 2015; Herrero et al. 2017)

	Advantages	**Disadvantages**
MAE	- Short treatment time and solvent consumption; - More efficient than conventional heating; - Reduction of extraction temperature using pressurized closed vessels; - Organic solvents and water can be used; - High extraction yields.	- Only solvents with high dielectric properties can be used; - Possible thermal degradation of the most thermolabile compounds when using open vessels; - High energy consumption.
UAE	- Short treatment time and solvent consumption; - High efficiency in cell disruption; - High extraction yields; - Suitable to extract thermolabile compounds; - Inexpensive.	- Solvents with low surface tension, low viscosity and low vapor pressure are preferable; - The presence of a dispersed phase contributes to the ultrasound wave attenuation; - Ultrasounds generate heat, being important to accurately control the extraction temperature; - Excess of sonication may damage the quality of extracts.
EAE	- Water can be used (Green technology); - The enzyme treatment can increase the recovery of bioactive compounds.	- The efficiency of enzymatic hydrolysis is very low if materials have low moisture content; - Enzyme treatment is usually a slow process, and it may take from hours to days.
SFE	- Green technology; - Higher selectivity because the solubility of a compound in a supercritical fluid can be manipulated; - It is possible to extract more polar compounds with the use of modifiers - Elimination of CO_2 is achieved without residues, yielding a solvent-free extract; - Suitable to extract thermolabile compounds.	- High costs for the high pressure equipment needed; - Can be more time-consuming than the other alternative techniques.
PLE / SWE	- Green technology in the case of pressurized water extraction (SWE); - Reduced solvent consumption; - Suitable to extract thermolabile compounds.	- High costs for the high pressure equipment needed; - Extractions performed at high temperatures may lead to degradation of thermolabile compounds.
GXL	- Can be consider as a half way from PLE to SFE by increasing the amount of compressed CO_2. - Requires lower working pressures (compared to SCFs) and the subsequent reduction in energy consumption and costs. - Suitable to extract compounds with intermediate polarity.	- Can be more time-consuming than the other alternative techniques.

Figure 1. Possibilities of increasing the production of valuable metabolites from algae.

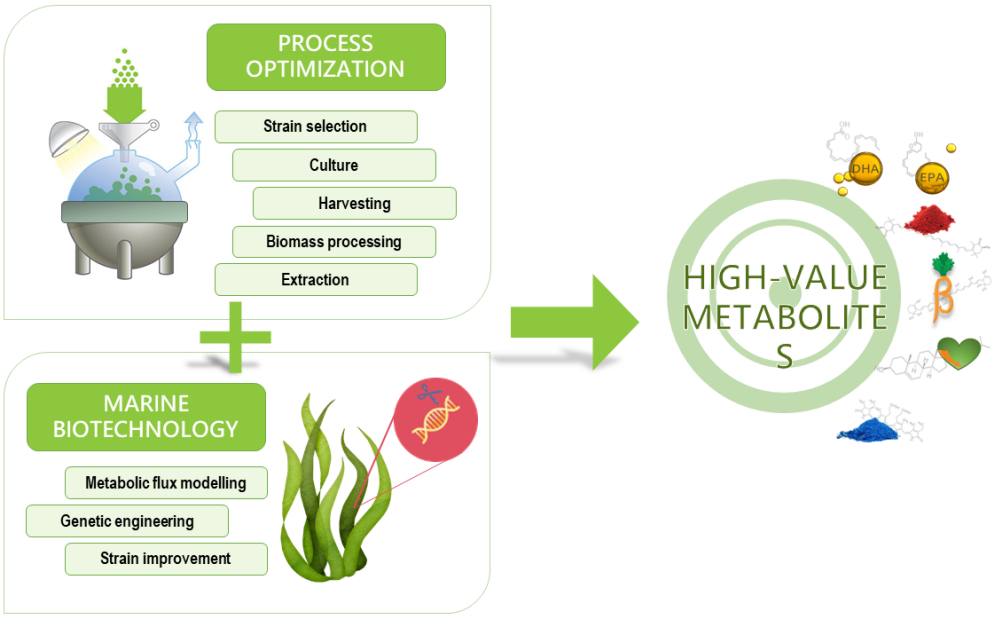

Figure 2. General scheme of equipment used for SFE, CXL and PLE.

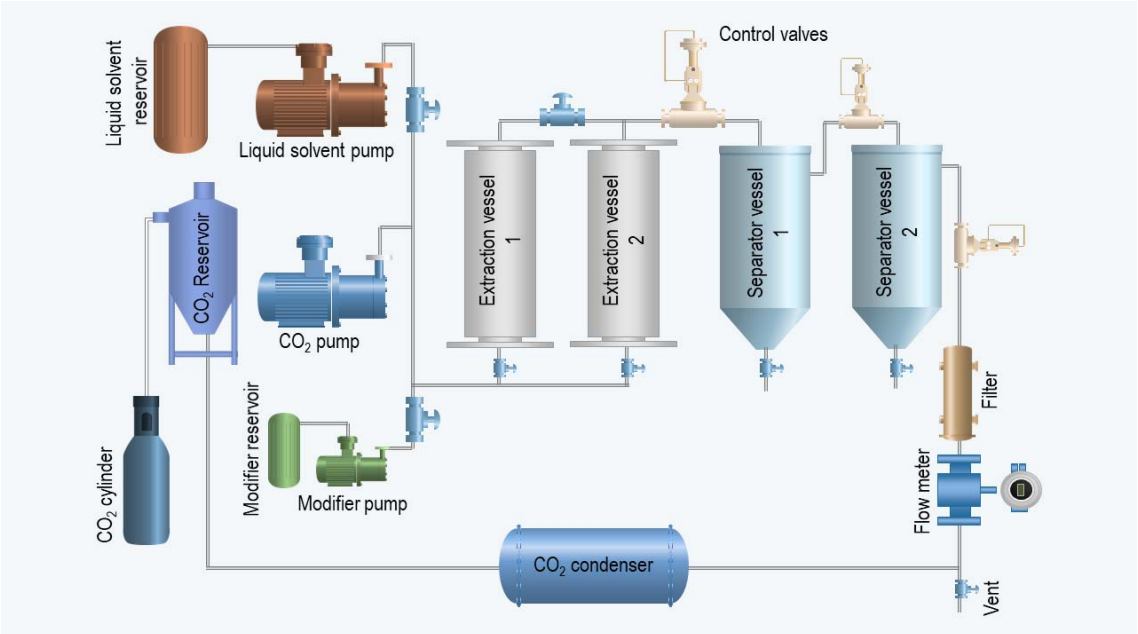

Figure 3. Downstream process for microalgae biorefinery