

Review

Supercritical Fluids Extraction of Valuable Compounds from Algae: Future Perspectives and Challenges

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Abstract. Algae (macro and micro) can be used to produce several high-value metabolites to supply industries as cosmetics, additives and pigments, among others. Those metabolites can have physiological and nutritional benefits for human and animal health. However, the availability of high-value metabolites from algae is still unaffordable due to traditional extraction techniques and their requirements of energy and use of pollutant solvents. Recently, green extraction technologies for the extraction of high-value metabolites have become more desirable due to their sustainability and environmental benefits. Supercritical fluids extraction, as green extraction techniques, has been widely applied for extraction of high-value metabolites from algae. Here, the highlight of supercritical CO₂ and subcritical water on the extraction of bioactive compounds from macro- and microalgae was presented. The perspective and challenge for using supercritical CO₂ and subcritical water on the algae extraction were also concluded.

Keywords: Extraction, macroalgae, microalgae, subcritical water, supercritical CO₂.

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1. Introduction

Algae, the aquatic biomass, are considered a promising sustainable feedstock for food and feed products, materials, chemicals, fuels and various high-value products [1–3]. Algae are diverse aquatic, photosynthetic organisms [4,5] characterized by many different phyla and different physiological attributes, representing 10% of the plant kingdom [6]. Algae are divided in two groups: macroalgae, such as red, green and brown algae and microalgae, such as blue-green algae [7].

Macroalgae, or seaweeds, are classified into 3 major groups based on the pigmentation: *Rhodophyceae* (red algae), *Phaeophyceae* (brown algae), *Chlorophyceae* (green algae). Some specific commercially important cultivated seaweeds and seaweed products include the brown seaweed *L. japonica*, wakame from the brown seaweed *Undaria pinnatifida*, Hizikia from *Hizikia fusiforme* and the high-value product Nori from the red seaweed *Porphyra* sp. [8]. Macroalgae provide a wide range of food and food ingredients with pharmaceutical, bio medicinal and nutritional importance [9]. They are generally fast growing and are able to reach sizes up to 20 m in length. Growth rates of macroalgae far exceed those of terrestrial plants. Cultivation of macroalgae at sea, which does not require arable land and fertilizer, are considered to be important feedstock for future bio refineries (co-producing biofuel and bioenergy). Macroalgae are mainly utilized for the production of food and the extraction of hydrocolloids [10]. Moreover, macroalgal biomass can be used in ethanol fuel production due to its high amounts content of sugars (at least 50%) [11].

Microalgae are the most primitive and simply organized members of the plant kingdom, with the majority existing as small cells of about 3 – 20 μ m, and a few species organized into simple colonies. They are extremely diverse and considered important producers of some highly bioactive compounds such as vitamins, pigments, fatty acids, sterols and polysaccharides [12–14]. There is great potential for use of microalgae in production of food ingredients, as they are photoautotrophic microorganisms that can grow on a very simple culture medium containing seawater, nitrate, phosphate, trace amounts of certain metals and carbon dioxide [15]. Some cultivated marine microalgae, such as *Spirulina*, *Dunaliella*, *Chlorella* and *Cryptocodinium cohnii*, have exhibited promising nutraceutical properties, including the presence of β -carotene, omega-3 fatty acids and antioxidants [16]. Some products obtained from microalgae include β -carotene, astaxanthin, vitamins C, A, E, H, B1, B2, B6 and B12, supplements in health foods, PUFAs and viscosifier gums from polysaccharides [15]. Commercially produced microalgal oils have been incorporated into infant milk formulations and used as dietary supplements and food additives [17]. In addition, microalgae can also provide several different kinds of renewable biofuel, including methane produced by the anaerobic digestion of the algal biomass [18], biodiesel synthesized from the micro algal oil [19] and biohydrogen produced by a photobiological mechanism [20].

The important aspect to be considered when dealing with obtaining bioactives from algae is the development of appropriate, fast, cost-effective and environmental-friendly extraction processes able to isolate the compounds of interest from these natural sources. Many extraction techniques have been used for the discovery of various bioactive compounds such as Soxhlet extraction, maceration and hydro-distillation. However, these techniques can be time consuming and costly, consume large quantities of highly pure organic solvent, poor selectivity and have a risk of decomposition of thermolabile compounds [21]. These difficulties have led to the development of the sub- and supercritical fluids extraction technique using supercritical CO₂ or subcritical water as a solvent. These solvents are safe and nonflammable.

In supercritical CO₂ extraction, the separation step to recover the target product may be avoided, since CO₂ is gaseous at ambient pressure. The selectivity is obtained by varying pressure and temperature. Moreover, the depressurization can be done step by step, allowing a fractionating of the extracted compounds, based on their solubility variation with density. The extract yields, which depend on experimental conditions, can be the same as (or even higher than) those obtained with extraction processes using organic solvents, for shorter extraction durations. The environmental benefit in using algae is also more significant if extraction is done using a nonpolluting solvent such as supercritical CO₂. In addition, the very low critical temperature of CO₂ allows its use for thermolabile compound extractions. Supercritical CO₂ solubilizes nonpolar compounds; and when the compound of interest is not soluble, the solvent power can be increased using a safe and polar modifier, such as ethanol [22].

Subcritical water extraction technique which is also known as pressurized hot water extraction and superheated water extraction is a green processing technology using water at high temperature (above its boiling point) and pressure enough to keep water at liquid state at the operating temperature. Water can be considered the greenest solvent to work with, it has negligible environmental effect, non-toxicity to health

and the environment and it is safe to work with and to transport. Therefore, water is the solvent to select in those applications in which polar protic solvents are needed, although considering the change in water properties with the temperature (for instance, the decrease in dielectric constant with increasing temperature), it is also able to extract medium polarity compounds [23].

Supercritical fluids technology is well-known today and is considered as a green process. Indeed, the use of organic solvent can be totally avoided, depending on the chemical nature of the extracted compounds. In supercritical CO₂ extraction, the major part of CO₂ is recycled, therefore decreasing the consumption per extracted mass. For the last three decades, a large number of industrial extraction plants using supercritical fluids have been constructed, attesting that this technology is economically viable for a large number of applications. Extraction of valuable compounds from algae with pharmaceutical, bio-medical and nutritional importance in a profitable manner can be quite challenging.

In this paper presents a recent progress in the extraction of valuable compounds from algae using supercritical fluids technology including supercritical CO₂ and subcritical water extraction for food, pharmaceutical and bio-medical application. The characterization of extracted bioactive compounds and their potential application were also summarized. Furthermore, future perspective and challenges on the production of bioactive compounds from algae were discussed.

2. Extracted Bioactive Compounds from Algae and Their Potential Application

In the following section, a brief description of the main potential of extracted bioactive compounds that may be isolated from macro- and microalgae was presented. They are antioxidants, polysaccharides, lipids and proteins.

Antioxidants. Generally, an antioxidant can be defined as any substance capable of inhibiting the oxidation of substrate significantly when present at low concentration, while antioxidant capacity can be expressed as the ability of a substance to reduce oxidation activity. It is more reactive than an oxidizable substrate. Antioxidants possess a positive effect on human health as they may protect the human body from harm by reactive oxygen species, which attack membrane lipids, proteins and DNA and cause many health disorders such as cancer, diabetes mellitus, neurodegenerative and inflammatory diseases with severe tissue injuries. Due the low of oxidative decay in the macro- and microalgae structural components and their stability to oxidation during storage, it indicated that the macro- and microalgae cells have antioxidative defense systems [24]. Hence, macro- and microalgae-derived pharmaceutically active compounds exhibit promising antioxidative effects. Antioxidant activity of macro- and microalgae-derived may arise from phenolic compounds, carotenoids and tocopherols (vitamin E). Directly or indirectly, these compounds participate to inhibition or suppression of free radical generation.

There are at least 8000 various phenolic compounds in various plant species including macro- and microalgae [25]. As the antioxidant components that have been isolated from macro- and microalgae, phenolic compounds are a class of chemical compounds consisting of a hydroxyl group (—OH) bonded directly to an aromatic hydrocarbon group and classified as simple phenols or polyphenols based on the number of phenol units in the molecule [26,27]. Based on their structure, phenolic compounds can be divided into several types, such as simple phenols, phenolic acids, hydroxycinnamic acids, coumarins, naphthoquinones, xanthenes, stilbenes, anthraquinones, flavonoids and lignins.

Similar to phenolic compounds, carotenoids can also act as antioxidants by scavenging and deactivating free radicals [28]. These substances are a family of pigmented compounds that are widely distributed in nature and synthesized by plants, algae, fungi and microorganisms, but not animals. In nature, carotenoids possess responsibility on the various colors of different photosynthetic organisms. The basic structure of carotenoid is constructed by eight isoprenoid units that form the symmetrical skeleton of the compound, along with a long chain with associated double bonds. Carotenoids have been considered to be responsible for beneficial properties in preventing human diseases including cancer, cardiovascular diseases, macular degeneration and other chronic diseases [29–33]. Other interesting natural antioxidants in macro- and microalgae is tocopherols. These compounds are also the most abundant groups of lipid-soluble antioxidants in chloroplasts [34]. Tocopherols are effective chain-breaking antioxidants as they may generate stable and relatively unreactive antioxidant radical. The tocopherols antioxidative action was indicated by the hydroxyl group in the chromanol ring, which transfer a hydrogen atom to inactivate free radicals.

Polysaccharides. Carbohydrates were defined as polyhydroxy aldehydes, ketones, alcohols, acids, their simple derivatives and their polymers having linkages of the acetal type. They can be divided into monosaccharides (simple sugars), disaccharides (two covalently linked monosaccharides), oligosaccharides (containing three to ten monosaccharides) and polysaccharides (containing ten or more monosaccharides). Of these, polysaccharides are relatively complex carbohydrates and already proved to have several important properties. These macro- and microalgae-derived active ingredients have been known as monosaccharides conjugated polymers by glycosidic bonds and applied in numerous commercial applications. Due to the biological properties of them, polysaccharides were known to possess antioxidative, antiviral, antibacterial, immunostimulatory, anticoagulant and anticancer effects. These macro- and microalgae-derived active ingredients were also used to combat obesity [35–37].

It has been known that marine algae including macro- and microalgae contain important polysaccharides which possess potential applications in medicine, food and pharmaceutical industry (cosmeceuticals, nutraceuticals). The major polysaccharides in macroalgae cell walls are carrageenans, agar and alginates. Other polysaccharides such as sulfated fucose, xylans, cellulose, laminarin and Floridean starch were also obtained in macroalgae as minor components. These marine algae-derived active ingredients can be found in brown, red and green seaweed [38]. However, different polysaccharides classes including carrageenan, agar and alginates were found in microalgae. In particular, sulphated exopolysaccharides as specific classes of polysaccharides were existed and found in many species of microalgae [35]. These polysaccharides can act as antiviral agents, health foods, antioxidants. On the other hand, they also possess a role in the immunomodulatory system and anti-inflammatory properties.

Lipids. It has been known that lipid is one of the characteristic ingredients in marine resources, and marine algae including macro- and microalgae have been considered to contain high-value chemicals including lipids. These substances are naturally occurring compounds that are soluble in nonpolar solvents and insoluble in polar solvents. These physical properties can be attributed solely to the long hydrophobic hydrocarbon chains which have saturated or unsaturated chains. Saturated chains comprise of all single bonds between adjacent carbons while unsaturated chains possess double or triple covalent bonds. In most cases, the lipid derived ingredients from macro- and microalgae include glyco- and phospholipids, fatty acids, sterols and others [39–43].

Currently, most of the studies are emphasized on the fatty acids extraction from macro- and microalgae which can be applied directly for producing biodiesel. However, due to macro- and microalgae sources are widely considered as possessing interesting lipid compositions, they were subjected as a source for lipid extraction that can be applied in various applications, such as food supplements or in the chemical, pharmaceutical and cosmetic industries [43–49]. Phospholipids and glycolipids that have been classified as polar lipids were known as the major classes of lipids found in macro- and microalgae. Phospholipids are structural components of cellular membranes, whereas glycolipids are major constituents of the thylakoid membrane in chloroplasts. Phospholipids consist of a phosphate group and glycerol attached to two fatty acid chains. The combination of the hydroxyl group in glycerol with phosphoric acid may generate a polar phosphate group (glycerophospholipids or sphingophospholipids). Similar to phospholipids, glycolipids also consisted of a carbohydrate group attached to the glycerol skeleton by a glycosidic bond. Consequently, they can be applied in medical, pharmaceutical and therapeutic areas [43–50].

Proteins. Proteins are large biomolecules, or macromolecules, consisting of one or more long chains of amino acid residues. Most of them are made up of 20 different amino acids that are conjugated by substituted amide bonds. Normally, proteins possess the folded compact structure, in which hydrophilic residues are located on the molecular surfaces and nonpolar amino acids residues are located in the molecular interior. The content of proteins in macro- and microalgae have been known vary species to species and the existence of them in macro- and microalgae were also in different forms. They can be found as simple or conjugated proteins. Simple proteins consist of only amino acids and conjugated proteins contain simple proteins linked to non-proteinous substance. In addition, macro- and microalgae may contain protein derivatives, such as enzymes or peptides, as well as free amino acids [50].

Proteins are existing in all living things and possess a key role in many biological processes. The main factors that affect to the protein behavior are the composition of amino acid, the residues sequence and the molecular weight. The involvement of the different structures protein in the source of raw materials is also important factor. Consequently, proteins and their derived compounds from macro- and microalgae can be characterized by their diverse structure, their functions and their cellular location. Most of these substances

have anticancer, antioxidant, antiinflammatory, antiaging and protective activities. These macro- and microalgae proteins are also used as moisturizing agents on skin and hair [51,52]. Hence, these proteins may be effectively applied in functional cosmetics and cosmeceuticals [53].

3. Supercritical Fluids Extraction of Bioactive Compounds from Algae

The extraction of macro- and microalgae bioactive compounds have been getting more research attention and interest due to their unique composition and possibilities of various industrial applications. The biodiversity of macro- and microalgae and the diverse chemical nature of their components cause the selection of a suitable extraction method a non-trivial process. Various extraction methods have been applied and used for extraction of macro- and microalgae bioactive compounds into extracts. The conventional extraction methods, such as solid-liquid extraction and liquid-liquid extraction, are often reflected by the use of organic solvents (most frequently toxic) in large amounts and long extraction times. These extraction methods also require labor-intensive and are highly dependent on the operator. Hence, the extraction methods that characterized by a lower consumption of organic solvents, a shorter extraction time, a higher extraction yield and more environmentally friendly than conventional extraction methods should be developed.

Supercritical fluid extraction, microwave assisted extraction, ultrasound-assisted extraction, enzyme assisted extraction and pressurized liquid extraction have been considered as the novel extraction methods to extract valuable bioactive compounds from macro- and microalgae. Currently, the key requirement that should be obeyed from a novel mentioned method is its contribution to a sustainable development in environmental impact. Accordingly, a novel extraction method should fulfill as many of the twelve principles of green chemistry as possible [54]. Of these, supercritical fluid extraction especially supercritical CO₂ is an appropriate and one of the most promising future methods. This technique possess many advantages such as minimal or no use of organic solvents [55-59].

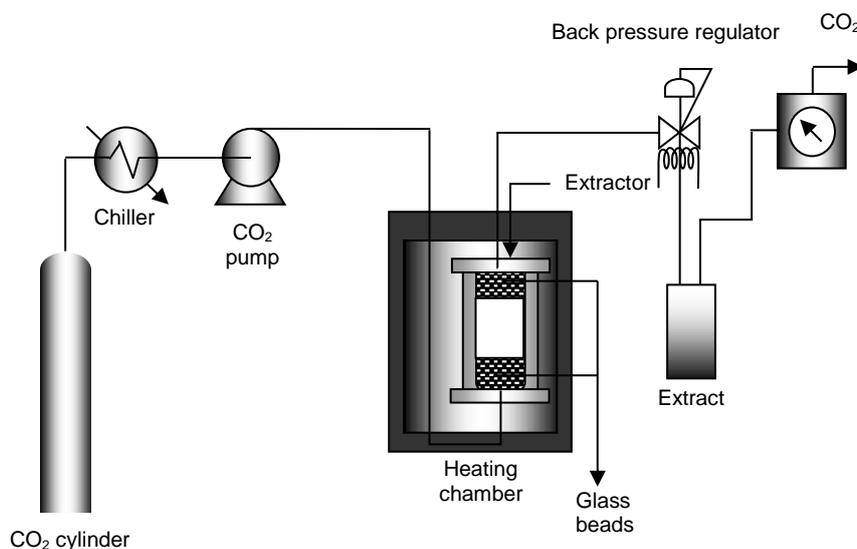


Fig. 1. Typical supercritical CO₂ extraction apparatus in laboratory-scale.

Figure 1 shows the schematic diagram of supercritical CO₂ extraction apparatus. The main apparatus consists of a high-pressure pump for CO₂, a heating chamber, extractor cell and back pressure regulator. The high-pressure pump was used to introduce CO₂ from CO₂ cylinder to the extraction cell that equipped with removable threaded covers on both sides included filters. To maintain the desired operating temperatures, the extractor was located in the heating chamber. In this system, the operating pressure was controlled by using back pressure regulator and monitored using a pressure gauge. Generally, the extraction procedures were conducted as follow. Initially, the matrix was loaded into the extraction cell and placed in the heating chamber. After the temperature at chamber heater reached to the desired temperature, CO₂ from a cylinder was firstly liquefied and then pumped into the extraction cell. Supercritical CO₂ extraction may be conducted

in three ways: static, dynamic and a combination of static and dynamic modes. In static extraction mode, the supercritical CO₂ circulates and contacts with sample matrix in extraction cell for a specified time before being released to the sample trap. In dynamic extraction mode, the sample matrix was continuously swept to the sample trap with fresh supercritical CO₂. Increasing the CO₂ flow rate may enhance extraction efficiency in this mode. In combination extraction mode, a static extraction mode is conducted in first for some period of time then followed by a dynamic extraction mode. In this mode, the static state may facilitate the analyte displacement from the sample matrix and followed by a dynamic sweep of the sample matrix cell to result a better extraction yield.

Table 1. Extracted valuable compounds from microalgae using supercritical CO₂.

Sample sources	Operating conditions	Extracted bioactive compounds	Reference
<i>Botryococcus braunii</i>	40 °C; 30.0 MPa	Hydrocarbons	[60]
<i>Chlorella vulgaris</i>	40 °C; 30.0 MPa	Carotenoids	[61]
<i>Chlorella protothecoides</i>	50 °C; 35.0 MPa	Neutral lipids	[62]
<i>Chlorella vulgaris</i>	40 °C; 30.0 MPa	Antioxidants, carotenoids, fatty acids	[63]
<i>Chlorococcum sp.</i>	60 °C; 30.0 MPa	Lipids	[64]
<i>Cryptobacterium cohnii</i>	40 – 50 °C; 20.0 – 30.0 MPa	Omega–3 fatty acid	[65]
<i>Dunaliella salina</i>	9 – 45 °C; 18.3 – 43.7 MPa	β -Carotene	[66]
<i>Dunaliella salina</i>	40 – 60 °C; 10.0 – 50.0 MPa	Carotenoids, Chlorophyll	[67]
<i>Haematococcus pluvialis</i>	40 – 90 °C; 30.0 – 64.0 MPa	Astaxanthin	[68]
<i>Nannochloropsis gaditana</i>	40 – 60 °C; 10.0 – 50.0 MPa	Carotenoids	[69]
<i>Nannochloropsis granulata</i>	50 – 90 °C; 35.0 – 55.0 MPa	Triacylglycerols	[70]
<i>Nannochloropsis sp.</i>	40 – 55 °C; 40.0 – 70.0 MPa	Polyunsaturated fatty acids	[71]
<i>Pavlova sp.</i>	60 °C; 30.6 MPa	Triglycerides	[72]
<i>Scenedesmus almeriensis</i>	32 – 60 °C; 20.0 – 60.0 MPa	β -carotene, Lutein	[73]
<i>Scenedesmus dimorphus</i>	100 °C; 40.8 MPa	Lipids	[74]
<i>Spirulina maxima</i>	20 – 70 °C; 10.0 – 18.0 MPa	Fatty acids and carotenoids	[75]
<i>Synechococcus sp.</i>	40 – 60 °C; 10.0 – 50.0 MPa	β -carotene, zeaxanthin, Chlorophyll	[76]
<i>Synechococcus sp.</i>	50 – 60 °C; 35.8 – 50.0 MPa	β -carotene	[77]
<i>Chaetoceros muelleri</i>	40 – 80 °C; 20.0 – 40.0 MPa	Fatty acids	[78]
<i>Tetraselmis chui</i>	60 °C; 25.0 MPa	Natural oil	[79]
<i>Spirulina platensis</i>	55 – 75 °C; 22.0 – 32.0 MPa	Antioxidants	[80]
<i>Spirulina platensis</i>	53 °C; 48.7 MPa	Chlorophyll a	[81]
<i>Spirulina</i>	40 °C; 31.6 – 48.4 MPa	γ -linolenic acid	[82]
<i>Spirulina platensis</i>	35 – 83 °C; 7.9 – 36.1 MPa	Vitamin E	[83]
<i>Spirulina maxima</i>	50 – 60 °C; 10.0 – 35.0 MPa	γ -linolenic acid	[83]

Sample sources	Operating conditions	Extracted bioactive compounds	Reference
<i>Chlorococcum littorale</i>	60 °C; 30.0 MPa	Carotenoids	[84]
<i>Schizochytrium limacinum</i>	40 °C; 35.0 MPa	Lipids, Oil, Fatty acids, DHA	[85]
<i>Nannochloropsis oculata</i>	50 °C; 35.0 MPa	Zeaxanthin, Carotenoids	[86]
<i>Nannochloropsis oculata</i>	50 °C; 35.0 MPa	Triglycerides, Astaxanthin	[87]
<i>Haematococcus pluvialis</i>	40 – 80 °C; 20.0 – 55.0 MPa	Astaxanthin	[22]
<i>Nannochloropsis gaditana</i> , <i>Synechococcus</i> spp. <i>Dunaliella salina</i>	40 – 60 °C; 20.0 – 50.0 MPa	Carotenoids	[88]
<i>Chlorella vulgaris</i>	40 – 80 °C; 20.0 – 40.0 MPa	Lutein	[89]
<i>Haematococcus pluvialis</i>	40 – 50 °C; 20.0 – 30.0 MPa	Lutein, astaxanthin, β -carotene and canthaxanthin	[90]
<i>Arthrospira platensis</i>	60 °C; 45.0 MPa	Carotenoids, Fatty acids, Tocopherols	[91]
<i>Chlorella vulgaris</i>	60 °C; 30.0 MPa	Carotenoids	[92]
<i>Chlorella</i> sp. T-89	50 °C; 30.0 MPa	Oil, Fatty acids	[93]
<i>Arthrospira platensis</i> , <i>Anabaena doliolum</i> , <i>Songiochloris spongiosa</i> , <i>Chlorella vulgaris</i>	40 °C; 10.0 MPa	Phenolic compounds	[94]
<i>Pavlova</i> sp.	60 °C; 30.6 MPa	Fatty acids methyl esters	[71]
<i>Scenedesmus obliquus</i> , <i>Chlorella protothecoides</i> , <i>Nannochloropsis salina</i>	45 °C; 15.0 MPa	Oil Fatty acids	[95]
<i>Nannochloropsis</i> sp.	40 °C; 30.0 MPa	Carotenoids, Fatty acids	[96]
<i>Monoraphidium</i> sp. GK12	60 °C; 20.0 MPa	Astaxanthin Chlorophyll	[97]
<i>Scenedesmus</i> sp.	60 °C; 30.0 MPa	Carotenoids	[98]
<i>Haematococcus pluvialis</i>	50 °C; 31.0 MPa	Astaxanthin, Carotenoids	[99]
<i>Nannochloropsis oculata</i>	60 °C; 40.0 MPa	Oil (Neutral lipids)	[100]
<i>Botryococcus braunii</i> , <i>Chlorella vulgaris</i> , <i>Dunaliella salina</i> , <i>Arthrospira maxima</i>	40 – 60 °C; 12.5 – 30.0 MPa	Alkadienes, Carotenoids, β -carotene, Fatty acids and lipids	[83]
<i>Chlorella vulgaris</i>	40 – 55 °C; 35.0 MPa	Carotenoids	[101]
<i>Arthrospira platensis</i>	180 °C; 20.7 MPa	Fatty acids	[102]
<i>Arthrospira platensis</i>	55 – 75 °C; 7.9 – 32.0 MPa	Vitamin E	[82]
<i>Arthrospira maxima</i>	50 – 60 °C; 25.0 MPa	Fatty acids, Lipids	[103,104]
<i>Synechococcus</i> sp.	50 – 60 °C; 30.0 – 50.0 MPa	Carotenoids, Chlorophylls	[75]
<i>Arthrospira platensis</i>	40 °C; 40.0 MPa	Fatty acids	[81]
<i>Chlorella vulgaris</i>	40 °C; 30.0 MPa	Carotenoids	[62]
<i>Haematococcus pluvialis</i>	70 °C; 50.0 MPa	Carotenoids	[105]
<i>Botryococcus braunii</i> , <i>Chlorella vulgaris</i>	40 °C; 30.0 MPa	Hydrocarbons, Carotenoids	[60]
<i>Arthrospira platensis</i>	48 °C; 20.0 MPa	Flavonoids, β -carotene, Vitamin A, α -tocopherol, Fatty acids	[106]

Sample sources	Operating conditions	Extracted bioactive compounds	Reference
<i>Haematococcus pluvialis</i> , <i>Arthrospira maxima</i>	60 °C; 30.0 MPa	Carotenoids	[107]
<i>Botryococcus braunii</i>	50 – 85 °C; 20.0 – 25.0 MPa	Fatty acids, Lipids	[108]
<i>Chlorella pyrenoidosa</i>	32 °C; 40.0 MPa	Antioxidants, Carotenoids	[109]
<i>Spirulina pacifica</i>	40 – 80 °C; 15.0 – 35.0 MPa	Carotenoids, β -carotene, β -cryptoxanthin, Zeaxanthin	[110]
<i>Chlorella pyrenoidosa</i>	50 °C, 25.0 MPa	Antioxidants, Carotenoids	[111]

Tables 1 and 2 list the applying supercritical CO₂ for macro- and microalgae processing to extract valuable compounds. Most of the supercritical CO₂ utilization on the microalgae was focused on the oil and its derived compound extraction; and it was deployed to extract carbohydrate based compounds from macroalgae when supercritical CO₂ was contacted with macroalgae. In supercritical CO₂ extraction process, the extraction of valuable compounds from macro- or microalgae proceeded in several steps [112]. Initially, the macro- or microalgae matrix absorbs the supercritical CO₂ solvent leads to the cellular structures expansion. This step may facilitate the CO₂ solvent flow via the declined resistance to mass transfer. Simultaneously, the extracted valuable compounds are dissolved in the CO₂ solvent and carried to the outer surface of macro- or microalgae matrix. Next, the dissolved compounds passed through the macro- or microalgae matrix outer surface then left the extractor cell. However, the specific valuable compounds as a target component may become the key factor to determine the selected operating condition (pressures and temperatures). Consequently, as listed in Table 1, a wider range of operating conditions has been applied on the microalgae matrix to extract valuable compounds. The most of operating conditions are 40 – 80 °C for the temperature and 10 – 60 MPa for the pressure. On the contrary, the narrow range of operating conditions was found when the supercritical CO₂ extraction was applied on the macroalgae matrix to extract their valuable components (see Table 2).

Table 2. Extracted valuable compounds from macroalgae using supercritical CO₂.

Sample sources	Operating conditions	Extracted bioactive compounds	Reference
<i>Dictyopteris membranacea</i>	40 °C; 9.1 MPa	Volatile oil	[113]
<i>Dilophus ligulatus</i>	35 – 55 °C; 8.0 – 25.0 MPa	Volatile oil	[114]
<i>Sargassum hemiphyllum</i>	40 – 50 °C; 24.1 – 37.9 MPa	Fatty acids	[115]
<i>Lessonia vadosa</i>	50 °C; 18.0 MPa	Fucosterol	[116]
<i>Sargassum muticum</i>	55 °C; 40.0 MPa	Fucoxanthin	[117]
<i>Undaria pinnatifida</i>	40 °C; 40.0 MPa	Fucoidan	[118]
<i>Gloiopeltis tenax</i>	45 °C; 30.0 MPa	Sesquiterpenes, fatty acids and their esters, phenols and sterols	[119]
<i>Fucus evanescens</i> , <i>Saccharina japonica</i> , <i>Sargassum oligocystum</i>	60 °C; 55.0 MPa	Fucoidans	[120]
<i>Hypnea charoides</i>	40 – 50 °C; 24.1 – 37.9 MPa.	Lipids	[121]
<i>Plocamium cartilagineum</i>	40 – 100 °C; 25.0 – 40.0 MPa	Halogenated monoterpenes	[122]
<i>Dunaliella bardawil</i>	40 °C; 44.8 MPa	Separation of <i>cis-trans</i> geometrical isomers of β -carotene	[123]

In supercritical CO₂ extraction, solubility is the most important factor in extraction process of particular substances contained in solid matrices. It may affect direct to the efficacy of extraction process parameters, i.e., the rate of extraction process and the yield of extract. In other words, solubility possess an impact on the extraction kinetics and efficiency of solid matrices performed under supercritical CO₂ conditions. Hence, the solubility information of substances in supercritical CO₂ is essential for the design and the operations of supercritical CO₂ extraction method [124]. In a supercritical CO₂ system, the solubility usually depends on the CO₂ density influenced by changes in pressures and/or temperatures. The influence of operating pressures obviously give a beneficial effect on yields of macro- and microalgae extraction [57]. At a constant temperature, the higher operating pressure resulted to the higher the CO₂ density and thereby improved yields and/or faster extraction kinetics. In some cases, high operating pressure can also inhibit the diffusivity of supercritical CO₂ into the macro- and microalgae matrices, therefore decreasing the extraction yield. The solubility dependence on temperature is somewhat more complex. An increase in operating temperature can cause a large decrease in the CO₂ density resulting in a decrease in solute solubility (retrograde behavior). Either a high solubility or extremely low solubility may be required depending on the desired process, however, high solubility is usually required in supercritical CO₂ extraction process.

Considering the low polarity of supercritical CO₂, this extraction method is restricted to the low or medium polarity substances and did not work well in the extraction of compounds with high polarity, for instance carotenoids. However, a change in supercritical CO₂ polarity can be attained by modifying CO₂ with cosolvents (polar modifiers) that enhance its solvating power resulting supercritical CO₂ able to extract compounds with high polarity. Ethanol is frequently used as a solvent modifier, where this solvent is efficient and less toxic. The addition of a small amount of ethanol cosolvent may increase the ability of supercritical CO₂ to dissolve relatively polar carotenoids. The addition of cosolvent can promote swelling of macro- and microalgae cells, promoting the rapid mass transfer of analytes from the macro- and microalgae matrices. In addition, ethanol may increase mass transfer by generating hydrogen bonding with analytes [90]. Several solvents such as methanol, propanol, butanol, acetone and vegetable oil also can be used as polarity modifiers for the efficient extraction of carotenoids from macro- and microalgae matrices. Despite the use of cosolvents enhances significantly the extraction yield, the presence of cosolvents can also decrease the extraction selectivity. Therefore, the use of a cosolvent should be considered and might be compromising to product purity.

Beside supercritical CO₂ extraction method has been known as a green processing technology to extract valuable compounds from macro- and microalgae matrices, nowadays, subcritical water extraction method also has been recognized and emerged as a useful media to substitute the conventional extraction methods to extract valuable compounds from solid matrix including macro- and microalgae. Subcritical water extraction method is conducted using water as an extractant at temperatures between 100 and 374 °C under pressure high enough to keep the liquid state leading to improved extraction efficiency via improvements in mass transfer and shifts in the polarity of water. Some advantages of the subcritical water extraction method are shorter extraction time, higher extraction yield, higher quality of the extracts and the low environment impact [125,126]. In addition, water is also easily available and inexpensive.

As a green extraction method, subcritical water extraction is being used in food, natural products and pharmaceutical applications when this extraction method was applied in macroalgae processing. Conversely, subcritical water treatment was usually used to result bio-oil from microalgae matrix [127]. Next, the application of subcritical water treatment in microalgae will not presented. At subcritical conditions, water possesses properties very different from those of ambient liquid water. Under subcritical water conditions, the ionization constant of water increases with temperature and the dielectric constant of water drops significantly. A low dielectric constant allows subcritical water to dissolve organic compounds, while a high ionization constant allows subcritical water to provide an acidic medium for hydrolysis reactions. These ionic reactions can be dominant due to the liquid-like properties of subcritical water. This can give large effect on the distribution of products, such as gas, liquid or solid, from hydrothermal biomass conversion [125,126]. Accordingly, the extraction and fractionation of valuable compounds may conduct by applying subcritical water treatment on the macroalgae matrix. Table 3 shows the extracted valuable compounds from macroalgae using subcritical water. As shown in this table, alginate, agar and carrageenan have been successfully extracted by using subcritical water treatment. The depolymerization of alginate from *Saccharina japonica* to produce antioxidants and other valuable compounds also can be carried out by applying subcritical water treatment. Furthermore, different compounds can be extracted depending on the macroalgae species and the subcritical water extraction conditions.

Table 3. Extracted valuable compounds from macroalgae using subcritical water.

Sample sources	Operating conditions	Extracted bioactive compounds	Reference
<i>Ulva spp.</i> <i>Monostroma latissimum</i>	Temperature: 100 – 180 °C Pressure: –	Sulfated polysaccharides (Rhamnan)	[128]
<i>Saccharina japonica</i>	Temperature: 180 – 420 °C Pressure: 1.3 – 52.0 MPa	Total organic carbon	[129]
<i>Saccharina japonica</i>	Temperature: 200 – 280 °C Pressure: 1.3 – 6.0 MPa	Antibacterial substance	
<i>Saccharina japonica</i>	Temperature: 180 – 260 °C Pressure: 1.5 – 6.5 MPa	Antioxidants Depolymerization of alginate	[130]
<i>Sargassum muticum</i>	Temperature: 150 – 240 °C Pressure: 1.4 – 4.0 MPa	Sulfated polysaccharides (fucoidan)	[131]
<i>Fucus vesiculosus</i>	Temperature: 172 – 180 °C Pressure: –	Sulfated polysaccharides (fucose)	[132]
<i>Undaria pinnatifida</i>	Temperature: 140 °C Pressure: –	Sulfated polysaccharides (degrade fucoidan)	[118]
<i>Sargassum muticum</i>	Temperature: 150 – 210 °C Pressure: –	Alginate	[133]
<i>Gracilaria changii</i> <i>Gracilaria salicornia</i>	Temperature: ambient Pressure: 0.1 MPa.	Agar	[134]
<i>Hypnea musciformis</i>	Temperature: 105 °C Pressure: –	Carrageenan	[135]
<i>Pterocladia capillacea</i> <i>Gelidium floridanum</i> <i>Gelidium serrulatum</i>	Temperature: 121 °C Pressure: –	Agar	[136]
<i>Eucheuma cottonii</i> <i>Gracilaria sp.</i>	Temperature: 120 – 200 °C Pressure: 1.0 – 10.0 MPa	Carrageenan	[137]

4. Future Perspective and Challenge

Nowadays, the demand for natural products is on the rise due to their application especially in the functional food and pharmaceutical industry. Public health, environmental impact and safety issues are the important parameters to develop separation method. To cope with this demand, the development of an appropriate extraction technology that is able to provide high-quality extracts is required. As challenges in the development of extraction process are to meet food and pharmaceutical regulation guidelines and to perform the extraction process effectively and economically. Supercritical CO₂ and subcritical water extraction are considered as green method and environmental friendly separation technologies. They have been developed as attractive alternatives to substitute the conventional methods for the extraction of valuable compounds from solid biomass matrices including macro- and microalgae.

The applying hydrothermal technologies by using subcritical water to extract valuable compounds from macroalgae seem to result in viable processes. By using this method, valuable compounds such as polysaccharides have successfully extracted from macroalgae with higher extraction yields, reducing extraction time and solvents used. This extraction process seems to allow the extraction of valuable compounds with different biological activities. Nevertheless, since the vaporization heat of water is relatively high, there is some difficulties in concentrating the valuable compounds in extract when subcritical water extraction was applied as an extraction media. The presence of water also may reduce the extract stability, freeze-drying is one way to remove water from the extract. However, freeze-drying is costly and time consuming and may also promote to the extract degradation due to light and oxygen contact during process.

Supercritical CO₂ extraction method, the most common application for supercritical fluid technologies, may accomplish the drawback of subcritical water extraction method. The major advantages of using supercritical CO₂ for the extraction of natural products are a low operating temperature and a low operating pressure. This is, of course, a beneficial effect in terms of natural products extraction and fractionation by using supercritical CO₂. Considering the low polarity of supercritical CO₂, the addition of a small amount of modifiers may increase the ability of supercritical CO₂ to dissolve relatively polar compounds. Although the existence of organic solvents as modifiers may cancel the benign advantages demonstrated by use of supercritical CO₂, the supercritical CO₂ coupled to modifier looks very promising. Finally, it could be said that supercritical CO₂ and subcritical water are suitable methods for natural products extraction from macro- and microalgae matrices.

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