Review Article

Supercritical Algal Extracts: A Source of Biologically Active Compounds from Nature

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The paper discusses the potential applicability of the process of supercritical fluid extraction (SFE) in the production of algal extracts with the consideration of the process conditions and yields. State of the art in the research on solvent-free isolation of biologically active compounds from the biomass of algae was presented. Various aspects related with the properties of useful compounds found in cells of microalgae and macroalgae were discussed, including their potential applications as the natural components of plant protection products (biostimulants and bioregulators), dietary feed and food supplements, and pharmaceuticals. Analytical methods of determination of the natural compounds derived from algae were discussed. Algal extracts produced by SFE process enable obtaining a solvent-free concentrate of biologically active compounds; however, detailed economic analysis, as well as elaboration of products standardization procedures, is required in order to implement the products in the market.

1. Introduction

The increase of public awareness, concerning potentially harmful ingredients present in commercial products, put the pressure on manufacturers to apply natural, environmental friendly materials of improved quality. Such requirements are met by algal biomass, the capacity of which, despite long history in daily living products, still remains largely unexplored [1]. Algae, both micro- and macrocellular (seaweeds), are known as a rich source of bioactive compounds, including proteins, minerals, vitamins, polysaccharides, polyphenols, phlorotannins, pigments, unsaturated fatty acids, sterols, and phytohormones [2, 3]. The properties of these compounds were used in various branches of industry, such as chemical,

pharmaceutical, human food, and animal feed production and integrated systems of plant cultivation [1, 4].

Among possible ways of enriching a given product with algal-derived material, application of extract is the most frequently reported. In order to extract bioactive substances from raw biomass, the proper solvent (e.g., water and organic solvents) should be chosen. The application of conventional methods of extraction (extraction in Soxhlet apparatus, solid-liquid extraction, and liquid-liquid extraction) has some disadvantages, for example, the use of high volumes of solvents and difficult medium separation [5]. Therefore, in the recent years, solvent-free methods of extraction have been developed. Alternatively to conventional procedures, supercritical

fluid extraction is usually proposed. This technique fulfills the market demand for both high quality and chemically safe products [6]. The term “supercritical” corresponds to substance behavior after exceeding the value of both critical temperature and pressure (the so-called “critical point”). Supercritical fluid (SCF) shows features bordering on characteristics of gaseous and liquid form of the compound and might be classified somewhere between these two states. Thus, supercritical fluids have unusual capacity to extract selected constituents from complex material, the efficacy of which is worth researching [7, 8].

In a present work, a review of SFE condition requirements, technological aspects, and obstacles is presented, as well as the application prospects of supercritical extracts from algae. Special attention was paid to the use of algae based products in agriculture, as a rich source of natural plant growth stimulators. Another section was also devoted to the novel analytical methods which are necessary to examine the organic and inorganic composition of algal extracts. On the basis of the composition of the new product, potential applications are sought.

2. Supercritical Fluid Extraction as an Efficient Method of Isolation of Valuable Compounds

Solvation properties of supercritical fluids were reported for the first time in 1879 by Hannay and Hogarth [9]. The idea of involving supercritical fluid extraction in industrial technologies was shown in public in 1969 by Zosel [10]. Due to the global concern of environmental damage caused by large-scale use of organic solvents in classical extractions, the implementation of new technologies, using minimum volumes of solvents, became the subject of great interest [11]. Currently, a lot of attention is paid to optimize processes under supercritical conditions, so that they can be used more extensively. However, there are still economic and energy issues (e.g., high investment cost and labor-intensive step of sample processing) that limit the use of SFE in commercial productions [12].

2.1. Fundamentals of SFE. In the literature, supercritical fluid extraction is usually compared with conventional processes in order to present advantages and disadvantages of both methods. Absence of the harmful or toxic chemicals in the final product is the most evident advantage of SFE. Fluids under supercritical state surpass organic solvents in reducing process time and required amount of the sample by an order of magnitude and in enhancing the yield of extraction [13]. Such differences resulting from the complex nature of supercritical fluids, lower viscosity and surface tension with higher compressibility and diffusivity (gas- and liquid-like features, alternately) enable more effectively penetrating the material and hence provide better mass transfer between phases [5, 14]. The high selectivity (ease of being modified by changing temperature or pressure value only, e.g., tuneable solvating power) and facility for fractionation of extracted compounds are also emphasized as the major benefits of

using supercritical fluids. For industrial application of SFE, the exclusion of oxygen and low processing temperature (depended on the type of fluid) is worth mentioning, since it gives an opportunity to obtain volatile or labile constituents without their damage [12, 13, 15].

A variety of compounds, both inorganic (carbon dioxide, nitrous oxide, ammonia, sulphur hexafluoride, and water) and organic (ethane, propane, *n*-pentane, fluoroform, and chlorodifluoromethane (Freon-22)) was subjected to tests under critical conditions [15]. Among examined fluids, supercritical CO₂ (SC-CO₂) has been reported as the most common choice. Benefits of using SC-CO₂ are well known and include such features as relatively low critical parameters ($T = 31^{\circ}\text{C}$, $p = 73.8$ bar), chemical inertness, no or low toxicity, nonflammability, noncorrosivity, and GRAS designation (Generally Recognized as Safe) from both American Food and Drug Administration and European Food Safety Authority [5, 15, 16]. Furthermore, under normal conditions, carbon dioxide is a gas, which can be easily separated from the extract and hence recovered, what reduces its cost [16]. Despite several advantages, SC-CO₂ is not a universal extractant, since it is nonpolar. Therefore, isolation of compounds with high polarity needs to be supported with modifiers, cosolvents, which if added at low concentration increase solvating power of the fluid towards the target compound [6]. In case of supercritical carbon dioxide, methanol and ethanol are the most frequently reported. The former is more efficient and the latter is less toxic [6, 11, 13].

Extraction under supercritical fluid requires equipment which involves a tank of the mobile phase (chosen solvent), a pump to pressurize the fluid, an oven comprising the extraction vessel with a matrix, a restrictor to maintain the high pressure inside the system, and a trapping vessel. Extracts are trapped during decompression of the analyte-containing SCF into an empty vial, through a solvent, or onto a solid or liquid material. There are three possible ways of SFE: dynamic mode, static mode, or a combination mode. In the former, the fluid flows continuously through the sample (extraction vessel) and out of the restrictor to the trapping vessel. In static mode, the fluid circulates in a loop within the extraction vessel for some period before being released through the restrictor to the trapping vessel. In combination mode, a static extraction is performed for some period of time, followed by a dynamic extraction [17].

2.2. Parameters in SFE. Crampon et al. reviewed the parameters that have an impact on the kinetics and efficiency of extraction of microalgae and seaweeds carried out under supercritical conditions (SC-CO₂) from dry biomass. Pressure seems to be the most important parameter [7]. At constant temperature, the higher the pressure, the higher the density and thereby enhanced yields and/or faster extraction kinetics might be noted [7, 15]. In the case of temperature, its effect on the strength of supercritical fluid depends on the pressure (retrograde behavior). Correlation between these two parameters varies and is determined by pressure value called “crossover point,” above which increasing temperature improves solvating power [15].

Another important parameter in SFE is solvating power (selectivity) of supercritical fluids. It increases with density. Such correlation was not observed for conventional liquid solvents. The density of extractant under supercritical conditions can be adjusted to the process needs by temperature, pressure, and/or composition (content of modifiers) [14, 18]. Efficiency of the extraction is also clearly related to molecular weight of analytes, their concentration in the sample, type and strength of binding to the matrix, and solubility in specific SFC. Considering extraction with supercritical carbon dioxide, it is advised to work with a high SC-CO₂/algae mass ratio [19].

Selection of the proper values of process variables is crucial for obtaining high degree of extraction. Since there are several variables to change, optimization of SFE might be performed through various approaches, which are generally classified as phase equilibrium strategies and experimental design with statistical modeling. The first approach considers limitation of stages that influence the final effect of the process. The second approach complements this knowledge by fitting statistical treatment to the results [6].

2.3. Preparation of the Biomass for SFE. Applying supercritical fluids to treat biological materials, including algae, in a profitable way is highly dependent on the proper pretreatment. In the first step, the biomass undergoes centrifugation, after which the concentrated algal suspension should be subjected to a drying process, freeze-drying or drying at low temperatures. High sample moisture might lead to a few disadvantages, such as limitation of the matrix-SCF contact [20] or, in case of applying supercritical CO₂, acidic hydrolysis of the analytes due to carbonic acid formation [21]. Therefore, there is a common practice to remove excess water during sample pretreatment. Finally, algae are crushed to break the cellular wall and thus increase extraction efficiency. Concerning the effect of crushing, results obtained by Crampon et al. indicated that the smaller the particle, the more rapid the kinetics of extraction and the higher the yields. Disintegration of cells is essential in the recovery of intracellular products from algae [7]. According to literature, the SFE can be coupled with cells disintegration techniques to obtain higher yields. Ultrasounds and microwaves are proved to facilitate extraction, hence the productivity, as well as reducing the time of the process [12]. Additionally, the following methods might be used: freezing, alkaline and organic solvents, osmotic shocks, sonication, homogenization at high pressure, and bead milling [22, 23]. Moreover, in the study of Valderrama et al., cells of *H. pluvialis* and *S. maxima* were crushed by cutting mills (coffee mill) and manually ground with dry ice [2].

3. Production of Algal Extracts by Supercritical Fluid Extraction

3.1. Extraction of Biologically Active Compounds from Algae by SFE. Algae form a diverse group of micro- and macroorganisms (seaweeds) containing a great amount of biologically active compounds, which participate in processes of growth,

development, and protection and therefore are considered to be capable of affecting other living organisms. The vast array of bioactive compounds in algae is the result of their adaptation to unfavorable environmental conditions. Production of these compounds increases when the environmental stress factors are occurring, for example, changes of temperature, salinity, drought stress, tidal flows, lack of nutrients, or presence of hostile organisms [24]. Algae are found in both marine and freshwater environments. Chemical composition of algae has not been known as well as terrestrial plants. On the other hand, algae contain unique compounds that are absent in higher plants [2, 3].

Supercritical fluids were first used for treating algal matrix to select biomolecules valuable in food processing industry [14, 17]. Currently, other functional compounds of proven activity on human health, plant growth, or livestock productivity and biofuels of new generation have been obtained from algae by using SFE [25–28].

Based on literature studies, microalgal cells are used in extraction with supercritical fluids more frequently than seaweeds. In the last 14 years, the words “supercritical fluid extraction and microalgae” appeared in the topic of the scientific papers 88 times, whereas “supercritical fluid extraction and seaweed” only 21 times (Web of Knowledge, December 12, 2014; <http://apps.webofknowledge.com/>). Adequate examples of applying supercritical conditions for microalgal biomass processing are collected in Table 1.

In the presented examples, SC-CO₂ was chosen as a solvent, occasionally supported by a modifier; ethanol and operational conditions ranged within 40–85°C and 78.6–500 bar. In general, SFE was particularly used for the extraction of pigments, lipids including polyunsaturated fatty acids (PUFAs) (e.g., omega-3 fatty acids: eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and omega-6 fatty acid: γ -linolenic acid (GLA) and arachidonic acid (AA)), polyphenols, and vitamins [23].

As it was mentioned above, there are fewer reports on the production of supercritical seaweed extracts. Most research on producing supercritical extracts from macroalgae focuses on comparing solvating power of SCFs with organic extractants. Marine red macroalgae *Hypnea charoides* were investigated as a nonconventional source of ω -3 fatty acids obtained by extraction with SC-CO₂. Tests were conducted under mild conditions: temperature range 40–50°C and pressure range 241–379 bar. Different conditions enabled observing their influence on product yield: the higher the temperature and pressure, the better the lipid recovery and ratio of unsaturated fatty acids. Moreover, solubility, hence extractability, of ω -3 fatty acids in supercritical CO₂ was proven to depend on the chain length [29]. SC-CO₂ was also used to extract fucoxanthin from brown seaweed *Undaria pinnatifida* [30]. A broad range of operational conditions was applied: temperature 25–60°C, pressure 200–400 bar, and CO₂ flow rate 1.0–4.0 mL/min, to investigate the variations of process efficiency. It was shown that the product recovery increased with decreasing temperature and increasing pressure and the highest yield of fucoxanthin (almost 80%) was achieved at 40°C and 400 bar during 3-hour extraction. More examples of the application of SFE of algal biomass (both

TABLE I: SFE extraction of biomass of microalgae: review of literature reports.

Extraction	Algae	Installation	Temp. [°C]	Pressure [bar]	Extract	Extraction yield	Reference
SFE with CO ₂	<i>Botryococcus braunii</i> , <i>Chlorella vulgaris</i> , <i>Dunaliella salina</i> , and <i>Arthrospira maxima</i> , whole, crushed, and slightly crushed	Flow	40–60	125, 200, and 300	<i>B. braunii</i> : alkenes; <i>C. vulgaris</i> : carotenoids (canthaxanthin, astaxanthin); <i>D. salina</i> : β -carotene (trans- and cis-isomer); <i>A. maxima</i> : GLA, C18:3 ω 6 (CO ₂ and CO ₂ + 10 mol% ethanol) and lipids	Total GLA: 45%; 35.0 MPa, 333.1 K with the mixture (CO ₂ + 10 mol% ethanol)	[26]
	<i>Haematococcus pluvialis</i> and <i>Arthrospira maxima</i> (<i>Spirulina</i>)	Column; flow rate: 1 mL/min	60	300	<i>H. pluvialis</i> : astaxanthin, <i>A. maxima</i> : phycocyanin	Astaxanthin: 1.7% mass (no effect of ethanol); phycocyanin: 1.1% mass (CO ₂), 1.7% mass (CO ₂ + ethanol)	[2]
SFE with CO ₂ and ethanol (9.4% mass)	<i>Haematococcus pluvialis</i>	Biomass 6.5 g; CO ₂ flow rate: 6.0 mL/min; time: 20 min	50	310	Pigment (astaxanthin)	Astaxanthin: 74% (11 mg/g dry cells), 8 extraction cycles	[5]
SFE with CO ₂	<i>Chlorella vulgaris</i>	Flow type	40, 55	350	Carotenoids, lipids	Carotenoids and lipids: improved for crushed cells and at higher <i>p</i>	[25]
SFE with CO ₂	<i>Botryococcus braunii</i>	Flow type	50–85	200–250	Fatty acids	Lipid yield decreased with temperature and increased with pressure	[36]
SFE with CO ₂	<i>Botryococcus braunii</i> and <i>Chlorella vulgaris</i>	Flow type	40	300 (<i>B. braunii</i>) and 350 (<i>C. vulgaris</i>)	<i>B. braunii</i> : hydrocarbons <i>C. vulgaris</i> : carotenoids (canthaxanthin and astaxanthin)	The extraction yield of carotenoids increased with the degree of crushing of the microalga	[37]
SFE with CO ₂	<i>Arthrospira platensis</i> (<i>Spirulina</i>)	Time: 4 h	48	200	85 g/kg of flavonoids; 78 g/kg of β -carotene; 113 g/kg of vitamin A; 3.4 g/kg of α -tocopherol; fatty acids: palmitic (35%), linolenic (22%), and linoleic (21%)	Yield of the extracts from <i>S. platensis</i> –10 g/kg	[38]
SFE with CO ₂ (soybean oil and ethanol as modifier)	<i>Chlorella vulgaris</i>	Flow type	40	300		Carotenoids: 69%, crushing strongly improved extraction recovery	[39]
SFE with CO ₂	<i>Haematococcus pluvialis</i>	Time: 4 h	70	500	Astaxanthin	The predicted amount of astaxanthin extracted was 23 mg/g	[40]
SFE with CO ₂ and ethanol (0.856 mL/g of biomass)	<i>Arthrospira platensis</i> (<i>Spirulina</i>)	Time: 1 h	40	400	γ -linolenic acid	A recovery of 102% GLA	[41]
SFE with CO ₂ and ethanol	<i>Arthrospira maxima</i> (freeze-dried)	Flow type	50–60	250	γ -linolenic acid and lipids	GLA and lipids: up to 45%	[27, 28]
SFE with CO ₂	<i>Synechococcus</i> sp.	Flow type	50 (for carotenoids and chlorophyll) 60 (for chlorophyll)	300 (for carotenoids and chlorophyll) 500 (for chlorophyll)	Carotenoids and chlorophylls	Carotenoids (1.5 μ g/mg dry weight of microalga); chlorophyll a (0.71 μ g/mg dry weight of microalga)	[42]
SFE with CO ₂ and CO ₂ : ethanol	<i>Arthrospira platensis</i> (<i>Spirulina</i>)	Pilot-scale plant	75 (CO ₂) 55 and (CO ₂ : EtOH) 78.6 (CO ₂ : EtOH)	320 (CO ₂) and 78.6 (CO ₂ : EtOH)	Vitamin E	CO ₂ : yield 0.85%; 16 mg of vitamin E/g of extract; CO ₂ : ethanol: yield 8.1%; 0.49 mg of vitamin E/g of extract; CO ₂ (w/w) 69%	[43]
PLE using mixture of ethanol : ethyl lactate	<i>Arthrospira platensis</i> (<i>Spirulina</i>)	Time: 15 min	180	207	γ -linolenic acid	Total yields up to 21% (w/w), for a solvent composition of ethanol : ethyl lactate (50 : 50, v/v), GLA recovery of 68%	[44]

micro- and macroalgae) are presented in a review paper of Michalak and Chojnacka [23].

3.2. Comparison SFE with Other Extraction Techniques from Biomass of Algae. Despite regulations limiting the use of chemicals and proven efficacy of using supercritical fluids as extractants, conventional solvent extraction still remains the leading technique to provide algae derived compounds for cosmetic or food industry [5, 26]. Applying organic solvents requires additional step of their recovery and posttreatment and might change physicochemical properties and functionality of isolated compounds [5]. There is an increasing number of reports focusing on comparison of conventional methods with SFE and some are summed in Table 2.

Halim et al. investigated a lab-scale biodiesel production by extracting lipids from green microalgae *Chlorococcum* sp. with the use of two different solvents: SC-CO₂ and *n*-hexane. Obtained results confirmed usability of supercritical conditions to algal lipid recovery. It was concluded that SFE generated comparable yield to Soxhlet extraction and shortened process time by over five times. Nevertheless, the time required to complete the extraction might be insufficient criterion [31]. In research of Crespo and Yusty, isolation of *n*-alkanes, C18, C19, C20, C22, C24, C28, C32, and C36, and the acyclic isoprenoid Pristane from brown seaweed *Undaria pinnatifida* was conducted by the use of *n*-hexane:dichloromethane mixture (Soxhlet mode) and SC-CO₂ with a modifier. Although SFE enabled hastening the process from days (conventional extraction) to 1 hour, the authors concluded that both methods are comparable, since the obtained yields of hydrocarbons were not significantly different. It was also noted that solvating power of SC-CO₂ is higher than organic solvent in case of longer-chain *n*-alkanes [32]. As opposed to investigation of Crespo and Yusty, supercritical CO₂ (with and without cosolvent) in experiments on isolation of astaxanthin (AXA) and chlorophyll from microalgae *Monoraphidium* sp. GK12 was proved to surpass ethanol. Efficacy of using chosen solvents was verified by performing bead beater extraction (BBE), the results of which were established as 100%. The yield of astaxanthin obtained by SFE was twice as high as in EtOH-extracts and this advantage increased with higher concentration of modifier to finally achieve similar level to the result of BBE. In case of extraction of chlorophyll, applying supercritical conditions was slightly more effective than both of the other methods [33]. Supercritical fluid extraction was also shown to be an efficient pretreatment method in the production of polysaccharides (fucoidan) from biomass of brown seaweeds *Fucus evanescens*, *Saccharina japonica*, and *Sargassum oligocystum*. It provided the equivalent yield as conventional (organic solvent) method [34].

3.3. General Application of Algae Derived Compounds. Extracted algal compounds are characterized by anticoagulant, anticancer, antiallergic, antiviral, antifungal, antioxidative, and immunomodulating activities [35]. These properties make that algal extracts have broad potential applications, for example, as components in cosmetics, medicines,

pharmaceuticals, nutraceuticals, feed additives, nutrition (feed) and food additives, aquaculture, plant growth biostimulants and bioregulators, biofuels, and pollution prevention [23].

It should be underlined that some algal-origin molecules are assigned for specific species or taxonomic groups. For example, in cyanobacteria typical bioactive compounds are malyngolide (*Lyngbya majuscula* (Dillwyn) Harvey), nostodione (*Nostoc commune* Vaucher), cyanobacterin (*Scytonema hofmanni* Kütz., and *Nostoc linckia* (Roth) Bornet & Flahault), aponin (*Gomphosphaeria aponina* Kütz.), fischerellin (*Fischerella muscicola* (Thuret) Gom.), and scytophycins (*Scytonema pseudohofmanni* Bharad.). It was shown that they demonstrate antibacterial, antifungal, and even antialgal properties that can be used in pharmaceutical industry [45]. In the work of Ramesh et al., the main attention was paid to active substances isolated from freshwater algae with pharmaceutical applications. This group of algae produces compounds with a vast array of properties: from antimicrobial and antiviral to cytotoxicity and immunomodulatory activity. Freshwater algae provide a diverse and unique source of bioactive compounds that can be used for the discovery of modern drugs (antibiotics, mycotoxins, alkaloids, and phenolic compounds) [46].

Another group of bioactive compounds constitute carotenoids (β -carotene, astaxanthin, and canthaxanthin) and phycocyanin (water-soluble phycobiliprotein) isolated from algal biomass. They can be used as natural pigments in nutrition of animals and humans [2]. Algae are a promising commercial source of carotenoids due to the relative fast growth rate (especially microalgae). Some species, such as unicellular *Dunaliella salina* (Dunal) Teod. or *Dunaliella bardawil* Ben-Amotz & Avron, demonstrate their capability to accumulate large amount of β -carotene in chloroplasts in the form of lipid globules. Adverse environmental conditions, such as high salinity, rapid change in temperature, and nutrient limitation acting as a stressor, may increase this capacity [47].

Other important compounds extracted from algae are unsaturated fatty acids [31, 48]. Fatty acid composition of marine algae species differs totally from higher plants. For example, cells of *Arthrospira* (*Spirulina*) *maxima* contain polyunsaturated γ -linolenic acid and α -linolenic acid (ALA), which can be the component of pharmaceuticals (schizophrenia, multiple sclerosis, diabetes, and rheumatoid arthritis) [26]. Fatty acids produced from algae, linolenic acid (from *Arthrospira* sp.), arachidonic acid (*Porphyridium* sp.), eicosapentaenoic acid (*Chlorella vulgaris* Beij.), and docosahexaenoic acid (*C. vulgaris*), have high biological activity and are mainly used in nutritional supplements [49, 50].

The extraction of the mentioned biologically active compounds from algae by SFE is especially recommended because this technique protects them from thermal or chemical degradation. Active ingredients in solvent-free environments are particularly important for their applications in medicines and nutraceuticals [26]. The industrial importance is due to not only the algal extract but also the postextraction residue which can be used as animal feedstock [12]. Algal

TABLE 2: Comparison of SFE with conventional extraction methods, on the example of algae processing; the results of independent experiments are shown separately and marked with (a)–(d).

Extraction	Biomass pretreatment	Sample and solvent	Conditions	Extraction yield	Reference
Solvent extraction with <i>n</i> -hexane at static and dynamic (Soxhlet) mode		(a) <i>Static mode</i> : (i) Microalgal powder: 4 g (ii) Microalgal paste: 13.3 g (iii) Solvents for performance on powder: pure <i>n</i> -hexane and mixture of <i>n</i> -hexane and isopropanol (3 : 2), separately, both 300 mL (iv) Solvent for performance on paste: pure <i>n</i> -hexane, 300 mL <i>Dynamic mode</i> (Soxhlet extraction): (i) Microalgal powder: 4 g (ii) Solvent: pure <i>n</i> -hexane, 300 mL	<i>Static mode</i> : Ambient conditions, agitation: 800 rpm, <i>t</i> : 7.5 h <i>Dynamic mode</i> : Rate of refluxes: 10 per hour, <i>t</i> : 7.5 h Posttreatment: Removal of solid residues from extract by separation on a filtration paper	<i>Results for microalgal powder</i> : Lipid yield [g lipid extract/g d.m.]: (i) Static mode: 0.015 and 0.048, with and without cosolvent, respectively (ii) Dynamic mode: 0.057 <i>Results for wet microalgal paste</i> : Lipid yield after 80 min [g lipid extract/g d.m.]: Static mode: 0.010	
		Microalgal powder: Oven-drying at 85 °C for 16 hours and grinding in a ring mill Microalgal paste (solid conc. 30%, by mass): centrifuging wet algae in benchtop centrifuge		<i>Results for microalgal powder</i> : Lipid yield after 80 min [g lipid extract/g d.m.]: (i) 60 °C, 300–500 bar: 0.058 (ii) 80 °C, 300–500 bar: 0.048 Effect of pressure: increase of the lipid yield almost twice in case of higher-pressure extractions, compared to lower-pressure experiments FAME content variations during 80 min of the process [g FAME/g d.m.]: (i) 60 °C, 300–400 bar: 0.23–0.29 (ii) 80 °C, 300–400 bar: 0.31–0.44 Effect of pressure: not significant <i>Results for wet microalgal paste</i> : Lipid yield after 120 min [g lipid extract/g d.m.]: 60 °C: 0.071	[31]
SFE with CO ₂		(i) Microalgal powder: 20 g (ii) Microalgal paste: 8 g Both powder and paste were formerly mixed with inert diatomaceous earth (d.e.) at ratios 2 : 1 w/w and 1 : 2 w/w, respectively Solvent f.r.: 400 mL/min	<i>T</i> : 60 or 80 °C, <i>p</i> : 100–300 or 300–500 bar (lower- and higher-pressure experiments, resp.), <i>t</i> : 80–120 min		

(b)

Extraction	Biomass pretreatment	Sample and solvent	Conditions	Extraction yield	Reference
Soxhlet extraction with mixture of <i>n</i> -hexane and dichloromethane	(1) Drying by lyophilization (samples number 1 and 2) or dehydration (sample number 3) (2) Pulverizing (3) Homogenization	(i) Samples: 1 g of each of 3 different matrixes collected from two locations in North-West Spain during two consecutive spring seasons) Each sample was mixed with 15 g of sea sand (ii) Solvent: <i>n</i> -hexane : dichloromethane (50 : 50), 250 mL	<i>t</i> : 7 h Extract posttreatment: Removal of solvent on a rotatory evaporator at 40 °C, oven-drying obtained extract at 75 °C for 90 min and then cooling to room temperature (20 °C) Providing fraction of hydrocarbons: Redissolving the extract in <i>n</i> -hexane (5 mL) and next passing through Sep-Pak silica solid-phase extraction (SPE) column, previously activated with <i>n</i> -hexane (4 mL); eluting hydrocarbons from SPE cartridge with 10 mL of <i>n</i> -hexane and then evaporating the eluent under a stream of air, redissolving the residue in <i>n</i> -hexane (1 mL) and subjecting it to analysis (GC-FID)	<i>Results for sample 1</i> (<i>n</i> = 6): Recoveries [%], RSD [%]: Pristane, sample at 5 mg/L: 74.1, 5.81; Pristane, sample at 40 mg/L: 90.5, 6.59; C ₁₈ , sample at 5 mg/L: 87.9, 5.36; C ₁₈ , sample at 40 mg/L: 97.8, 6.88; C ₁₉ , sample at 5 mg/L: 86.0, 1.52; C ₁₉ , sample at 40 mg/L: 96.7, 3.20; C ₂₀ , sample at 5 mg/L: 69.1, 4.90; C ₂₀ , sample at 40 mg/L: 96.5, 3.31; C ₂₂ , sample at 5 mg/L: 69.6, 4.98; C ₂₂ , sample at 40 mg/L: 99.4, 4.69; C ₂₄ , sample at 5 mg/L: 78.4, 4.42; C ₂₄ , sample at 40 mg/L: 99.8, 3.39; C ₂₈ , sample at 5 mg/L: 90.4, 7.58; C ₂₈ , sample at 40 mg/L: 101, 1.65; C ₃₂ , sample at 5 mg/L: 98.8, 1.05; C ₃₂ , sample at 40 mg/L: 99.7, 1.74; C ₃₆ , sample at 5 mg/L: 97.9, 0.58; C ₃₆ , sample at 40 mg/L: 97.4, 3.10 Aliphatic hydrocarbon yield [μg/g.d.m.]: C ₁₈ : 4.32 ± 0.08, C ₂₀ : 5.35 ± 0.34, C ₂₂ : 3.99 ± 0.20, C ₂₄ : 2.65 ± 0.06, C ₂₈ : 1.06 ± 0.06, total: 14.9 ± 0.5 <i>Results for sample 2</i> (<i>n</i> = 2): Aliphatic hydrocarbon yield [μg/g.d.m.]: C ₁₈ : 5.14 ± 0.19, C ₂₀ : 5.88 ± 0.27, C ₂₂ : 5.03 ± 0.14, C ₂₄ : 2.50 ± 0.11, C ₂₈ : 0.64 ± 0.00, and total: 17.9 ± 0.3 <i>Results for sample 3</i> (<i>n</i> = 2): Aliphatic hydrocarbon yield [μg/g.d.m.]: C ₁₈ : 4.25 ± 0.25, C ₂₀ : 5.10 ± 0.30, C ₂₂ : 5.32 ± 0.44, C ₂₄ : 2.88 ± 0.25, C ₂₈ : 1.03 ± 0.15, and total: 17.0 ± 1.0, <i>Results for sample 1</i> (<i>n</i> = 6): Recoveries [%], RSD [%]: Pristane, sample at 5 mg/L: 36.8, 7.53; Pristane, sample at 40 mg/L: 52.3, 8.11; C ₁₈ , sample at 5 mg/L: 66.4, 9.28; C ₁₈ , sample at 40 mg/L: 76.0, 3.49; C ₁₉ , sample at 5 mg/L: 77.6, 4.69; C ₁₉ , sample at 40 mg/L: 89.1, 4.71; C ₂₀ , sample at 5 mg/L: 84.6, 4.01; C ₂₀ , sample at 40 mg/L: 94.3, 4.42; C ₂₂ , sample at 5 mg/L: 87.9, 3.04; C ₂₂ , sample at 40 mg/L: 98.4, 5.18; C ₂₄ , sample at 5 mg/L: 90.8, 2.46; C ₂₄ , sample at 40 mg/L: 100, 3.85; C ₂₈ , sample at 5 mg/L: 88.6, 5.75; C ₂₈ , sample at 40 mg/L: 100, 3.35; C ₃₂ , sample at 5 mg/L: 87.8, 8.40; C ₃₂ , sample at 40 mg/L: 98.6, 3.86; C ₃₆ , sample at 5 mg/L: 84.8, 5.76; C ₃₆ , sample at 40 mg/L: 98.1, 4.09 Aliphatic hydrocarbon yield [μg/g.d.m.]: C ₁₈ : 6.28 ± 0.66, C ₂₀ : 6.66 ± 0.79, C ₂₂ : 4.21 ± 0.44, C ₂₄ : 2.56 ± 0.28, C ₂₈ : 0.71 ± 0.07, and total: 19.1 ± 2.0 <i>Results for sample 2</i> (<i>n</i> = 2): Aliphatic hydrocarbon yield [μg/g.d.m.]: C ₁₈ : 4.54 ± 0.23, C ₂₀ : 4.25 ± 0.10, C ₂₂ : 2.99 ± 0.04, C ₂₄ : 2.30 ± 0.18, C ₂₈ : 0.59 ± 0.05, and total: 13.6 ± 0.6, <i>Results for sample 3</i> (<i>n</i> = 2): Aliphatic hydrocarbon yield [μg/g.d.m.]: C ₁₈ : 7.90 ± 1.07, C ₂₀ : 6.73 ± 0.63, C ₂₂ : 4.49 ± 1.26, C ₂₄ : 2.99 ± 3.55, C ₂₈ : 1.15 ± 5.17, and total: 21.7 ± 0.2	[32]
		SFE with CO ₂ (+ methanol as modifier)			

(c)

Extraction	Biomass pretreatment	Sample and solvent	Conditions	Extraction yield	Reference
Solvent extraction with ethanol		(i) Sample: 1 g (ii) Solvent: 20 mL	<i>t</i> : 30 min (soaking matrix in ethanol) Posttreatment: Separation of extract from solid residues by centrifuging (4000 ×g, 20°C, 10 min, extract = supernatant)	<p><i>Results of AXA extraction:</i> Yield ± δ [mg AXA/g d.m]: 1.16 ± 0.18 Recovery [% of yield obtained with BBM method]: 48</p> <p><i>Results of chlorophyll extraction:</i> Yield ± SD [mg chlorophyll/g d.m]: 16.1 ± 1.91 Recovery [% of yield obtained with BBM method]: 56</p>	
SFE with CO ₂ (+ ethanol as modifier)	Freeze-drying	(i) Sample: 1 g (ii) Solvent: (a) Pure solvent: SC-CO ₂ (b) Cosolvent: EtOH, 0.1, 0.2, 0.5, 1.0, 2.0, or 20 mL	<p><i>T</i>: 60°C, <i>p</i>: 200 bar, <i>t</i>: 60 min</p> <p>Providing extract after SFE: soaking obtained biomass in 20 mL of ethanol for 30 min and then separation of supernatant by centrifugation (4000 ×g, 20°C, 10 min); in case of samples treated with 0.1–2.0 mL of cosolvent, proper volume of ethanol had to be added to complement it to 20 mL</p> <p>Extract posttreatment: Removal of chlorophyll to reduce the saturation of green color by treating extract with several acids, such as H₂SO₄, HCl, H₃PO₄, and CH₃COOH, at the concentration range of 0.002–0.1 N</p>	<p><i>Results of AXA extraction:</i> Yield ± δ [mg AXA/g d.m]; recovery [% of yield obtained with BBM method]:</p> <p>Pure SC-CO₂: 2.02 ± 0.20, 83; SC-CO₂ + 0.1 mL of EtOH: 2.13 ± 0.36; 87; SC-CO₂ + 0.2 mL of EtOH: 2.33 ± 0.62, 96; SC-CO₂ + 0.5 mL of EtOH: 2.40 ± 0.37, 98; SC-CO₂ + 1.0 mL of EtOH: 2.32 ± 0.43, 95; SC-CO₂ + 2.0 mL of EtOH: 2.41 ± 0.49, 99; SC-CO₂ + 20 mL of EtOH: 2.46 ± 0.23, 101</p> <p>Results for samples treated with pure SC-CO₂ and SC-CO₂ + cosolvent at the highest concentration showed statistically significant difference (<i>p</i> < 0.05 by Student's <i>t</i>-test) with AXA yield of both: conventional method and the rest of supercritical extracts</p> <p><i>Results of chlorophyll extraction:</i> Yield ± SD [mg chlorophyll/g d.m], recovery [% of yield obtained with BBM method]:</p> <p>Pure SC-CO₂: 29.4 ± 1.70, 103; SC-CO₂ + 0.1 mL of EtOH: 28.8 ± 0.91, 104; SC-CO₂ + 0.2 mL of EtOH: 30.8 ± 2.31, 108; SC-CO₂ + 0.5 mL of EtOH: 28.7 ± 1.24, 104; SC-CO₂ + 1.0 mL of EtOH: 28.4 ± 0.91, 103; SC-CO₂ + 2.0 mL of EtOH: 29.1 ± 0.36, 102; SC-CO₂ + 20 mL of EtOH: 29.5 ± 1.04; 103</p> <p>Results for all samples showed statistically significant differences (s.s.d., <i>p</i> < 0.05) with chlorophyll yield obtained using conventional method and no s.s.d. between each other</p>	[33]

(d)

Extraction	Biomass pretreatment	Sample and solvent	Conditions	Extraction yield	Reference
Solvent extraction with aqueous ethanol	(1) Grinding beforehand using a laboratory mill in equal short time intervals in order to avoid overheating (2) Passing the material through laboratory sieves (diameter 3 mm), periodically, and collecting the fine fraction after each sieving (ethanol as modifier)	(i) Sample: 30 g (ii) Solvent: aqueous ethanol at the concentration of 70% Solvent: sample ratio (w/w): 1:1	<i>t</i> : 10 days Providing extract (fraction) of polysaccharides: Drying EtOH extract in air and then treating it twice with 0.1 M HCl, at ratio (w/w) 1: 20, at 60° C for 120 min; neutralizing newly obtained extract and centrifuging to separate supernatant, from which WSPS fractions were isolated (concentrating supernatant in a rotary evaporator → dialyzing against distilled H ₂ O → lyophilization) HCl extract posttreatment: Separation of fucoidans from the polysaccharide fractions by anion-exchange chromatography (elution by a linear gradient of H ₂ O and 2 M NaCl solution), which resulted in providing one, two, or three fucoidan subfractions of different degree of sulfation (marked as F1–F3, according to an increasing order of sulfated group content) fractions	<i>Results for F. evanescens extracts:</i> Yield of fucoidans [% by d.m.]; content of total sugar [% by mass]: 5.11; 48.0 Content of SO ₃ Na and polyphenols [% by mass]: 34.6 and 0.5 <i>Results for S. japonica extracts:</i> Yield of fucoidans [% by d.m.]; content of total sugar [% by mass]: F1: 0.38; 46.4; F2: 1.28; 45.2 Content of SO ₃ Na and polyphenols [% by mass]: F1: 14.0 and 0; F2: 26.3 and 0.1 <i>Results for S. oligocystum extracts:</i> Yield of fucoidans [% by d.m.]; content of total sugar [% by mass]: F1: 0.34; 46.0; F2: 0.65; 48.1; F3: 0.55; 44.0 Content of SO ₃ Na and polyphenols [% by mass]: F1: 17.4 and 0.4; F2: 24.0 and 1.1; F3: 32.0 and 0.1	[34]
		(i) Sample: 30 g (ii) Solvent: Treating <i>F. evanescens</i> : pure SC-CO ₂ Treating <i>S. japonica</i> and <i>S. oligocystum</i> : both pure SC-CO ₂ and SC-CO ₂ + EtOH (5%) Tested fluid: sample ratios (w/w): from 10:1 to >30:1; fluid: sample ratio (w/w) chosen for experiments: 30:1	<i>T</i> = 60° C, <i>p</i> : 550 bar, <i>t</i> : 60 min Providing fraction of polysaccharides and posttreatment of HCl extract were as described in case of solvent extraction	<i>Results for F. evanescens extracts:</i> Yield of fucoidans [% by d.m.]; content of total sugar [% by mass]: Pure SC-CO ₂ : 3.02; 49.2 Content of SO ₃ Na and polyphenols [% by mass]: Pure SC-CO ₂ : 39.2 and 1.5 <i>Results for S. japonica extracts:</i> Yield of fucoidans [% by d.m.]; content of total sugar [% by mass]: Pure SC-CO ₂ , F1: 0.35; 48.5; pure SC-CO ₂ , F2: 1.26; 44.2; SC-CO ₂ + EtOH (5%), F2: 1.35; 45.1 Content of SO ₃ Na and polyphenols [% by mass]: Pure SC-CO ₂ , F1: 11.8 and 0.1; pure SC-CO ₂ , F2: 27.0 and 0.1; SC-CO ₂ + EtOH (5%), F2: 27.3 and 0.1 <i>Results for S. oligocystum extracts:</i> Yield of fucoidans [% by d.m.]; content of total sugar [% by mass]: Pure SC-CO ₂ , F1: 0.38; 47.4; pure SC-CO ₂ , F2: 0.57; 45.2; pure SC-CO ₂ , F3: 0.27; 42.0; SC-CO ₂ + EtOH (5%), F2: 0.55; 49.0; SC-CO ₂ + EtOH (5%), F3: 0.32; 34.9 Content of SO ₃ Na and polyphenols [% by mass]: pure SC-CO ₂ , F1: 16.4 and 2.1; pure SC-CO ₂ , F2: 23.4 and 1.7; pure SC-CO ₂ , F3: 34.0 and 0.1; SC-CO ₂ + EtOH (5%), F2: 28.8 and 1.5; SC-CO ₂ + EtOH (5%), F3: 32.0 and 0.5 Effect of different fluid: sample ratios: 10:1 ratio enabled to provide about 95% by mass of all extractable substances, while increasing ratio over 30:1 did not result in significant enhancement of SFE efficacy	

proteins have significant nutritional value to the animal organism [51].

3.4. Algae Based Products in Agriculture. Nowadays, due to future changes in European Union legislations, there is a growing interest in the use of supercritical algal extracts as natural foliar biostimulants for crop production. Plant growth stimulators which are known as phytohormones are the next important group of compounds, which can be extracted from algal biomass by SFE. From a chemical point of view, plant hormones are structurally diverse groups of compounds, which include auxins, gibberellins, cytokinins, salicylic acid, jasmonates, and brassinosteroids [52]. Plant hormones are the promoters of many essential physiological processes such as cell division, growth, and differentiation, organogenesis, sleep and seed germination, aging, and leaves pigments and for the response to biotic stress and abiotic factors [53, 54].

The effect induced on plants, by the treatment with products of algal origin, is mainly determined by the content of different types of plant hormones and their concentrations [55]. The functional importance is that these products should be applied in high dilutions. In many bioassays, researchers proved that products made from seaweeds stimulate the growth of many plants. The concentration of used extract and the method of application play an important role in such phenomena. As far as plant hormones and other biologically active compounds affect positively the plants in small concentrations, in higher doses they may cause inhibitory effect on some processes [56].

Algae are also rich in mineral compounds and trace elements. Their role in enhancing the plant growth should be underlined [57]. Möller and Smith investigated the importance of mineral components in suspensions made from seaweeds. Two brown algae extracts were tested on lettuce seedlings. The results showed that extracts were promoting the growth of cotyledon of lettuce. The experiment has led to the conclusion that mineral components were mainly responsible for this effect. Additionally, it was noticed that seaweed suspension was less effective than ashed extract. There is a possibility that suspensions contained some inhibiting organic compounds [58]. Cyanobacteria and eukaryotic algae have also the ability of phosphorus accumulation in the form of polyphosphates which as the reserve of phosphorus can significantly enrich the algal biomass used for the purposes of soil fertility and better plant growth. Seaweed extracts tested on *Vigna sinensis* stimulated the growth of this plant but only at concentration smaller than 20%. At higher concentration, the effect was the opposite [59]. The use of algal biostimulants may improve seedling growth, shoot and root length and weight, chlorophyll content, and in consequence total protein content. In another bioassay, the information about the influence of seaweed extract on spinach (*Spinacia oleracea* L.) was presented. Spinach seeds were irrigated with different concentrations of extract from *Ascophyllum nodosum*. Total flavonoids and phenolic compounds content and antioxidant activity were measured at a certain time after application which confirmed that the use of seaweed extract enhanced all of the tested parameters. Total flavonoids content increased 1.2 and 1.5 times compared to control and the upswing

depended on concentration of seaweed extract. Since, total content of phenolic compounds increased, the antioxidant activity also has been improved. The optimal concentration of extract, which showed the desired activity, was determined as 1 g/L [60].

Plants treated with algal extracts showed more intense growth of their roots, which significantly improved the uptake of the nutrients from soil. This phenomenon seems to be crucial, especially if regarding the habitats poor in mineral compounds [1]. Field experiment on soybean showed that application of seaweed extract from *Kappaphycus alvarezii* enhanced yield parameters. Researchers observed also better nutrient uptake by this crop after foliar spraying. The maximum straw yield was obtained after using the extract at a concentration of 15% [61].

Besides growth promoting effect, seaweed extracts also show antibacterial and antifungal properties. Carrot plants were treated with seaweed extract (0.2%) from *Ascophyllum nodosum* 6 h after the conidial suspension of *A. radicina* or *B. cinerea* was inoculated. After 25 days, the results were measured and the plants treated with seaweed extract exhibited reduced infection by around 50%. Molecular analysis showed the accumulation of defense gene transcripts, phenolics, and phytoalexins [62].

Due to a wide spectrum of positive influence on many aspects of plant growth, several commercially available products derived from algae are being used worldwide. Brown and red algae are the most popular in biofertilizers production, because of their availability throughout all the seasons of the year and high content of bioactive substances. The most known plant growth stimulants manufactured by BASF are Kelpak and Profert, manufactured from *Ecklonia maxima* and *Durvillaea antarctica*, respectively. Many manufacturers utilize also brown algae *Ascophyllum nodosum* for their biostimulating properties [56].

4. Composition of Algal Extracts: Analytical Methods

Determination of the full chemical profile of algae is a complicated task, even under unfavorable growth conditions, which significantly enhance the synthesis of active compounds in algal cells. Complexity of the matrix is the major obstacle needed to be overcome.

Furthermore, there are problems in the preparation of biomass samples suitable for the analysis, whereas this pretreatment is the key step in the whole test. This procedure is time consuming and requires several steps including tissue fragmentation (mechanical, using radiation or ultrasounds) and extraction (different types of solvent techniques, supercritical fluid extraction). Algal extract obtained using the above methods needs the specific sample preparation before qualitative and quantitative analysis. The most popular techniques used for this purpose are solid-phase extraction, membrane microextraction, immunoaffinity extraction, vapor-phase extraction, extract filtration, evaporation, and in many cases sample fractionation and derivatization. Working with plants is also hindered considering vulnerability. In

some conditions, content of chemical compounds might be changing during the extraction and sample preparation. Consequently, the total concentrations of desired compounds might be different compared to the whole plant [63]. A variety of analytical methods are available to determine chemical composition of biological material, depending however on the chemical properties of desired compounds: analytes.

Instrumental methods, combined with various detectors, made it possible to determine several hormones simultaneously in fresh plant material, as well as in products (mostly food) that are used in many bioassays [3, 64–66]. Among these methods, chromatographic techniques seem to be the best and the most accurate for measuring trace amounts of phytohormones in seaweeds and extracts from plants.

Since the early 1970s of the last century, liquid chromatography and high pressure liquid chromatography have become more popular in plant hormones analysis. Good resolution and relatively low limit of detection allowed for simultaneous qualitative and quantitative determination of various classes of plant hormones [63]. Liquid chromatography used for plant hormone analysis does not require sample derivatization. Popular methods of detection connected with LC or HPLC are UV–V is detectors, diode array and fluorescence detection, and especially MS that allow determining the chemical structure of the analytes [67]. The potential of this method is being multiplied when tandem mass detector is used [68]. Other instrumental methods like capillary electrophoresis or spectral and electrochemical methods and especially biosensors are rather used for the analysis of phytohormones.

For the analysis of nonpolar or volatile compounds from algae extracts, gas chromatography is widely used. This technique, especially combined with mass detectors, is efficient for the structural identification and accurate quantification in multiple phytohormone analysis. Nevertheless, the requirement for sample volatility limits its application to only few plant hormones and potential volatile biostimulants. Sometimes derivatization is needed in case of obtaining better and more reliable results; nevertheless, this step significantly extends the time of analytical procedure [69].

Chromatography assays might be combined with supercritical fluid extraction, resulting in analysis defined as supercritical fluid chromatography (SFC). This method enables performing measurements in automated system under online control, providing shorter time of the assay and decreased level of contamination [70]. Depending on particular need, different ways of collecting SF-extracted analytes might be suitable: (1) solvent collection in a solvent containing vessel, (2) solid-phase collection: highly selective separating analytes on packed-bed column, filled with inert or adsorbing material, from which they are eluted by the use of appropriate solvent, (3) online collection: using a connection of collected device with chromatograph, and (4) alternative collection: (4a) solid-liquid phase collection: recommended for highly volatile analytes, which are trapped in a system of a solid-phase and solvent containing vessel (catching losses), (4b) collection inside fused-silica capillaries, and (4c) empty vessel trap collection: a way excluding solvent-sample separation step [71, 72]. Besides the mentioned volatile compounds,

SFC is suitable for testing wide spectrum of molecules with diverse characteristic, for example, polarity and molecular mass, including fat-soluble vitamins (without necessity of former derivatization). In case of algae derived constituents, supercritical fluid chromatography was applied to determine content of isoflavones from seaweeds (brown algae: *Sargassum muticum*, *Sargassum vulgare*, and *Undaria pinnatifida*; red algae: *Hypnea spinella*, *Porphyra* sp., *Chondrus crispus*, and *Halopytis incurvus*), freshwater green algae (*Spongiochloris spongiosa*), and cyanobacteria (*Scenedesmus* and *Nostoc* 17). The whole experiment involved biomass pretreatment (sonication) and dynamic extraction with SC-CO₂ (modified with aqueous methanol) at 40°C and 350 bar for 60 minutes, followed by fast chromatography analysis and tandem mass spectrometry detection [73]. Abrahamsson et al. investigated supercritical fluid chromatography for quantitative determination of carotenoids from microalgae *Scenedesmus* sp. SFE was performed on pretreated sample (freeze-drying and grinding with liquid nitrogen) using CO₂ (with or without co-solvent – ethanol) at flow rate 2 mL/min, at 60°C and 300 bar for 60 minutes, and the obtained extract was analyzed with a series of two columns: C18 and 2-ethyl pyridine. Research proved validation of the method to separate and quantify carotenoids to be comparable to standard approach [74].

5. Conclusions

Supercritical fluid extraction gives the possibility to isolate biologically active compounds from the biomass without their degradation. Solvent-free extracts can be used in many branches of industry: as active ingredients in cosmetic products, as components of biostimulant formulations in order to increase crop production, or as feed additive allowing for the production of healthy animal dietary feed supplement. The implementation of new algal-derived products in the market coincides with the public demand for natural products. There is also a need to replace classical extraction methods with innovative technologies based on bioresources. Limited use of environmental friendly CO₂ solvent and the possibility of the reuse of waste byproducts produced by SFE are the main advantages of this process, instead of the high costs of the SFE installation. Ingredients derived from raw algal material in SFE process ensure no residues of organic solvents. Therefore, algal extracts have promising future prospects as products for humans, animals, and plants. In this review, special attention was paid to the application of algal extracts in plant cultivation, since this issue is rarely studied in the literature. In order to properly apply algal extract, detailed characteristics should be provided by the use of novel analytical methods.

The potential of algae as the source of many specific substances of biological activity, as well as growing interest and possibilities of using these organisms, creates favorable conditions in many areas of research and development.

Abbreviations

AA: Arachidonic acid

GLA: γ -Linolenic acid

ALA:	α -Linolenic acid
PLE:	Pressurized liquid extraction
AXA:	Astaxanthin
PUFA:	Polyunsaturated fatty acids
BBE:	Bead beater extraction
SCF:	Supercritical fluids
DHA:	Docosahexaenoic acid
SC-CO ₂ :	Supercritical carbon dioxide
EPA:	Eicosapentaenoic acid
SFC:	Supercritical fluid chromatography
FAME:	Fatty acid methyl esters
SFE:	Supercritical fluid extraction.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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