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Microalgae biomass as an alternative source of biocompounds: New insights and future perspectives of extraction methodologies

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ABSTRACT

Microalgae have characteristics that make them unique and full of potential. Their capacity to generate interesting bioactive molecules can add value to various industrial applications. However, most of these valuable compounds are intracellular, which makes their extraction a major bottleneck. Conventional extraction methodologies have some drawbacks, such as low eco-friendly character, high costs and energy demand, long treatment times, low selectivity and reduced extraction yields, as well as degradation of extracted compounds. The gaps found for these methods demonstrate that emergent approaches, such as ohmic heating, pulsed electric fields, ionic liquids, deep eutectic solvents, or high-pressure processing, show potential to overcome the current drawbacks in the release and extraction of added-value compounds from microalgae. These new processing techniques can potentially extract a variety of compounds, making the process more profitable and applicable to large scales. This review provides an overview of the most important and promising factors to consider in the extraction methodologies applied to microalgae. Additionally, it delivers broad knowledge of the present impact of these methods on biomass and its compounds, raising the possibility of applying them in an integrated manner within a biorefinery concept.

1. Introduction

1.1. *Microalgae: An alternative source of biocompounds*

One of the greatest challenges that modern society faces is related to the exponential growth of worldwide population observed over the last century. The world population has doubled and it is expected to continue to increase in number in the following decades. In this context, finding new ecological and functional ways to meet human nutritional needs and obtain products of interest through sustainable and functional ways becomes critical (Buchmann, Brändle, Haberkorn, Hiestand, & Mathys, 2019). One possible solution for this problem is the biotechnological use of certain microorganisms, such as microalgae (**Fig. 1**). With relatively simple procedures, small spaces, and short production periods, high yields can be achieved when using microrganisms to obtain important macronutrients and bioactive compounds, representing an interesting strategy to (partially) overcome overpopulation's challenges (Geada et al., 2018).

Microalgae are photosynthetic microorganisms and one of the oldest forms of life on Earth; however, they remain rather unexplored (Cunha et al., 2023; Geada et al., 2018). It is estimated the existence of hundreds of thousands to several million of species. Among these, only approximately 73,000 are currently identified, being a small fraction cultivated at industrial scale for commercial purposes (Cunha et al., 2023). Recently, this group of microorganisms has gained biotechnological interest due to a set of characteristics that make them unique and full of potential: i) their growing conditions are easy to reproduce, which facilitates their study; ii) they are highly adaptable to extreme conditions, allowing climatic adaptation and generation of new or rare bioproducts; iii) their biodiversity opens the possibility of discovering new products or exploring alternative sources of the existing ones (e.g., carotenoids); and iv) they are a promising microbial protein source for human nutrition (Geada et al., 2018, 2021; Varshney, Mikulic, Vonshak, Beardall, & Wangikar, 2015).

Microalgae can be divided into two types, considering their cell complexity: prokaryotic or cyanobacteria and eukaryotic. Prokaryotic microalgae cells, such as *Microcystis aeruginosa* and *Arthrospira platensis*, are characterized as cells lacking membrane-bound organelles, reproduction using asexual processes – like binary fission, multiple fission, and fragmentation –, and not having structured DNA in chromosomes. On the contrary, eukaryotic microalgae, such as *Chlorella vulgaris* and *Dunaliella salina*, are characterized by having organelles – such as the

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Golgi apparatus, chloroplast, mitochondria or nucleus (with structured DNA in chromosomes) – and the reproductive process occurs mostly asexually by cell division, fragmentation or production of spores, although almost all species are also able to reproduce sexually. The nutrition mode of microalgae can be classified, depending on the origin of the carbon source, in: (i) autotrophic, when using an inorganic carbon source like carbon dioxide, and (ii) heterotrophic, when an organic carbon source, such as glucose, is consumed. Some microalgae can combine autotrophy and heterotrophy pathways the so-called mixotrophic conditions – to satisfy their nutritional needs, using both organic and inorganic carbon sources during growth (Cunha et al., 2023; Geada, Vasconcelos, Vicente, & Fernandes, 2017).

The maximization of valuable biocompounds production through microalgae frequently depends on culture conditions. Under different growth environments, microalgae may exhibit distinct metabolic responses, resulting in the synthesis of several bioproducts – as pigments, proteins, fatty acids, or carbohydrates – with interest to multiple industrial branches (Buchmann et al., 2019; Carullo et al., 2018; Geada et al., 2018; Guihéneuf & Stengel, 2017; Jacob-Lopes et al., 2019; Luengo, Martínez, Bordetas, Álvarez, & Raso, 2015). Indeed, the annual production of dry microalgae biomass is around 19,000 tons, generating 5.7 billion US$ (Jacob-Lopes et al., 2019). Worldwide, *Arthrospira*, *Chlorella*, *Dunaliella*, and *Haematococcus* are the most used genera for industrial purposes. Several bioproducts derived from these photosynthetic microorganisms are already in the market, including carotenoids or polyunsaturated fatty acids (PUFAs) (Guihéneuf & Stengel, 2017; Luengo et al., 2015). By 2026, carotenoids market is estimated to worth around 2.0 billion $US (Market (Astaxanthin, Beta-Carotene, Lutein, Lycopene, Canthaxanthin and Zeaxanthin), Application (Feed, Food & Beverages, Dietary Supplements, Cosmetics and Phamaceuticals), Source, Formulation, and Region - Global Forecast ,2020, 2026). Likewise, PUFAs market is expected to grow, having a current total value of 10.6 billion $US (Market, Industry Analysis, Size, Share, Analysis, & Forecast, 2020, 2021, 2026). In this case, microalgae also offer advantages as sources of PUFAs when compared to conventional sources, such as fish and fish oil, since conventional sources have drawbacks that include the possible accumulation of toxins, fish odour, unpleasant taste, poor oxidative stability, and the presence of mixed fatty acids, which turn them unsuitable for vegetarian diets (Brennan & Owende, 2010).

Most of the products resulting from microalgae are intracellular, which is a disadvantage since the presence of cell walls often limits the access to such constituents(Buchmann et al., 2019; Carullo et al., 2018; Geada et al., 2019; Luengo et al., 2015). Therefore, the capacity to generate compounds of interest is not a factor that contributes to the valorisation of microalgae by itself. It is necessary to make them accessible to allow their recovery and usage. To overcome this problem, a set of cell extraction methodologies are currently being applied. Though, the conventional methodologies have some drawbacks, including low eco-friendly character, high processing costs and energy demand, long treatment times, low selectivity and/or extraction yields, and exposure of the extracts to excessive heat, light or oxygen (Azmi, 2020; Moreira, Alexandre, Pintado, & Saraiva, 2019). Furthermore, most of the extracted fractions must be further purified before employed in different industrial applications, since they are frequently contaminated with organic solvents used throughout the extraction process. Such additional treatments are time-consuming and entail extra costs, which is not appealing at industrial scale (Azmi, 2020; Carullo et al., 2018; Cha, Lee, Koo, Song, Lee, & Pan, 2010; Moreira et al., 2019). Based on these facts, there is an opportunity to create an effective alternative. Processing technologies based on the application of electric fields, deep eutectic solvents, supercritical fluids, ionic liquids or high-pressure processing are some examples of emergent extraction technologies, which allow combining the main steps of downstream processing: cell disruption and product separation, concentration, and purification (Azmi, 2020; Buchmann et al., 2019).

In this review, particular focus is given to recent developments in extraction methodologies applied to microalgae and cyanobacteria, as well as how emergent technologies can be used to improve process cost-effectiveness and extraction yield through more sustainable/environmentally friendly approaches.

2. High-value biocompounds from microalgae

Pharmaceutical, cosmetics or food industries are just some examples of industrial branches that use microalgal metabolites in their products (Table 1) (Geada et al., 2018; Jacob-Lopes et al., 2019).

Microalgal proteins are an excellent alternative to their animal-based counterparts, presenting advantages such as carbon–neutral emissions,

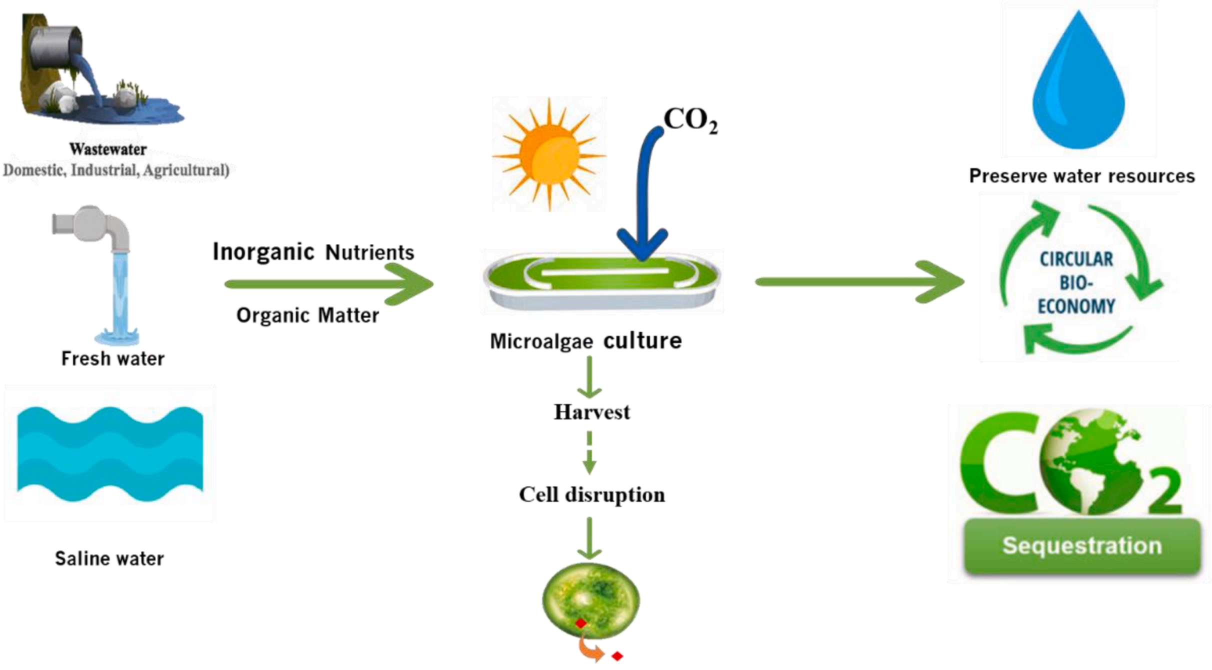

Fig. 1. Microalgae: an alternative and eco-friendly source of several biocompounds.

Table 1
Biocompounds derived from different microalgae with application in several industrial branches.

Microalgae fraction	Biocompound		Purpose/Activity	Industrial application	Microalgae species	Conventional sources	References
Whole biomass	———		Protein supplementation	Food	*Arthrospira platensis; Chlorella vulgaris; Dunaliella salina*	No similar alternative	(Jacob-Lopes et al., 2019)
Proteins	EAAs		Protein supplementation	Food	*Arthrospira platensis; Chlorella vulgaris; Dunaliella sp*	Meat	(Geada et al., 2021; Jacob-Lopes et al., 2019)
	Bioactive peptides		Antihypertensive; Antioxidant; Anticoagulant; Antimicrobial; Anticancer	Nutraceutical; Pharmaceutical	*Arthrospira platensis; Chlorella vulgaris*	Synthetic, transgenic, and recombinant therapeutic peptides	(Ovando et al., 2018; Suetsuna & Chen, 2001)
Pigments	β-carotene		Colorant; Additive; Pro-vitamin A UV protection; Oxidative stress protection; Anti-carcinogenic; Anti-heart diseases; Cholesterol control	Food; Feed; Pharmaceutical; Cosmetics	*Dunaliella salina*	Chemical synthesis	(Jacob-Lopes et al., 2019; Monte et al., 2020)
	Astaxanthin		Supplement; Additive; UV protection; Oxidative stress protection, Hormone precursor; Anti-inflammatory; Immune system enhancement	Food; Feed; Pharmaceutical; Cosmetics	*Haematococcus pluvialis*	Chemical synthesis	(Jacob-Lopes et al., 2019; Praveenkumar et al., 2015)
	Lutein		Prevent retinal degeneration; Anti-cancer; Prevent cardiovascular diseases; Antioxidant	Nutraceutical	*Chlorella sp; Scenedesmus sp*	Marigold flowers	(Low et al., 2020; Luengo et al., 2015)
	Fucoxanthin		Antioxidant; Anti-diabetic; Anti-obesity; Anti-cancer; Anti-inflammatory; Hepatoprotective; Skin protection	Pharmaceutical; Cosmetics	*Isochrysis galbana; Phaeodactylum tricornutum*	Brown macroalgae	(Zarekarizi, Hoffmann, & Burritt, 2019)
Lipids	Triglycerides and hydrocarbons		Third generation biofuels	Biofuels	*Chlamydomonas reinhardtii; Dunaliella salina; Chlorella sp; Botryococcus braunii*	Fossil fuels	(Brennan & Owende, 2010; Dragone et al., 2010)
	PUFAs	α-linolenic acid	Preventative effect against cardiovascular diseases	Food; Nutraceutical	*Nannochloropsis sp; Nodularia harveyana; Rhodomonas baltica; Pseudokirchneriella subcapitata*	Walnuts, flaxseed oil, and soybean	(Guihéneuf & Stengel, 2017; Jacob-Lopes et al., 2019; Sathasivam et al., 2019)
		Eicosapentaenoic acid	Nutritional supplements; Aquaculture; Prevention of chronic inflammation-linked metabolic diseases	Food; Feed; Pharmaceutical	*Nannochloropsis; Phaeodactylum nitzschia; Pavlova lutheri*	Fatty fish (salmon, mackerel, tuna) and fish oils	
		Docosahexaenoic acid	Infant formulas; Nutritional supplements; Aquaculture; Prevention of chronic inflammation-linked metabolic diseases	Food; Feed; Pharmaceutical	*Schizochytrium sp; Crypthecodinium cohnii; Pavlova lutheri*	Fatty fish (salmon, mackerel, tuna) and fish oils	
		Linoleic acid	Anti-inflammatory; Acne reductive; Moisture retentive properties	Food	*Scenedesmus obliquus; Chlorella vulgaris; Chroococcus sp; Rhodomonas baltica*	Corn, safflower, soybean, cottonseed and sunflower oils	
		Arachidonic acid	Supplements; Aquaculture; Prevention of chronic inflammation-linked metabolic diseases	Food; Feed; Pharmaceutical	*Porphyridium sp*	Meat, poultry and eggs	
		γ-linolenic acid	Supplements; Aquaculture; Prevention of chronic inflammation-linked metabolic diseases	Food; Feed; Pharmaceutical	*Arthrospira sp*	Evening primrose oil, borage oil and black current seed oil	

(*continued on next page*)

Table 1 (*continued*)

Microalgae fraction	Biocompound	Purpose/Activity	Industrial application	Microalgae species	Conventional sources	References
Carbohydrates	Polysaccharides	Third generation biofuels	Biofuels	*Chlamydomonas reinhardtii; Dunaliella salina; Chlorella sp; Botryococcus braunii;*	Fossil fuels	(Brennan & Owende, 2010; Dragone et al., 2010)
		Viscosifiers, lubricants, and flocculants; Antiviral; Immunostimulatory; Anticancer; Anti-inflammatory and immunosuppressive	Food; Pharmaceutical; Nutraceutical	*P. cruentum, C. pyrenoidosa, C. stigmatophora*	Conventional crops; Chemical synthesis	(Jacob-Lopes et al., 2019; Sathasivam et al., 2019)
	Sulfonated polysaccharides	Anticoagulant; Antiviral; Antioxidative; Anticancer	Pharmaceutical; Nutraceutical	*Chlorella vulgaris; Scenedesmus quadricauda*	Conventional crops; Synthetic drugs	(Sathasivam et al., 2019)
Toxins	Microcystins	To investigate neurodegenerative diseases; Antialgal; active principle in anticancer drugs production	Pharmaceutical; Antifouling agents	*Microcystis aeruginosa*	Synthetic drugs	(Geada et al., 2019)
	Okadaic acid	To investigate the therapeutic effects of atypical, antipsychotic drugs; Treatment of cognitive impairment and schizophrenia	Pharmaceutical	*Dinophysis sp*	Synthetic drugs	(Sathasivam et al., 2019)
Antioxidants	Phenolic compounds	Antioxidant; Extend the shelf-life of foodstuffs	Food	*Chlamydomonas nivalis; Botryococcus sp; Chlorella sp; Dunaliella sp; Nostoc sp; Phaeodactylum sp; Spirulina sp; Haematococcus sp; Chaetoceros sp*	Terrestrial plants; Synthetic antioxidants	(Goiris, Muylaert, & Fraeye, 2012)

no need for arable land, higher productivity rates, and low water consumption (Geada et al., 2021). Certain microalgae species can accumulate over 50 % of their dry weight in proteins – as the case of *Arthrospira maxima* (60–71 %), *Chlorella vulgaris* (41–58 %) or *Euglena gracilis* (39–61 %) (Carullo et al., 2018; Dragone, Fernandes, Vicente, & Teixeira, 2010; Jacob-Lopes et al., 2019; Niccolai, Chini Zittelli, Rodolfi, Biondi, & Tredici, 2019). Additionally, these proteins contain numerous peptides and amino acids that can be used as food supplement – as these might present fractions that are rich in essential amino acids with a similar profile to the animal protein sources – or with nutraceutical function – due to some peptides exhibiting antihypertensive, immunomodulatory, anti-inflammatory, or anti-diabetic activity, as shown in Table 1 (Geada et al., 2021; Ovando et al., 2018; Suetsuna & Chen, 2001). Consequently, there are products already in the market that incorporate microalgae in their formulation, such as snacks, drinks, pasta or chocolates (Geada et al., 2021). However, despite several microalgae genera (*Arthrospira*, *Chlorella*, *Aphanizomenon*, *Dunaliella*, and *Haematococcus*) are currently used for human consumption and fully commercialized, a strict food regulation, particularly in Europe, is limiting their wider consumption (Buchmann et al., 2019; Geada et al., 2018, 2021; Niccolai et al., 2019). Nevertheless, the approval of more species is expected to occur in the next few years due to the increasing consumers' demand for more sustainable alternatives (and, especially, non-animal-based products).

Nowadays, microalgae biomass is used as source of three classes of pigments: chlorophylls, carotenoids, and phycobiliproteins (Jacob-Lopes et al., 2019). Some species are well established at commercial scale – for instance, *Dunaliella salina* and *Haematococcus pluvialis* as a source of β-carotene and astaxanthin, respectively (Brennan & Owende, 2010; Monte, Bernardo, Sá, Parreira, Galinha, Costa, Casanovas, Brazinha, & Crespo, 2020). Both pigments can be used in the food industry, as a source of pro-vitamin A, or for fish feed (Geada et al., 2018; Jacob-Lopes et al., 2019). Recently, microalgal pigments have been explored by the pharmaceutical, nutraceutical, and cosmetics industries due to their antioxidant activity and potential health benefits against obesity, diabetes, cancer or cardiovascular diseases (Guihéneuf & Stengel, 2017; Jacob-Lopes et al., 2019; Luengo et al., 2015). Astaxanthin and β-carotene have already been used in cosmetics as protection agents against UV radiation and oxidative stress. Regarding pharmaceutical applications, astaxanthin is being used to boost the immune system and as a hormone precursor, having anti-inflammatory properties. On the other hand, β-carotene is mainly applied as a cholesterol controller, anti-carcinogenic agent, and to act in the prevention of cardiovascular diseases (Geada et al., 2018). Usually, commercial pigments are produced either by chemical synthesis or extracted from other biological organisms, such as flowers. Pigments deriving from microalgae present several advantages when compared with conventional sources. For example, when considering β-carotene, about 90 % of this pigment found in the market results from chemical synthesis; however, its natural form produced by microalgae exhibits higher bioaccessibility (Monte et al., 2020). Likewise, microalgae offer benefits when compared with the conventional source of lutein – marigold flowers – since they have higher lutein content and productivity and do not need arable land to grow (Luengo et al., 2015).

Several microalgae species are known for their capacity to generate high amounts of lipids. The first interest in microalgal lipids relied on the potential to be a promising source of renewable biodiesel. This situation was related to the accumulation of great amount of fatty acid methyl esters that could be converted into biodiesel through a process called transesterification. By using this renewable biodiesel, a significant reduction of CO_2 emissions (up to 78 %) would be possible when compared to the conventional source –petroleum (Brennan & Owende, 2010; Dragone et al., 2010). Recently, the interest in the lipidic fraction of microalgae gained new momentum due to their ability to accumulate

PUFAs. This group of compounds includes both omega-3 (such as eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA,22:6n-3), and α-linolenic acid (ALA,18:3n-3)) and omega-6 fatty acids (such as γ-linolenic acid (GLA,18:3n-6), arachidonic acid (ARA,20:4n-6) and linoleic acid (LA,18:2n-6)). PUFAs are commonly used in numerous industrial branches, such as pharmaceutical, food and animal feed. Among other applications, these compounds can be employed in the treatment of rheumatism or skin diseases (Geada et al., 2018; Guihéneuf & Stengel, 2017; Jacob-Lopes et al., 2019).

As with lipids, the carbohydrates fraction from microalgae can be used to produce a new generation of biodiesel as well (Geada et al., 2018). Although biomass composition varies from species to species, certain microalgae can accumulate high amounts of carbohydrates – around 55 % in dry weight (Dragone et al., 2010; Fernandes et al., 2013). In addition, starch – one of the carbohydrates accumulated by microalgae as a storage product – can be applied in human supplementation (Fernandes et al., 2013; Lai, Puspanadan, & Lee, 2019). Beyond that, it can be incorporated in conventional plastics, contributing to their greater biodegradability without compromising the mechanical properties of the materials (Lai et al., 2019). Microalgal carbohydrates can also target other potential applications due to the presence of specific groups (e.g., glucuronic acid, sulfate groups) in their structure, which can impart with antioxidant, antiviral, or anticancer activities to bioactive molecules (Jacob-Lopes et al., 2019; Sathasivam, Radhakrishnan, & Hashem, 2019).

Besides the aforementioned metabolites, microalgae biomass is a potential source of other interesting bioactive compounds, such as vitamins (e.g., A, B_{12}, C, E, K), minerals, enzymes, sterols, and antioxidants (Geada et al., 2018, 2019; Jacob-Lopes et al., 2019; Sathasivam et al., 2019). Given the biodiversity and increasing interest in microalgae, it is expected that new compounds will be discovered, opening possibilities of developing new products for industrial applications.

Table 2
Most widely used conventional extraction methodologies in microalgae and the corresponding advantages and drawbacks.

Methodology	Advantages	Drawbacks	References
Bead milling	Low energy inputs; Mild conditions; Industrial scale	Non-selective extraction; Energy conversion into heat	(Günerken et al., 2015; Postma et al., 2017)
Chemical treatment	Low energy inputs; Good scalability; Selective extraction	Use of organic solvents; Non-food grade	(Kim et al., 2016; Show, Lee, Tay, Lee, & Chang, 2015)
Ultrasonication	Improve algal substrate solubility; Able to be combined with other methodologies	Low cell disruption efficiency for some microalgae species; High energy demand; Extreme treatment conditions (high temperatures and pressures; Not applicable at large scale	(Grimi et al., 2014; Günerken et al., 2015; Halim, Rupasinghe, Tull, & Webley, 2013; Show et al., 2015)
Microwaves	Temperature increases more homogeneously when compared to conventional heating; Combined effect of temperature and microwaves increase the extraction yield for 37.5–44 % when compared to conventional heating; Easy scale-up	Limited to combined use with polar solvents; Non-applicable for volatile target compounds; Unfavourable methodology to include on a microalgae cascade extraction strategy; May cause protein aggregation and denaturation	(Günerken et al., 2015; Kim et al., 2016; Show et al., 2015)

3. Extraction methodologies

Despite the great biotechnological potential, most microalgae compounds are intracellular, as mentioned previously. Therefore, separation and recovery processes need to be optimized to make them economically viable, resulting in more competitive approaches at industrial scale (Jacob-Lopes et al., 2019). Based on these facts, there is an opportunity to establish other extraction methodologies that offer potential advantages regarding conventional options; these alternatives tend to be more environmentally friendly and easily applied at larger scale, not requiring purification steps and presenting low operational costs (Khanra et al., 2018).

The most widely used conventional techniques to release biocompounds from microalgae biomass are mainly bead milling, chemical treatments, ultrasonication, and microwaves. Despite the advantages displayed by these methods, they also have serious shortcomings (Table 2), revealing the pressing need for new effective alternatives. The major drawbacks of such commonly used methodologies are: i) great environmental impact due to the use of organic solvents (e.g., chemical treatments); ii) low selectivity, which does not allow for the implementation of a microalgae cascade extraction approach (e.g., bead milling); iii) difficulty to apply at large scale (e.g., ultrasonication); or iv) possibility to degrade certain fractions of the extracted compounds as consequence of the harsh conditions used (e.g., microwave). The selective and efficient release of the target bioproduct, and consequent high recovery yield, is a key aspect for a successful extraction process (Fig. 2), along with the reduction of contaminants – both in terms of number and content – and minimization of cell debris micronization (Grimi et al., 2014).

Usually, microalgae are cultivated to obtain a single product. This cultivation strategy is adopted mainly due to the conventional extraction methodologies that are already established, also focused in a specific compound (Jacob-Lopes et al., 2019). Ultimately, this type of approach does not meet the full potential of microalgae biomass. Recently, a paradigm shift has been observed with the growth of microalgae considering the sequential multi-stage extraction of biocompounds from biomass (i.e., cascade extraction concept), also as consequence of the rise of new extraction methodologies (Ferreira-Santos et al., 2021; Jacob-Lopes et al., 2019).

Microalgae cascade extraction consists in the valorisation of the leftover biomass after the extraction of a desired product or, in other words, multiple extractions of different fractions of the microalgal biomass. For instance, Ferreira-Santos et al. (2021) performed a sequential multi-stage extraction procedure to make full use of *Spirulina platensis* biomass. In their study, ohmic heating (OH), conventional heating, and enzyme-assisted extraction were used for the disintegration and/or disruption of *Spirulina platensis* cells aiming a selective recovery of phycobiliproteins as a first stage, which was then followed by the extraction of phenolics, other pigments, and lipids (in subsequent steps) from the remaining biomass (Ferreira-Santos et al., 2021). This strategy allows a fully valorisation of the microalgae by harnessing multiple compounds, improving the economic feasibility of the whole production process and contributing to a minimal waste generation (Bhattacharya & Goswami, 2020; Ferreira-Santos et al., 2021; Jacob-Lopes et al., 2019). Another important contribution of the application of the sequential extraction concept is associated with the products that require intensive downstream processing. Costs related to processing of the target biocompounds can represent between 50 % and 60 % of the overall cost of the product, depending on the desired purity and biochemical properties (Eppink, Ventura, Coutinho, & Wijffels, 2021). Considering that many of these products are generated in low amounts inside microalgae cells, the

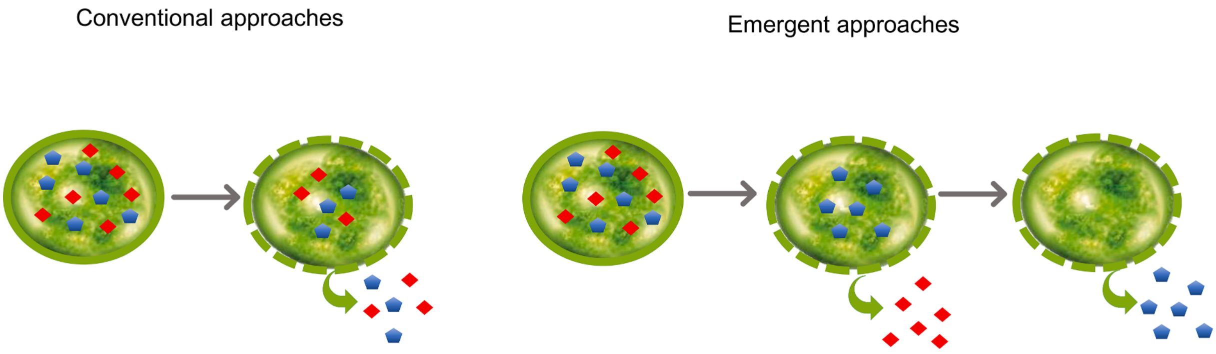

Fig. 2. Diagram representing the differences between the traditional and emergent approaches for biocompounds recovery.

extraction and purification processes are, therefore, quite expensive, contributing significantly to increase the total production costs. Thus, the widespread commercialization of novel speciality chemicals from microalgae (e.g., carotenoids, PUFAS, proteins) is deeply dependent on the application of an efficient cascade extraction processing approach. This strategy will allow reduced production costs – through the use of the remaining microalgal fractions – leading, in turn, to greater economic and environmental valorisation, as mentioned previously (Jacob-Lopes et al., 2019; Santos, 2018).

In the present review, an assessment of the extraction methodologies that are most widely used in microalgal processes (conventional methodologies) was carried out, being their main drawbacks highlighted in Table 2. Based on this information, a set of emerging methodologies was identified considering the potential to (partially) overcome the problems presented by the conventional methodologies. Given the wide range of alternatives found and the fact that a detailed comparison of all the methodologies would be quite difficult to accomplish, those with greater potential to be integrated into a cascade extraction (of bioactive compounds) approach were chosen for further analysis and discussion of the present manuscript. For this propose, the Sciencedirect, Scopus, Pubmed, and Google Scholar databases were used in order to identify the information to be discussed using keywords such as microalgae biomass extraction, novel extraction methodologies, downstream processing, innovative extraction approaches, emerging extraction methodologies, sustainable extraction techniques, biocompound recovery from microalgae, and green extraction processes.

3.1. *Electrical processing*

The alternative extraction methodologies based on the application of electric fields (EFs) (i.e., electrotechnologies), are considered eco-friendly, with low energy demand, and have (potentially) the ability to extract different kinds of compounds within a short treatment time. They also allow the combination of thermal, electrical and electrochemical effects and are already applied at industrial scale (e.g., food pasteurisation and sterilisation). The exposure of cells to EFs can alter the structure of their cellular membranes due to a transmembrane charge exchange. Consequently, the membrane loses its barrier function and becomes permeable, a phenomenon often referred to as electroporation or electropermeabilization (Buchmann et al., 2019; Carullo et al., 2018; Jaeschke, Menegol, Rech, Mercali, & Marczak, 2016; Luengo et al., 2015). The electroporation induced by the application of EFs can be reversible or irreversible, depending on the intensity of the EF applied. Irreversible electroporation occurs when the pores created in the cell wall during the application of EFs stay permanent after treatment, whereas reversible electroporation allows cells to restore the original form after electrical treatment(Azmi, 2020; Buchmann et al., 2019; Jaeschke et al., 2016; Luengo et al., 2015). The reversibility and irreversibility of the electroporation phenomenon are related to the size and number of pores generated in the phospholipid layer due to the perturbation caused by the application of the EF (Luengo et al., 2015). The presence of an EF may result in different outcomes and practical applications. Therefore, some parameters, such as the intensity of the EF applied, type of electrical waveform (sinusoidal, pulse or square), electrical frequency, total treatment time, specific treatment energy, and inherent electrical properties of the sample (i.e., electrical conductivity), are important to bear in mind when exploring electrical processing approaches (Geada et al., 2018). Within EFs, different extraction approaches might be performed: i) application of electrical pulses with high voltages for a short period of time (micro or nano-seconds), ii) application of moderate electric fields (<1000 V/cm) allowing to maintain a controllable increase/maintenance of temperature (Fig. 3), iii) application of high temperature for a short-time (HTST) – due to a controllable dissipation of internal heat, known as Joule heating or OH effect, or iv) application of different solvents during the electrical treatment to increase the extraction rate and improve extraction selectivity (without compromising the integrity of the extracted compounds), thus facilitating the subsequent purification process (if necessary). These different approaches will be responsible for different disturbances at the microalgae cells membrane level, being expected to result in the extraction of different compounds (Table 3).

3.1.1. *Pulsed electric fields*

Pulsed electric fields (PEFs) treatment consists of the application of electrical pulses – in the range of kV – for a short time – nano to micro-seconds – to promote the release of target compounds, while reducing the need for thermal effects. The EF applied is usually operated under a square wave and alternate directional pulses. The use of square waves is a more effective and energy-efficient approach, whereas the use of alternate directional pulses prevents/reduces some operational problems, namely electrolytic reactions and electrode erosion. The most important factors to consider, using PEFs as an extraction methodology, are the pulse duration and number of pulses applied, being the correct conjugation of these factors a key to successful extraction procedures.

The application of PEFs treatment induces the electroporation phenomenon, which affects cells membrane integrity and promotes the release of intracellular ionic compounds and small molecular weight organic compounds, such as aminoacids and small proteins (Carullo et al., 2018; Grimi et al., 2014). Some studies showed a substantial increase of electrical conductivity after PEFs treatment, indicating the release of ionic intracellular components (Carullo et al., 2018; Silve et al., 2018). However, extraction is frequently limited to molecules with low molecular weight because, in some cases, only cell membrane is affected by PEFs treatment, while the outer cell wall remains unchanged ('t Lam et al., 2017; Lam, 2017). This fact presents itself as a disadvantage compared to more effective cell disruption methodologies, such as high-pressure homogenization or bead milling, which allow obtaining higher extraction rates in this type of compounds (Buchmann et al., 2019; Carullo et al., 2018; Grimi et al., 2014). As an example, Buchmann et al. (Buchmann et al., 2019) demonstrated a low rate of protein

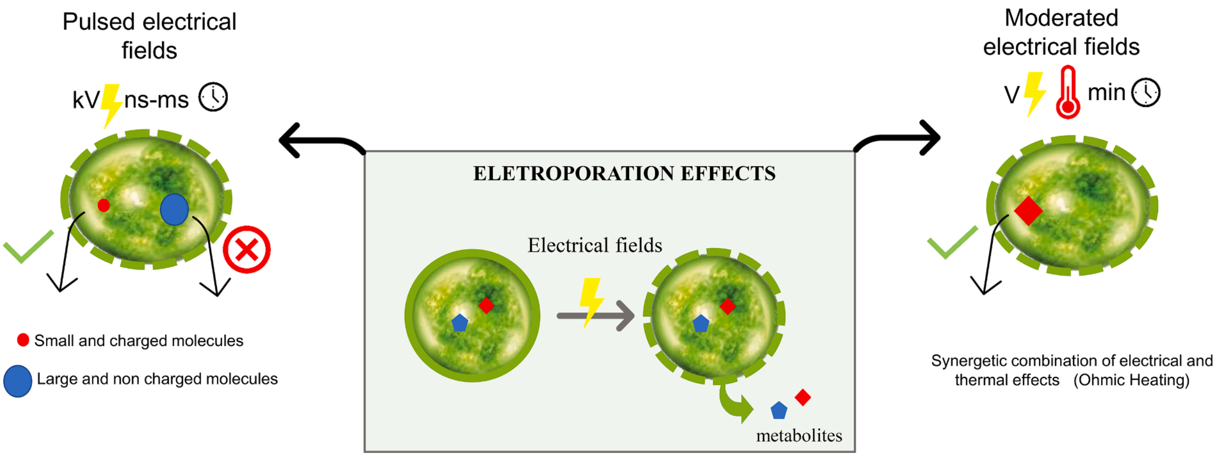

Fig. 3. Different extraction approaches through the application of electrical fields on microalgae ssbiomsass.

extraction using PEFs in comparison to the high-pressure homogenizer. The same authors also suggested that PEFs are only able to release free protein, not acting upon structural proteins of membranes and organelles. In another study, Carullo et al. (Carullo et al., 2018) also highlighted that high-pressure homogenization is capable of extracting over 10 times more proteins than PEFs from *Chlorella vulgaris* biomass, since this electrotechnology can only release proteins with a molecular weight up to 20 kDa. Despite the higher extraction rate verified for high-pressure homogenizers, there is still the presence of cellular debris in suspension, which complicates the downstream processing (Carullo et al., 2018). In fact, although extraction rates are sometimes low, PEFs processing has the potential to allow a selective extraction of compounds through a cascade extraction approach (Buchmann et al., 2019; Carullo et al., 2018; Grimi et al., 2014). This capacity can be very useful for downstream processing, by decreasing the number of purification steps and concomitantly improving, at the same time, the cost-effectiveness of microalgae-based products.

A possible solution for the lower extraction rates observed for some compounds can be the combination with other treatments – for example, pre-enzymatic weakening of the outer cell wall can result in substantially higher yields after PEFs treatment (Lam, 2017). Another possibility is to perform several extraction cycles in order to increase extraction yield of a given metabolite, as demonstrated by Buchmann et al. (2019). According to this study, the authors were capable to perform a continuous extraction of proteins from *Chlorella vulgaris* biomass, by applying treatments of 1.94 ± 0.01 kJ/kg of cells suspension. With this strategy, it was possible to reach extraction rates of free proteins of 29.1 ± 1.1 % without compromising microalgae growth, ensuring full recovery of the culture after 168 h from the first extraction (Buchmann et al., 2019). The extraction efficiency of some compounds using PEFs can also be improved by establishing a synergistic combination of this technology with other factors, such as temperature or the use of solvents. At high temperatures, the organization of the lipid bilayer is compromised. This phenomenon results in the need of a lower EF intensity to generate the same number of pores (or even larger pores) when compared to the intensity that would be necessary at lower temperature (Luengo et al., 2015). Based on this approach, Luengo et al. (Luengo et al., 2015) reported an increased lutein extraction yield of 3.5–4.2 fold in comparison to the control, using an EF of 25 kV/cm during 100 μs and a treatment temperature of 25–30 °C. The combination of PEFs with temperature results in suitable treatment conditions for the extraction of some compounds, as the case of pigments. Similar to the synergistic combination of PEFs with temperature, the joint effect of this electrotechnology with solvents showed interesting results. In this approach, PEFs treatment induces cell permeabilization, facilitating the penetration of solvents that will enable better extraction rates and purity due to the great affinity with the target metabolites (Leonhardt et al., 2020; Silve, Papachristou, Wüstner, Sträßner, Schirmer, Leber, Guo, Interrante, Posten, & Frey, 2018).

Over the past few years, several positive indications have been reported about the prospects of PEFs processing as a promising pre-treatment for the extraction of biocompounds from microalgae biomass. Some studies suggested that extracts with more antioxidant capacity and purity are obtained after PEF treatment when compared to bead milling processing – one of the most frequently used methods for microalgae cell disruption (Jaeschke et al., 2019). The low levels of energy input of PEFs technology are a key aspect of their application in the industry. The current state of the art showed that bead milling has an energy demand in the range of 1.6–3.6 MJ/kg of dry weight, while PEFs present values around 1.5–2.0 MJ/kg of dry weight (Silve et al., 2018). Despite these promising expectations, several aspects are not completely understood. Most of the literature focuses essentially on the electrical aspects of the methodology. Nonetheless, some other aspects, such as; biomass concentration of suspension; the time needed for diffusion step of target compounds to be extracted after treatment; underlying mechanisms of the solvents-assisted extractions; and the most susceptible growth phase to attain better extraction rates. Some of these aspects have already been proven to play a significant role on the extraction efficiency of PEFs, but they need to be systematically investigated in order to reach a complete validation and optimization of this innovative technology (Leonhardt et al., 2020; Silve, Papachristou et al., 2018).

3.1.2. *Moderate electric fields and ohmic heating effect*

Moderate electric fields (MEFs) consists of the application of low to moderate EFs, between 1 and 1000 V/cm, generally in the form of sinusoidal or square waves (Jaeschke et al., 2016). The application of MEFs induces some effects on cells, such as permeabilization or microbial inactivation. Although EF intensity and electrical frequency are some of of the most relevant parameters in this kind of treatment. The use of low frequencies (typically between 50 and 60 Hz) usually demonstrates greater efficiency in the permeabilization of biological tissues; however, it also contributes to the occurrence of electrochemical reactions that lead to degradation (i.e., corrosion) of the electrodes, which can contaminate the product due to the leakage of metals. The use of high electrical frequencies (>17 kHz) prevent occurrence of these electrochemical phenomena (Geada et al., 2018; Rocha, Genisheva, Ferreira-Santos, Rodrigues, Vicente, Teixeira, & Pereira, 2018).

As mentioned previously, electrical processing allows the synergistic combination of electrical and thermal effects due to occurrence of OH. The heat generation in the medium is a consequence of the exposure of biomass to a given EF for a certain time. In this process, commonly referred to as OH, heat is produced directly within the material itself as electric current passes through the (semi-)conductive material. In other words, heating occurs due to molecular friction and molecular agitation

Table 3
Recent advances in extraction techniques applied to microalgae.

Treatment	Conditions applied	Microalgae	Target compound	Major Findings	Reference
PEFs	10, 15, 20 kV/cm	*Chlorella vulgaris*	Proteins	PEF processing allowed the extraction of up to 96.6 ± 4.8 % available free protein at an electrical field of 20 kV/cm.	(Buchmann et al., 2019)
	10, 20, 30 kV/cm	*Chlorella vulgaris*	Proteins and carbohydrates	PEF promoted the selective extraction of carbohydrates (36 %, of total carbohydrates), and low molecular weight proteins (5.2 %, of total proteins) at an electrical field of 20 kV/cm	(Carullo et al., 2018)
	10, 15,20, 25 kV/cm, at several temperatures 10, 20, 30, 40 °C	*Chlorella vulgaris*	Lutein	Increasing temperature increased the sensitivity of *C. vulgaris* cells to irreversible electroporation and a treatment of 25 kV/cm during 100 µs at 25–30 °C increased the lutein around 3.5–4.2-fold in comparison with the control.	(Luengo et al., 2015)
	5 and 15 kV/cm	*Chlorella sorokiniana*	Pigments, proteins, and lipids	Application of PEF increased protein concentration by ≈ 4 mg/g of BSA per dry weight, which corresponds to ≈ 1 % of the total protein. Differences in chlorophyll and lipid extraction of untreated and PEF treated were minimal.	(Leonhardt et al., 2020)
	Incubation in the dark either at 25 °C or on ice after PEF at 40 kV/cm	*Auxenochlorella protothecoides*	Carbohydrates and lipids	PEF-treatment specific energy can be reduced down to 0.25 MJ/kg of dry weight when coupled with 20 h incubation at 25 °C and still leads to high extraction yields.	(Silve et al., 2018; Silve, Papachristou et al., 2018)
	1 to 15 pulses, from 0.5 to 15 kV/cm, with a duration of 0.05 and 0.2 ms	*Chlamydomonas reinhardtii*	Proteins	PEF is an energy-efficient cell disruption method for selective release of water-soluble proteins, after the microalgal outer cell wall is removed.	(Lam, 2017)
	40 kV/cm	*Arthrospira platensis*	Phycocyanin and proteins	The antioxidant capacity of the extracts obtained after PEF-treatment was higher than of those obtained after bead milling.	(Jaeschke et al., 2019)
OH	Conventional heating and OH during 10 min at 50, 60 and 70 °C	*Coelastrella* sp. LRF1	Pigments and proteins	Application of MEF in combination with temperature in microalgae biomass contributes to an increase in the extraction rate of chlorophyll and proteins with a lower energy demand	(Sousa et al., 2022)
	Temperature from 30 to 70 ∘C and frequency from 2 to 20 kHz)	*Cyanobium* sp.	Carotenoids and phycobiliproteins	OH showed a better extraction of pigments in ethanolic extracts (rich in carotenoids) and higher antioxidant capacity in either, ethanol and aqueous extracts (rich in extracts phycobiliproteins) than the continuous pressurized extraction	(Pagels et al., 2021)
	OH using 7 V/cm at 37 °C using different periods of time (10, 20, 30, 40, 50 and 60 min)	*Arthrospira platensis*	Phycobiliproteins, phenolics, lipids, carotenoids and chlorophyll.	OH can be used in a cascade extraction approach as a treatment that favours the disintegration and rupture of the microalgae cell wall and significantly improves the selectivity and recovery of intracellular compounds	(Ferreira-Santos et al., 2021)
	Conventional heating and OH using at 44 °C during 30 min with a solid:liquid ratio (w:v) of 1:20	*Arthrospira platensis*	C-phycocyanin	The C-phycocyanin was recovered with high yield (45.54 ± 1.93 mg/g of dry weight) when compared to the conventional heating (maximum of 29.36 ± 10.12 mg/g of dry weight)	(Ferreira-Santos et al., 2020)
	Electrical fields of 0–40 V/cm and 25 mL/100 mL of ethanol/water solution during 10 min	*Heterochlorella luteoviridis*	Carotenoids and lipids	Application of MEF combined with ethanol as solvent (40 V/cm, 75 mL/100 mL of ethanol solution) yielded up to 73 % of carotenoid extraction, and provide the highest lipid extraction yield (83 %)	(Jaeschke et al., 2016)
HPP	Applied pressures (100–600 MPa) under different cycles (1–3) using different extraction solvents (water, ethanol, ethyl acetate or ethanol/D-limonene)	*Haematococcus pluvialis* and *Porphyridium cruentum*	B-phycoerythrin, carotenoids, and PUFAs	The ultra-high-pressure extraction has been demonstrated as a fast and viable eco-friendly alternative using GRAS solvents for the simultaneous cell disruption and extraction of these bioactive compounds	(Bueno et al., 2020)

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Table 3 (*continued*)

Treatment	Conditions applied	Microalgae	Target compound	Major Findings	Reference
	The first step, the mixture (microalga and ethanol) was pressurized to 100, 300, and 600 MPa at 50 °C during 1 (20 min) or 3 extraction cycles (7–6 − 7 min). The second step the remaining residue was dried, and limonene was added	*Nannochloropsis oceanica*	Carotenoid and lipids	The use of pressure based processes has been demonstrated as a feasible environmentally green alternative for the valorisation of the microalgae biomass. The study showed that HPP technique did not improve carotenoids extraction compared to pressurized liquid extraction, although the recovery of polyunsaturated fatty acids was significantly better.	(Gallego et al., 2021)
	Samples were exposed to pressures of 300, 450 and 600 MPa at 24 °C for 15 min	*Chlorella vulgaris* and *Arthrospira platensis*	———————	Pressurization gradually transformed the liquid-like algal suspensions into a gel-like structure by unfolding the protein structure and, subsequently, agglomeration. The obtained information could be useful for food product development and tailoring the gel rigidity of the designed food products.	(Ahmed & Kumar, 2022)
IL	Ionic-Liquid-Based Aqueous Biphasic Systems	*Isochrysis galbana*	Proteins, arabinans, and glucans	The approach using IL-ABS appears as a simple and efficient method of purification with industrial potential, which can also be easily integrated into continuous flow process.	(Santos, 2018)
	Imidazolium-based ionic liquid–water mixture using dry and fress microalgae biomass	*Chlorella* sp.	Lutein	The maximum lutein extraction efficiency was 98.06 % after three times extraction for dried microalgae and it was 97.73 % for fresh microalgae.	(Zhu et al., 2021)
	Extraction using 1-butyl-3-methylimidazolium hydrogen sulfate with microwave irradiation	*C. sorokiniana*, *N. salina* and *Galdieria sulphuraria*	Lipids	The combination of ionic liquid and microwave irradiation for lipid extraction showed higher efficiency than the conventional methods using organic solvents	(Pan et al., 2016)
	Three classes of ILs (1-ethyl-3-methylimidazolium, 1-butyl-3-methylimidazolium, and 1-butyl- 3-methylpyridinium, were tested	*Haematococcus pluvialis*	Astaxanthin	This study conceptualized an energy-efficient biotechnological *H. pluvialis* hard-cyst pretreatment alternative that utilizes the process of germination to facilitate the extraction of astaxanthin using ILs as green solvents.	(Praveenkumar et al., 2015)
	Hydrated phosphonium ionic liquid	*Nannochloropsis oculata* and *Chlorella vulgaris*	Lipids	This study has clearly demonstrated that the ionic liquid procedure can be used to extract lipid directly from wet microalgae under mild conditions	(Olkiewicz et al., 2015)
DES	Specifications of the studied DES: HBA using was Choline chloride and the HBD using was oxalic acid, urea and glycerol	*Dunaliella salina*	Carotenoids and lipids	This study generally found that DESs enhanced the solvent permeability in the microalgae cell wall and the efficiency and sustainability of the extractive process can be increased depending on the chosen strategy.	(Asevedo, 2023)
	Different aqueous DES were used: aqueous choline chloride-oxalic acid, aqueous choline chloride-ethylene glycol, and aqueous urea-acetamide	*Chlorella* sp.	Lipids	DESs are a promising alternative of conventional ionic liquids for microalgal biomass pre-treatment to enhance lipid extraction	(Lu et al., 2016)
	Specifications of the studied DES: HBA using was choline chloride and sodium acetate and the HBD using was lactic acid, ethylene, and glycerol. Compared then with the conventional methods with organic solvents	*Nannochloropsis gaditana*	PUFAs	In the case of EPA extraction, and under optimal conditions, DES were capable of recovering over 18 % more quantity than the obtained with HCl: methanol. This study demonstrate that DES are effective at both recovering total fatty acids from pre-treated biomass and at selectively recovering EPA using both unpretreated and pre-treated biomass.	(Moreno Martínez et al., 2022)
	Conditions investigated: the ratio of powder weight to solvent volume (0.05 g, 0.1 g, 0.15 g, 0.2 g, 0.3 g in 5 mL NADES), mole ratio of HBA to HBD (0.25:1, 0.5:1, 1:1, 1.5:1, 2:1, 3:1, 4:1, 5:1), extraction temperature (25–80 °C) and time (20–100 min)	*Scenedesmus* sp.	Lutein	The NADES could increase lutein yield compared with the conventional methodology using organic solvents. Concomitantly, this methodology significantly enhanced the lutein stability under various conditions.	(Fan et al., 2022)
	DES and microwaves were used as pre-treatments for lipids extraction using	*Phaedactylum tricornutum*	Lipids	The combination of this pre-treatment with environmentally friendly	(Tommasi et al., 2017)

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Table 3 (*continued*)

Treatment	Conditions applied	Microalgae	Target compound	Major Findings	Reference
	dimethyl carbonate and supercritical CO_2. DESs were formed by choline chloride and different hydrogen-bond donors (oxalic acid, levulinic acid, urea, ethylene glycol, and sorbitol).			solvents such as dimethyl carbonate and supercritical CO_2 allows highly purified lipid extracts to be obtained, along with a total fatty acid yield comparable to that of the benchmark Bligh and Dyer method, while avoiding toxic and dangerous solvents.	

provoked by the motion of charged molecules within the material. OH presents high heating rates with a precise temperature control, allowing a mild processing approach that helps preserving the nutritional, functional, and structural properties of biocompounds. Heat is generated inside of the material not depending on the traditional heat transfer mechanisms between phases and interfaces, which allows heating uniformity and extremely rapid heating rates (Jaeschke et al., 2016). The very first studies of MEFs (and its attendant OH effect) were directed towards the extraction of compounds in food materials, proving EFs could affect the permeability of cell membranes (Kulshrestha & Sastry, 2003, 2010; Sensoy & Sastry, 2004). Sensoy and Sastry (Sensoy & Sastry, 2004), for example, demonstrated that the application of MEFs promoted a total solids extraction of 0.241 g/L from fresh mint leaves, a higher value than the one observed under conventional heating at 80 °C, which was 0.131 g/L. This increase was explained by the electro-permeabilization effects induced by the application of EFs, which may contributed to the creation of pores thus enhancing the molecular transport of biocompounds across the membrane. Most of the published works regarding the extraction of biocompounds using electrical technologies are based on PEFs application, although MEFs starts now to be considered a less expensive procedure for the same purpose (Jaeschke et al., 2016).

The interesting results obtained for vegetable tissues, as well as the cost-effectiveness of OH processing, place this methodology among the most promising to be applied in microalgae biomass. In fact, the application of OH in microalgae has been consistent with the expectations so far. As an example, this methodology frequently results in a more efficient recovery of intracellular compounds with less energy demand than conventional heating (Ferreira-Santos et al., 2021; Ferreira-Santos et al., 2020; Jaeschke et al., 2016; Jaeschke, Merlo, Mercali, Rech, & Marczak, 2019; Pagels et al., 2021; Sousa, Loureiro, Carvalho, & Pereira, 2022). Supporting this, Jaeschke et al. (Jaeschke et al., 2019) demonstrated that a MEF pre-treatment under 40 °C contributed to the extraction of 1431 mg/g of dry weight of carotenoids – representing a yield of 84 % –, while conventional heating was able to extract 733 mg/g of dry weight at the same temperature – corresponding to a yield of 43 %. Likewise, Ferreira-Santos et al. (Ferreira-Santos et al., 2020) also demonstrated the great potential of OH to extract compounds over conventional heating. The authors reported a high extraction yield (45.54 mg/g of dry weight) of C-phycocyanin from *Spirulina platensis* using OH, while conventional heating attained 29.36 mg/g of dry weight. The extraction rate observed for OH was even higher than freeze-thawing (41.90 mg/g of dry weight), one of the most widely used methods to promote cell wall disruption. In addition, OH showed higher efficiency in protein and pigments extraction compared to conventional heating, allowing better preservation of the extracted compounds and mitigating the negative effects caused by the high temperatures commonly applied during the extraction process. This can be easily explained by the higher extraction rates OH attains using lower temperatures and shorter treatment times, when compared to conventional heating (Ferreira-Santos et al., 2020; Jaeschke et al., 2019). The greater extraction and preservation of compounds using OH may also rely upon the capacity of this processing technique to extract the same (or higher) amounts of organic matter using milder treatment conditions due to the occurrence of electroporation effects, promoting a more efficient migration of metabolites (Sousa et al., 2022). Regarding the impact on compounds' bioactivity, OH treatments presented higher antioxidant activity for ethanol and water extracts (8.0 and 8.3 mg/g of total extract per dry weight, respectively) obtained from the marine cyanobacterium *Cyanobium sp*. when compared with the homogenization procedure (a standard technique) – 6.5 and 6.6 mg/g of total extract per dry weight, respectively (Pagels et al., 2021).

The interesting extraction yields provided by OH can be enhanced by its combination with other approaches – for instance, by combining with an optimized use fo certain solvents (Ferreira-Santos et al., 2021; Jaeschke et al., 2016). According to Jaeschke et al. (2016), the combination of MEFs (40 V/cm) with a diffusive step in ethanol 75 % (v/v) yielded the extraction of up to 73 % and 83 % of carotenoids and lipids from *Heterochlorella luteoviridis*, respectively. The combination of this electrotechnology with diffusive steps in solvents makes this process suitable for a sequential multi-stage extraction, allowing a greater valorisation of the microalgae biomass according to the concept of cascade extraction (Ferreira-Santos et al., 2021). However, additional studies must be carried out to integrate green and/or food grade solvents in this kind of approach, maintaining or improving (if possible) the effectiveness of the overall process.

Despite all the positive indications that OH presents, including the possibility of scaling up without significant loss of efficiency (Yodsuwan, Kamonpatana, Chisti, & Sirisansaneeyakul, 2018), certain mechanisms are still not fully understood and should therefore be further investigated, as the case of: i) interaction of EFs and bioactive molecules; ii) effect of EFs over the extraction kinetics and solvent selectivity (Jaeschke et al., 2016; Jaeschke et al., 2019); iii) impact of extraction routes on bioaccessibility of biomolecules; and iv) impact of OH on cell viability and cell's structure (Sousa et al., 2022).

3.2. High-pressure processing

High-pressure processing (HPP) treatments are non-thermal procedures consisting of the application of pressures between 100 and 1000 MPa where turbulence or cavitation is created in the pressure chamber (Ahmed & Kumar, 2022; Ferreira, Almeida, Delgadillo, & Saraiva, 2015). During HPP treatment, the pressure is applied uniformly, instantly, and independently from the size and shape of microalgae – the so-called Isotactic Principle. This methodology is widely used in the food industry to inactivate spoilage and pathogenic microorganisms and enzymes, contributing to the extension of shelf life and safety level of treated food with low energy demand. It is already used in several food products placed in the market (e.g., fruit jellies, guacamole, sauces, rice, cakes, desserts), without compromising their quality, safety, and nutritional value. In recent years, HPP has been explored in order to extract bioactive compounds from microalgae biomass, showing promising results. Nonetheless, there is limited information regarding the real impact of HPP on cell's structure and extracted biocompounds (Ferreira et al., 2015).

As opposed to high-pressure homogenization, which is responsible for a complete disruption of the cell walls, HPP usually presents a more controlled disruption process (Ahmed & Kumar, 2022). Indeed, this feature makes HPP a promising disruption methodology – like electrotechnologies – to be integrated into cascade extraction strategies, as demonstrated by Gallego et al. (Gallego et al., 2021). Targeting a sequential extraction of lipids and carotenoids from *Nannochloropsis oceanica*, the authors performed a green strategy divided into two steps. In the first step, aiming carotenoids extraction, an extraction cycle was conducted using ethanol at 50 °C and 300 MPa, resulting in an extraction yield of 84.7 ± 13.81 mg/g (total carotenoids per mass of extract). With the remaining biomass, the authors performed a second (lipid-oriented) extraction step – using limonene as solvent – and reached a recovery efficiency near 100 % for PUFAs. In this lipid extraction process, no significant differences were observed within the range of pressures tested (100–600 MPa); on the other hand, significant differences were revealed when increasing the temperature from 50 to 70 °C, improving the lipid extraction yield from 4.8 ± 0.4 to 5.5 ± 0.5 % (Gallego et al., 2021). In another study, carried out by Bueno et al. (Bueno et al., 2020), the authors presented the ultra-high pressure (100–600 MPa) as an alternative extraction methodology – through the application of 1 cycle of 20 min. The conditions applied allowed greater extraction carotenoids (119.35 ± 7.34 mg/g of total carotenoids per mass extracted) from *Haematococcus pluvialis* biomass than the pressurized liquid extraction (91.57 ± 6.83 mg/g), a methodology that is frequently used to extract bioactive compounds from microalgae biomass. Previous studies demonstrated the impact of different pressures on cells (Gallego et al., 2021) (Bueno et al., 2020). At lower pressures (100 MPa), cells were affected but cell walls' integrity was not compromised, pointing this treatment as a good strategy for damaging cell membrane without causing complete disruption of the cell wall. On the contrary, when exposed to higher pressures (600 MPa), some cell walls appear to be completely broken (Bueno et al., 2020).

Although HPP has recently been used for the extraction of microalgae compounds and promising results were reported, considering this technology as a viable option (Table 3), further studies are needed to clarify some operational factors, the impact of the methodology on the extracted compounds or even the potential to be applied at industrial scale.

3.3. *Green solvents*

The use of solvents for the extraction of value-added compounds from microalgae biomass is one of the most widely used methodologies. Organic solvents, such as methanol, ethyl acetate, dichloromethane, hexane, acetone, or chloroform, have been currently used for that purpose (de Jesus, Ferreira, Moreira, Wolf Maciel, & Maciel Filho, 2019; Ferreira-Santos, Zanuso, Genisheva, Rocha, & Teixeira, 2020; Mehariya, Fratini, Lavecchia, & Zuorro, 2021). However, several international organizations, including the European Union and the United Nations, are paying particular attention to the need of reducing the use of such dangerous substances (Clarke, Tu, Levers, Bröhl, & Hallett, 2018; Ferreira-Santos et al., 2020). These solvents have been progressively replaced by greener alternatives since many of them, especially organic solvents, present environmental problems, are toxic, carcinogenic, and/or flammable, and are not compatible with some industrial applications (de Jesus et al., 2019; Eppink et al., 2021; Ferreira-Santos et al., 2020; Häckl & Kunz, 2018). A clear definition of green solvent is not yet available, being only established in relative terms. A solvent can be considered green when there is evidence that it is "greener" compared to a conventional solvent used (Häckl & Kunz, 2018). The ideal solvent is a commitment between several requirements, such as (i) effectiveness: cost, selectivity, solvent power or availability; (ii) environmental restrictions: volatile organic compounds emissions, biodegradability, toxicity or greenhouse effects; and (iii) safety and legislation: permission of products, flammability, explosion risk or recycling (Häckl & Kunz, 2018). Water can be an alternative to the use of organic solvents since it presents a set of interesting characteristics, such as low hazard potential, high availability, low cost, and potential application in biphasic systems. Despite these advantages, water usage requires large amounts of energy to promote its separation from the extracted product as consequence of its significant heat capacity, which makes obvious the need for alternative green and innovative solvents (Häckl & Kunz, 2018). Based on this assumption, several options have emerged as potential solutions for the extraction of added-value compounds from microalgae biomass. Ionic liquids (ILs) and deep eutectic solvents (DES), described in the following sections (Table 3), are some examples of green solvents that might turn extraction into a more eco-friendly and effective process (Ferreira-Santos et al., 2020).

3.3.1. *Ionic liquids*

Recently, ILs have attracted attention due to their capacity to be a green alternative to organic solvents, commonly employed in conventional extraction processes. In fact, the use of organic solvents,as well as the necessary extreme conditions to promote the extraction, can compromise the structure and bioactivity of the desired compounds. Considering the selective partitioning promoted by ILs and their eco-friendly character, it is possible to consider them a better option for extracting biomolecules from microalgae biomass (Eppink et al., 2021; Pan et al., 2016; Praveenkumar, Lee, Lee, & Oh, 2015). ILs are liquid molten salts at temperatures below 100 °C, composed of a large organic cation and small organic or inorganic anions, with exclusive and adjusted physicochemical properties. Since ILs have an extraordinary capacity for solvation, they are capable to swell or dissolve a wide range of biomass matrices, thus allowing easier access to the target compounds. Additionally, ILs aqueous solutions also display improved and unique solvation performance, as demonstrated by their outstanding hydrotropic nature or when applied as surface-active ingredients, allowing enhanced extractions (Choi et al., 2014; Eppink et al., 2021; Praveenkumar et al., 2015).

ILs can extract diverse biocompounds from microalgae biomass, such as lipids (Choi et al., 2014; Olkiewicz et al., 2015; Pan et al., 2016), pigments (Praveenkumar et al., 2015; Zhu, Li, Wang, Ren, & Zhao, 2021), polysaccharides (Santos et al., 2018), and proteins (Santos et al., 2018). Furthermore, they can be used in a biorefinery approach, contributing to the full use of microalgae biomass with minimal waste generation. The efficient extraction process, mediated by ILs, is due to the disruption of the hydrogen-bond network of the cell wall (Zhu et al., 2021). The use of ILs in extraction processes involving microalgae biomass contributes to a viable bioeconomy as this approach is more sustainable and cost- and energy-effective, allowing a selective extraction and purification of the desired biocompounds. Studies have already demonstrated the effectiveness of ILs compared to organic solvents. Pan et al. (Pan et al., 2016), for example, showed that the total lipid extraction was higher using the IL 1-butyl-3-methylimidazolium hydrogen sulfate than the conventional mixture of chloroform–methanol (1:1) commonly used. The authors reported a lipid extraction of 0.23, 0.10, and 0.19 g/g of dry weight using this IL in three different microalgae species – *Chlorella sorokiniana*, *Nannochloropsis salina,* and *Galdieria sulphuraria*; on the other hand, the organic solvent was responsible for 0.087, 0.064, and 0.095 g/g of dry weight, respectively.

Interestingly, ILs can be adjustable solvents, allowing a large number of ion combinations and the possibility to design specific fluids for particular tasks (Choi et al., 2014; Eppink et al., 2021). The ability of some ILs to swell or dissolve the cellulose of microalgae's cell wall may avoid the use of extraction methodologies, reducing the energy demand and need to use organic solvents associated with the conventional processes (Choi et al., 2014; Eppink et al., 2021; Olkiewicz et al., 2015; Pan et al., 2016; Praveenkumar et al., 2015). Indeed, Praveenkumar et al. (Praveenkumar et al., 2015) have disclosed the capacity of the IL 1-ethyl-3-methylimidazolium ethylsulfate to extract (in just 1 min) 19.5 pg of astaxanthin per cell, corresponding to approximately 82 % of the total astaxanthin extracted using high-pressure homogenization and ethyl acetate.

Although ILs can be applied individually, they also offer the possibility of being combined with other methodologies or integrating liquid biphasic systems, in order to achieve higher extraction rates and/or selectivity and purity yields (Eppink et al., 2021; Pan et al., 2016; Santos et al., 2018). In addition, unlike organic solvents, ILs do not present a volatile character, have excellent chemical and thermal stability, low vapour pressure and high boiling point, a wide range of miscibility, and the potential to be recycled and reused (Choi et al., 2014; Olkiewicz et al., 2015; Pan et al., 2016; Praveenkumar et al., 2015). ILs can be recycled – reducing the environmental impact – maintaining their stability, as demonstrated by Olkiewcz et al. (Olkiewicz et al., 2015). The authors studied the stability of the tetrakis(hydroxymethyl)phosphonium chloride IL by ^{1}H NMR spectroscopy after recycling and realized its quality was preserved, not compromising lipid extraction (Olkiewicz et al., 2015).

The major problem associated with IL is related to their potential toxicity, at least in certain cases (Ventura et al., 2012, 2014), the cost associated with manufacturing, and the environmental impact (e.g., biodegradability). ILs toxicity is mainly dependent on the length of their alkyl chains, well-known for their toxicity, as the case of guanidium-based ([TMGC4]I and [TMGC7]I), phosphonium-based ([P6,6,6,14]Br and [P6,6,6,14]Cl) or cholinium-based ILs ([Chol][Bit]) (Eppink et al., 2021; Ventura et al., 2012, 2014). Therefore, the use of these solvents in the food industry is restricted. During the past few years, some efforts have been done in order to overcome this major problem, being the design of ILs based on natural biocompounds a possible solution (Toledo Hijo, Maximo, Costa, Batista, & Meirelles, 2016). Currently, there is evidence of ILs with low or nontoxic effects, which presents a promising perspective for future applications in the food industry (Toledo Hijo et al., 2016). As already mentioned, the manufacturing cost of ILs can also be a problem. In some cases, it can be 2–100 times higher than that of organic solvents, which can hinder ILs industrial implementation. However, the possibility of being reused might mitigate high costs impact and contribute to reduce the environmental impact of ILs (Eppink et al., 2021).

During the last few years, several studies have highlighted the potential of ILs in the extraction of various biocompounds from microalgae, including better performance than the already established methodologies. The performance of life cycle assessment (LCA) studies focused on processes and products, as well as the development of scalable and economically viable separation processes, with reduced use of solvents and energy consumption, are though some of the challenges that still need to be addressed in a near future.

3.3.2. *Deep eutectic solvents*

The valorisation of bioactive compounds from microalgae is very interesting for several applications and DES are an alternative to extract them. DES consist of homogeneous liquids that have lower melting points than their individual constituents. ILs and DES are very similar. However, ILs are solely able to extract compounds through ionic interaction, while DES extraction is a joint result of both ionic interactions and hydrogen bonds. Hydrogen bonds are promoted by the presence of hydrogen bond acceptors (HBA) and hydrogen bond donors (HBB), which integrate the DES (Huang et al., 2017; Rodrigues et al., 2020; Torres-cornejo, Gerardo, & Mendiola, 2021). Depending on the HBA and HBB, they display more affinity for certain compounds, contributing to more efficient extraction processes, as demonstrated by Tommasi et al. (Tommasi et al., 2017). Herein, the authors reported that, when using a polyol or urea as HBB, the treatment does not significantly affect lipids extraction and selectivity; on the contrary, DES having choline chloride or carboxylic acids presented better lipid extraction.

In addition to their eco-friendly features, low or no toxicity, and biodegradability, DES can be easily combined with other methodologies (e.g., supercritical carbon dioxide, microwaves) to enhance and promote a selective extraction process (Fernandes & Cunha, 2018; Lu et al., 2016; Tommasi et al., 2017). Authors already demonstrated the ability of DES to extract compounds from microalgae biomass. Although the extraction capacity is lower than that of conventional options, the fact is that most DES extracts present higher amounts of valuable bioactive compounds (Tommasi et al., 2017). The application of DES in microalgae cells contributes to a significant structural modification of the cell wall's morphology, as shown by Lu et al. (Lu et al., 2016) through scanning electron microscopy (SEM). The authors reported that cells treated with DES presented clear wrinkles, significant flaws, cracks, crevices, or pores, in contrast with the untreated cells that maintained normal spherical structures without structural changes. As opposed to ILs, DES have an essential characteristic that makes them an excellent alternative to conventional solvents: their low toxicity. Several authors have already pointed out this key aspect, which is allowing the direct application of these solvents in the food and pharmaceutical industries (Huang et al., 2017; Rodrigues et al., 2020; Torres-cornejo et al., 2021).

Based on the abovementioned advantages, Lu et al. (2016) proved the ability of DES to enhance the efficiency of lipid extraction from *Chlorella* sp. biomass when used as a pre-treatment. The authors reported an increase from 52.03 %, for untreated conditions, to 80.90, 66.92, and 75.26 % using the following DES: aqueous choline chloride-oxalic acid, aqueous choline chloride-ethylene glycol, and aqueous urea-acetamide, respectively (Lu et al., 2016). Positive indications were also reported for the extraction of carotenoids (Asevedo, 2023; Fan, Liu, Shan, & Cao, 2022), PUFAs (Moreno Martínez, Ortiz-Martínez, Sánchez Segado, & Salar-García, 2022), proteins (Xu, Wang, & Hou, 2020), and phenolic compounds (Wan Mahmood, Lorwirachsutee, Theodoropoulos, & Gonzalez-Miquel, 2019). Considering the positive indications related to the extraction of compounds from microalgae biomass (i.e., selective extraction, extraction of the most valuable compounds, potential to combine with other methodologies, low toxicity), the application of DES and natural DES (NADES) is expected to increase in a near future. The formulation of new DES – with different polarities – and exploration of different combinations of DES with other emergent and eco-friendly technologies, will be definitely evaluated in forthcoming studies, leveraging a more widespread use of such solvents in extraction processes.

3.4. *Liquid biphasic systems*

Liquid biphasic systems (LBS) are a novel technique for downstream processing. Conventional methodologies used to recover biomolecules from microalgae biomass exhibit problems at both separation and purification level, as some industrial applications require the desired compounds in their purest form (Khoo et al., 2020; Krishna Koyande et al., 2020; Low, Idris, & Mohd Yusof, 2020). Membrane separation, chromatography-based methods, ultrafiltration, and precipitation are some techniques already established for this purpose. However, all of them show operational problems, such as long processing time, need for multiple steps, high energy demand, and numerous challenges during scale-up (Khoo et al., 2020; Krishna Koyande et al., 2020).

LBS are a well-known to promote an efficient separation and purification of several biomolecules (e.g., proteins, lipids, pigments) (Krishna Koyande et al., 2020; Low et al., 2020; Sankaran et al., 2018; Santos et al., 2018). As an example, Chang et al. (Chang, Show, Lan, Tsai, & Huang, 2018) evaluated the C-phycocyanin recovery from *Arthrospira platensis* biomass using an ILs-based aqueous two-phase system. The authors reported that the use of 1-octyl-3-methylimidazolium bromide/salt LBS resulted in an extraction efficiency, a partition coefficient, and a separation factor of 99 %, 36.6, and 5.8, respectively. These results indicate that this is a simple, fast, and green methodology suitable for the isolation of biocompounds from microalgae biomass. LBS consists of two liquids separated by an interfacial layer, occurring when the mixture of two incompatible liquids is beyond the critical condition. The LBS approach allows an eco-friendly, inexpensive, scalable, rapid, and single-step process when compared to conventional techniques, which normally require multiple operations/steps and are time-consuming (Khoo et al., 2019; Khoo et al., 2020; Khoo et al., 2020; Krishna Koyande et al., 2020). In LBS, there are different types of phaseforming components, namely polymer/salt, alcohol/salt, ILs, DES, and surfactant/detergent based (Chang et al., 2018; Khoo et al., 2020; Krishna Koyande et al., 2020; Santos et al., 2018). Depending on the characteristics of the solvents used, the system will have more affinity for some compounds than others, increasing the extraction rate and purity of the target molecules. These systems can be part of an integrated process with other methodologies in order to optimize the extraction rate of specific compounds from microalgae biomass. They can be combined either with physical (e.g., microwaves, ultrasounds, OH, PEFs) or non-physical processes (e.g., osmotic shock), using the previously mentioned techniques as pre-treatment – for cell disruption or permeabilization – and the LBS to promote selective extraction. This synergistic combination allows higher separation efficiencies compared with conventional liquid–liquid LBS extraction. In a recent study, Khoo et al. (Khoo et al., 2020) assessed the efficiency of a single-step disruption approach through the combination of ultrasounds and LBS, aiming the extraction of astaxanthin from *H. pluvialis* biomass. The authors reported an astaxanthin recovery of 95.08 ± 3.02 %, while the extraction efficiency and partition coefficient were 99.74 ± 0.05% and 185.09 ± 4.78, respectively. The results obtained were better when compared to those obtained by the same authors in a previous study, where LBS was used alone (Khoo et al., 2019). In this case, astaxanthin recovery and partition coefficient suffered a decrease (78.83 ± 0.93 and 142.58 ± 3.87 %, respectively), whereas the extraction efficiency kept stable at 99.86 ± 0.05%. As demonstrated, the combination of LBS with ultrasounds contributed to the mitigation of some of the problems observed when this methodology (LBS) is used alone, namely due to the increased access to intracellular biocompounds as a consequence of the cell disruption induced by the application of the physical method.

4. Technological level of the emergent extraction methodologies

Patents are an early indicator of future technologies. Many works are often published on this subject, but the evaluation of technological trends involving patents is poorly investigated. Considering the extraction methodologies reported in the present review, they are at different stages of implementation. In order to evaluate the technological level of the emergent extraction methodologies, current patents were identified based on the Europen Patent Office and Google Patents databases. Some of them– namely PEFs, ILs, and DES – are already in an advanced state of innovation, with a considerable number of existing patents linking the use of such technologies to the extraction of compounds from microalgae biomass. Particularly for PEFs, there is a significant number of patents either referring to the apparatus itself or the process applied for the extraction of compounds from macro-/microalgae (Golberg & Robin, 2021; Hulshoff & Jager, 2019). A similar trend is observed for ILs and DES since several patents can be found addressing the use of these green solvents to extract microalgal compounds. The intellectual property displayed by these methodologies covers the extraction of various compounds, such as lipids (Angelis & Castaldo, 2022; Guo, Gao, Wang, & Liu, 2012; Salvo, Reich, Dykes, & Teixeira, 2011; Weidong, Zhongming, Shaohuan, & Zhenhong, 2017; Wen, Pengmei, Zhongming, Zhenhong, Huiwen, Lingmei, & Weizheng, 2014), pigments (Liyun, Tianyou, Qingpeng, Xiaoqian, Wenbin, Ping, & Le, 2023; Minceva & Bauer, 2020), or proteins(Wang, 2021). As in the case of PEFs, OH technology also presents several patents. Some of them are associated with the OH apparatus, while others include the extraction process of compounds. However, as opposed to the observed for PEFs technology, none of the patents based on OH is specific for microalgae. Nevertheless, there is, for instance, a method for oils extraction from chia seeds with the assistance of OH (Jingzhang, Yinku, & Sanqiao, 2015). Therefore, considering the recent advances in studies involving OH and microalgae, as well as the existing patents of extraction processes from plant tissues, the number of patents related to the OH-aided release of compounds from microalgal biomass is expected to increase in a near future.

Regarding HPP, there are no patents addressing the use of this technology as an extraction step. Existing patents are mainly associated with the HPP apparatus or other processes for which HPP processing can be used, as mentioned in section 3.2 (i.e., food preparation or sterilization/preservation) (Hernando Saiz, Tonello Samson, González-Angulo, & Queirós, 2023; Jianqiang, Fen, Li, Dan, Shuzhen, & Linjie, 2022). In line with OH, HPP technology is also expected to grow in number of patents aiming the extraction of compounds from microalgae biomass since several works have been carried out in this research field in recent years, showing promising results (Bueno et al., 2020; Gallego et al., 2021).

5. Future perspectives

The exponential growth of the population has had significant impact over food production capacity owing to: i) increasing demand for food; ii) higher pressure upon natural resources; iii) agricultural intensification; iv) food security risks; and v) great environmental impact of conventional food production processes. To address these challenges, it is important to adopt sustainable approaches of food production, where microalgae emerge as a potential alternative. The bioactive compounds contained in microalgae have attracted increasing interest due to their potential impact on human health and biomedical industry. Furthermore, the COVID-19 pandemic allowed understanding that the widespread use of these biocompounds might be relevant to prevent/attenuate similar events or other deadly diseases worldwide. For example, some microalgae pigments (e.g., fucoxanthin, β-carotene, astaxanthin) have antioxidant and/or anti-inflammatory activities, which can act against oxidative stress and promote cellular health (Geada et al., 2018; Jacob-Lopes et al., 2019). Additionally, micoalgal fatty acids, such as EPA and DHA (omega-3 fatty acids), are important for cardiovascular and brain health (Geada et al., 2018; Guihéneuf & Stengel, 2017; Jacob-Lopes et al., 2019). Consequently, these features open up the possibility of integrating these bioactive compounds found in microalgae in the development of dietary supplements, functional foods, and pharmaceuticals, in order to improve human health and well-being. On the other hand, microalgae contain a wide variety of other essential nutrients, including high-quality proteins, vitamins, and minerals. Considering the latest series of events with impact at global scale (i.e., pandemics, wars), causing feedstock/food shortage and production limitations, microalgae can be seen as a promising alternative of safe and nutritious food, commercialized as a single ingredient or incorporated into food products (e.g., bread, pasta, cereal bars) as means of increasing their nutritional value.

Despite all these potential and exciting perspectives, the commercial exploitation of microalgal biomass is currently facing several major challenges, namely process scalability, high production costs, energy consumption, environmental impact, especially when using chemicals, and the lack of mild-processing technologies to prevent non-target metabolites damage and enable exploring multiple biomass fractions. Additionally, the fact that most of the bioactive compounds are intracellular, as well as the presence of a cell membrane (and, in some cases, a cell wall), frequently limits the access to microalgae's bioactive compounds, hindering their widespread use. Since some of these identified obstacles are interconnected and related to the extraction processing, emerging technologies – such as PEFs, OH, HPP, IL, and DES – arise as viable alternative routes to overcome several drawbacks and bottlenecks of the conventional processes. Some of them, as the case of electrotechnologies, are low-energy demandind methodologies and have potential to extract all kinds of compounds. Additionally, the possibility of combining several approaches – for instance, electrotechnologies or HPP with green solvents as IL and DES – offers great advantages in terms of process harshness and effectiveness, since it would enable exploring multiple biomass fractions. Furthermore, it would allow a better environmental performance – due to the use of eco-friendly solvents – and increased market value of microalgae biomass, as consequence of lower production costs and wider range of compounds commercialized.

Although numerous efforts have been made recently, there is still a long way to go until the complete validation and consolidation of the abovementioned emerging methodologies, consequently hampering their implementation at industrial scale. In addition to the limitations identified in Section 3 for each of these technologies, which will require further and deeper research, there is an alarming lack of assessment of their real impact – both environmentally and economically – of these strategies on the extraction of microalgae compounds when compared to widely used conventional methods. Therefore, LCA, recognised by the European Commission (2003) as the best framework for assessing the potential environmental impacts of products, is a powerful and reliable tool to support the transition from conventional downstream processes to the widespread use of emergent technologies. The number of studies determining this type of parameters on microalgae processing is still scarce, as most of the literature found is focused on biodiesel production or the use of different cultivation modes. Thus, the use of LCA to evaluate the extraction processes of compounds from microalgae biomass will allow a better understanding of the environmental impacts of these emerging technologies, being a very important tool for their industrial validation. Likewise, the performance of techo-economic analyses, following the same rationale, could play a significant role on shifting the paradigm of extraction methodologies applied to microalgae biomass as well.

6. Conclusions

Microalgae biomass is a noble source of high-value products but their commercialization is dependent on the effectiveness of the extraction methodologies applied. Conventional approaches present several drawbacks, such as non-eco-friendly character, long extraction or treatment times, non-selective extraction, and exposure of the extractives to excessive heat, light, and oxygen, which can cause the destruction or damage – and subsequent loss – of several high-added value metabolites/fractions. Therefore, the widespread use of emergent technologies (e.g., electrotechnologies, HPP, green solvents) may shift this paradigm and allow the extraction of multiple high-value compounds, also attaining highly efficient and selective extraction steps. Ultimately, these emergent technologies may enable the potential for the implementation of a biorefinery approach with a green character, establishing a landmark to decrease production process costs without compromising its environmental performance.

CRediT authorship contribution statement

Vítor Sousa: Conceptualization, Writing – original draft. **Ricardo N. Pereira:** Conceptualization, Writing – review & editing. **António A. Vicente:** Writing – review & editing. **Oscar Dias:** Writing – review & editing. **Pedro Geada:** Conceptualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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