Cultured microalgae and compounds derived thereof for food applications: Strain selection, cultivation, drying, and processing strategies

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Abbreviations:

PEFs: Pulsed electric fields; HPH: High pressure homogenisation; US: Ultrasounds; DHA: docosahexaenoic acid; EPA: Eicosapentaenoic acid; EFSA: European Food Safety Authority; HSH: High pressure homognisation; HVEF: high-voltage electrostatic fields; HVED: High-voltage electrical discharges; WHC: Water-holding capacity; OHC: Oil-holding capacity; ACE-I: Angiotensin-I-converting enzyme.

Abstract

Currently, global demand for microalgae foods is growing and their functional potential is now evident in several books and scientific studies. One of the aims of the current paper was to review the most important food ingredients that can be obtained from microalgae, focusing on pigments and proteins which are both industry relevant and top-trends in the food industry. In addition, this review also highlights the importance of key aspects that need to be considered when using microalgae for food. These include strain selection and cultivation, harvesting, and drying conditions. Recent advances on extraction technologies were also reviewed and discussed.

Keywords: Microalgae; novel ingredients; functional foods; proteins; pigments; extraction techniques

1. Introduction

Marine resources are gaining increased importance in the context of European bioeconomy, where microalgae are particularly attractive because they are sources of a wide variety of valuable molecules with potential for being used in diverse applications [1]. These include, but are not limited to, high quality proteins, carbohydrates, lipids, pigments, and other bioactive compounds. Valuable microalgae-derived compounds can be used as ingredients for the development of functional foods, which is one of the top trends in the food industry [2]. Microalgae have been part of the human diet for thousands of years. However, microalgae biotechnology is a relatively new area which has grown exponentially over the last two decades, especially since 2005 [3]. With an increased demand for sophisticated and innovative products, microalgae are positioning themselves firmly in the food market [4].

Microalgae composition depend on several factors including specie, climate, growth phase and cultivation conditions. For example, Finkel, Follows, Liefer, Brown, Benner and Irwin [5] reported important differences in the major macromolecular molecules across different phyla of microalgae and between different growth stages. Differences can also be seen in terms of composition, i.e., while the carotenoid fraction of green vegetative cells of *Haematococcus pluvialis* consist of mostly lutein (75-80%) and β-carotene (10-20%), during the red stage, the carotenoid pattern is replace by secondary carotenoids, mainly astaxanthin at quantities ranging between 80 and 99% of total carotenoids [6]. Therefore, the production of a certain compound of interest can be enhanced under environmental stress factors and by choosing the most suitable strain or the optimum moment for harvesting.

Most of the products recently launched into the market contain a microalgae-derived compound rather than the whole biomass. Main reasons include a strong biomass colour

(generally green), a powdery consistency, and a fishy taste and odour [7]. One of the main problems to obtain valuable compounds from microalgae is the difficulty to release them from their intracellular location in energy-efficient and economically sustainable ways. Microalgae have different cell wall structures, which limit the extraction step and generally result in ineffective extractions [8]. In order to overcome this problem, several novel physical and chemical strategies have been developed. These include, but are not limited to pulsed electric fields (PEFs), high-pressure homogenization (HPH), or ultrasounds (US) [9-11].

The aim of this article was to review the current state of the art of microalgae biotechnology for food applications. Main aspects covered include strain selection and microalgae biomass composition as well as cultivation and harvesting strategies. In addition, this paper also reviews valuable food ingredients that can be obtained from microalgae focusing on proteins and pigments as well as recent advances in cell disruption technologies.

2. Strain selection: Main species and general composition

As mentioned previously, global demand for microalgae foods is growing and their functional potential is evident in numerous recent books and review papers [12-15]. Commercially cultivated microalgae for food use is a mature industry which primarily focuses on the cultivation of *Chlorella*, *Spirulina*, *Dunaliella*, *Nannochloris*, *Nitzschia*, *Crypthecodinium*, *Schizochytrium*, *Tetraselmins*, and *Skeletonema* species [16], some of them shown in Figure 1. Most common food products containing microalgae can be broadly divided into two main groups: (i) dried microalgae, mainly *Chlorella* and *Spirulina* species and (ii) products enriched in a certain compound derived from microalgae. The former products can be directly sold as dietary supplements, or used as ingredients in the manufacture of other innovative products, and the latter include products enriched in pigments, such as astaxanthin, antioxidants such as β-carotene, or fatty acids including omega-3, docosahexaenoic acid (DHA), or eicosapentaenoic acid (EPA) [1]. Examples of foods and beverages which containing microalgae or microalgae-derived compounds and have been recently launched onto the market are listed in Table 1. These include Plancton Marino Veta la Palma® (Fitoplancton Marino SL, Spain), which is a freeze-dried *Tetraselmis chuii* product authorized by the European Food Safety Authority (EFSA) to be marketed as a novel food in accordance with Article 3(1) of Regulation (EC) No 258/97 [17].

The general composition of microalgae biomass is shown in Table 2. Generally, microalgae contain 40–70% proteins, 12–30% carbohydrates, 4–20% lipids, 8–14% carotene, and substantial amounts of vitamins B1, B2, B3, B6, B12, E, K, and D [16]. In a recent study, Finkel, Follows, Liefer, Brown, Benner and Irwin [5] provided a new estimate of the macromolecular composition of microalgae using a hierarchical Bayesian analysis of data compiled from the literature and reported the median

93 macromolecular composition of nutrient-sufficient exponentially growing microalgae as
94 32.2% proteins, 17.3% lipids, 15.0% carbohydrate, and 17.3% ash. It is important to
95 highlight that the composition of microalgae depends on several factors and change
96 during culture ageing. For example, as shown in Table 2, a big difference can be
97 observed between the protein content of *Dunaliella salina* when harvested at their
98 exponential growth phase (38.0 g/ 100 g) or at early or late phases (9.9 g/ 100 g).
99 Similar differences can be observed for other constituents and other species. In addition,
100 when microalgae are cultivated under sub- or supra-optimal conditions, they react
101 changing their metabolic pattern and strategies resulting in fluctuations in the relative
102 content of the biomass compounds [18]. Growth characteristics and composition of
103 microalgae depend on cultivation conditions [19]. Therefore, in order to obtain high
104 quantities of a certain compound, the optimization of the cultivation and harvest
105 strategy is of key importance.

3. Edible microalgae: Advances in cultivation, harvesting and drying technologies

3.1 Cultivation of microalgae

Cultivation conditions for microalgae can be divided into four major types: photoautotrophic, heterotrophic, mixotrophic, and photoheterotrophic cultivation. The former, which is the most commonly used cultivation condition for microalgae growth occurs when microalgae use light (generally sunlight) as the energy source, and inorganic carbon (for example, carbon dioxide) as the carbon source to form chemical energy [19]. Heterotrophic cultivation is called when microalgae can not only grow under phototrophic conditions but also use organic carbon in the absence of light, and has also attracted increased interest for production of pigments and other valuable compounds [20]. Moreover, mixotrophic cultivation is when microalgae are able to live under either phototrophic or heterotrophic conditions or both [21].

The core of the biomass production process is the photobioreactor in which microalgae are produced [22]. Different production systems have been designed for the large-scale production of microalgae. These are usually operated under phototrophic conditions [23] and can be divided into two main groups, open or closed systems – Figure 2. Within the open systems, the best choices are generally open shallow ponds, made of levelled raceways (2-10 m wide and 15-30 cm deep), running as simple loops or as meandering systems, where turbulence is provided by rotating paddle wheels [24]. Scaling up these reactors is easy and investment and operational costs are relatively low. Main disadvantages of open cultivation systems include the difficulty of process control, the need to operate in batch or semi-continuous regimes, poor light utilization efficiency, and the low population densities obtained. Moreover, contamination control in open reactors, which is important for food applications, is not possible. Closed systems allow operating in a continuous regime and offer higher biomass concentration,

productivity, and quality as well as higher photosynthetic efficiency. Closed reactors are certainly more expensive and difficult to scale up. Advances on closed photobioreactors have been reviewed by Wang, Lan and Horsman [25]. As mentioned previously, microalgae are still expensive to produce. For this reason, a lot of efforts are being made in the design of more efficient photobioreactors as well as in optimizing their control and modelling to maximize biomass yields and reduce costs [22].

3.2 Harvesting and drying of microalgal biomass

One of the main challenges in downstream processing of microalgae is separating the microalgae from their growth medium. Because of the shading effect of microalgae (when high concentrations lead to mutual shading and decreased productivity), biomass concentrations in microalgae cultures are low, ranging from 0.5 g/L in open reactors to 5.0 g/L in closed photobioreactors [26]. Flocculation has been suggested as an initial step to concentrate the dilute suspension to a 10-50 g/L slurry, which can be further concentrated by centrifugation to a 25% dry matter content [26]. However, the low energetic cost of flocculation operations can be partially cancelled by the additional cost of flocculants together with the impact of flocculants in the water recycle stream [27]. Although it is energy intensive, centrifugal recovery of microalgal biomass is the preferred harvesting method mainly because it can be rapid, and because of the small size of microalgae and their colloidal stability in suspension, which renders harvesting by sedimentation not feasible. Filtration operating under pressure or vacuum has been also used for recovering relatively "large" microalgae such as *Coelastrum proboscideum* and *Spirulina platensis* [28].

The harvested biomass needs to be further processed rapidly, especially in hot climates where it can spoil easily. Show, Lee, Tay, Lee and Chang [29] reviewed the main drying strategies, namely freeze-drying, rotary drying, spray drying, solar drying, cross-

flow drying, vacuum-shelf drying, flash drying, and incinerator drying. Except for solar drying, which is a simple and inexpensive method, these operations heavily affect the overall energy consumption and production cost of microalgae. Indeed, Fasaei, Bitter, Slegers and van Boxtel [27] recently assessed the techno-economic performance of 28 scenarios for large scale microalgae harvesting and dewatering and concluded that harvesting and dewatering contribute to approximately 3-15% of the production costs of microalgae biomass [27]. Main disadvantages of solar drying include overheating of the biomass, risk of fermentation and spoilage under prolonged drying, and a high dependence on the weather making the process unreliable. Overall, the selection of a certain drying methodology will depend on both, production scale and purpose for which the biomass is intended.

4. Recent advances in cell wall disruption and extraction techniques

Cell disruption methods can be divided into mechanical or non-mechanical processes, which include enzymatic cell disruption. This review will only focus on mechanical processes. Different mechanical or physical pre-treatments or extraction methods including PEFs, HPH, or US processing have been evaluated as novel strategies to improve extraction yields. These operations can be also used alone or one after another in order to improve the disruption efficiency.

4.1 Solid shear cell disruption: Bead milling

Bead milling is a purely mechanical disruption method, which can be used at industrial scale applications due to its efficiency in single-pass operations, low labour intensity, availability of large scale devices, and ease of scale up [11]. An example of a bead mill can be seen in Figure 3. The main disadvantage of this disruption method, besides heat production, is an extreme cell disruption, complicating downstream purification steps. However, mild disintegration can be achieved by carefully controlling and optimizing the process conditions. This was done, for example, by Postma, Miron, Olivieri, Barbosa, Wijffels and Eppink [30] who utilised a response surface methodology to optimise soluble protein yield, energy consumption, and processing time. Similar results were obtained by Suarez Garcia, van Leeuwen, Safi, Sijtsma, Eppink, Wijffels and van den Berg [31] who obtained a protein extract after developing a mild and simple one-step extraction process using bead milling (Table 3).

4.2 Liquid shear cell disruption: High pressure homogenization and sonication

HPH is a mechanical process, during which a liquid dispersion is forced by high pressure, generally ranging between 50 and 300 MPa, through a micrometric disruption chamber [32]. This chamber increases the velocity and as a result, microalgae cells are subjected to extremely intense fluid-mechanical stresses such as shear, elongation,

turbulence, and cavitation, resulting in physical disruption of cell walls and membranes [33]. This strategy has irrupted as the main method to obtain a complete disruption of biological cells. However, one of the main limitations of HPH is the non-selective release of intracellular compounds, complicating downstream separation steps. HPH conditions should be determined based on the target cell component and the properties of the cell suspension. These can depend on factors including concentration and age of the culture [34]. Several studies have highlighted the potential of HPH for recovering valuable compounds from microalgae [33-35]. One of the most relevant ones, recently published by Safi, Cabas Rodriguez, Mulder, Engelen-Smit, Spekking, van den Broek, Olivieri and Sijtsma [35] evaluated the efficacy of multiple cell disruption methods and concluded that HPH was not only the most effective (together with bead milling) but also the least expensive. In that study, the authors obtained over 95% cell disintegration, approximately 50% (w/w) release of total proteins and the lowest energy input, calculated as under 0.5 kWh per kg of biomass and 0.15-0.25 € per kg of protein. Another cell disruption method is high speed homogenisation (HSH), which is based on hydrodynamic cavitation produced by a stirring device at high speed. It is probably the most simple and one of the most effective cell disruption methods. Main advantages include short contact times and potential to disrupt suspensions with relatively high concentrations [11].

Sonication has also been studied as a strategy to increase extraction yields. The destructive effects of ultrasounds on microalgal cells have been attributed to acoustic cavitation. One of the main advantages of this technology is that large-scale ultrasonic devices are currently available and they allow operating in continuous mode [36]. Yamamoto, King, Wu, Mason and Joyce [37] recently demonstrated that high-frequency sonication is more effective than conventional low-frequency for the

disruption of *Chlamydomonas concordia* and *Dunaliella salina*. Wang and Yuan [36] also reported that increasing ultrasound intensity resulted in improved cell disruption efficiency.

4.3 Other methods: Microwave-assisted extraction, pulsed electric fields, and other electro-technologies

PEFs consist on a short time (from nanoseconds to milliseconds) electrical treatment with pulse electric field strength from 100 to 300 V/cm to 20-80 kV/cm [38]. The application of PEFs can cause lethal damage to cells or induce sub-lethal stress by transient permeabilization of membranes and electrophoretic movement of charged species between cellular compartments [8]. When utilized at high electric fields, PEFs can constitute an alternative to thermal processing by inactivating alterative microorganisms and human pathogens as well as inactivating quality-related enzymes [39]. When used at low electric fields, cell membranes lose their semi-permeability either temporarily or permanently, allowing a selective recovery of valuable compounds from the intracellular matrix [38]. One of the main advantages of this technology is that it could be potentially used in a biorefinery concept, where soluble ingredients are extracted before solvent extraction of lipids is performed [9]. **Table 3** summarizes the main outcomes observed after utilizing PEFs and other novel processing techniques to improve the extraction yields of bioactive or functional compounds from microalgae. Electric field strengths and pulse durations most commonly used ranged between 7.5 to 45.0 kV/cm and 2 μs to 6 ms, respectively. PEFs processing demonstrated to be an effective method to increase lipid recovery yields. Zbinden, Sturm, Nord, Carey, Moore, Shinogle and Stagg-Williams [40] suggested that combination of PEFs with organic solvents could reduce processing times and facilitate lipid extraction. Similar results were reported by Sheng, Vannela and Rittmann [41]. However, overall, no major

242 increases in protein or carbohydrate recovery yields were reported by Postma, Pataro,
243 Capitoli, Barbosa, Wijffels, Eppink, Olivieri and Ferrari [42], who observed that over
244 95% of proteins are retained inside the microalgal cell after PEFs processing. The
245 authors of that study reported an increased carbohydrate recovery yield after PEFs
246 processing (20-25%), especially when PEFs were performed at 55 ºC (32-40%).
247 However, yields were still lower when compared to benchmark bead milling (40-45%
248 for protein and 48-58% for carbohydrates). Overall, the required energy input for PEFs
249 processing is higher and bioactive compound recovery yields are generally lower when
250 compared to traditional extractions techniques. However, this technology does show
251 potential for further research and development and, as it promoted the selective release
252 of small water-soluble compounds, it could be used in a multi-stage biorefinery as an
253 initial stage or pre-treatment for selective recovery of small valuable cytoplasmatic
254 compounds.
255 Barba, Grimi and Vorobiev [8] recently suggested that high-voltage electrostatic fields
256 (HVEF) and high-voltage electrical discharges (HVED) could also be used to improve
257 the extraction of food additives and nutraceuticals from microalgae. However, to the
258 best of our knowledge, these technologies have not yet been used for promoting the
259 extraction of valuable compounds from microalgae. Both technologies have been shown
260 to be promising methods for intracellular extraction of valuable compounds from food
261 sources [43-46] and could be potentially used in cultured microalgae.
262 Microwaves are non-ionising electromagnetic radiations, with frequencies ranging from
263 300 MHz to 300 GHz, that cause heating by dipolar rotation and ionic conduction [47].
264 Microwaves can also be used to promote the mitigation of compounds and to increase
265 the penetration of a certain solvent into a food matrix, thus, facilitating the collection of
266 a target compound [48]. Microwave-assisted extraction has been proposed as a quick

and efficient process for the extraction of several valuable compounds from different food sources such as antioxidants, pigments, or oils from plants. More recently, microwaves were applied to microalgae to facilitate the extraction of valuable compounds. For example, Lee, Yoo, Jun, Ahn and Oh [49] compared the efficiency of various methods namely autoclaving, bead-beating, sonication, microwaves, and a 10% (w/v) NaCl solution to extract lipids from the microalgae *Botryococcus* sp., *C. vulgaris*, and *Scenedesmus* sp. and showed the microwave oven method showed the highest lipid extraction efficiency for all the tested species (especially for *Botryococcus* sp., with an oleic acid productivity of as 5.7 mg per litre and per day). Table 3 lists other recent studies where microwaves were utilised to facilitate extraction of valuable compounds from microalgae.

5. Bioactive and techno-functional ingredients derived from microalgae

Microalgae biomass or valuable compounds derived thereof can be used as ingredients with both, techno-functional or bioactive properties which could be used for the development of novel foods. Most important compounds for food applications, obtained from microalgae biomass, include proteins, pigments, oils, and other compounds such as polyphenols with high antioxidant activity. Several companies, mainly based in Australia, Israel, Germany, Spain, the Netherlands, and the United States are currently commercializing high-valuable compounds from microalgae [50]. The current paper will only focus on pigments and proteins, which are the most industry relevant ingredients obtained from microalgae.

5.1 Proteins: High quantity and high quality

Table 2 lists the average protein content of several microalgae. Protein content differs widely across groups of microalgae. Phylogenetic differences in protein content reflect differences in cell wall composition, the light harvesting apparatus, and storage reserve strategies [5]. For example, the phyla with the highest protein content on a dry weight basis are the Cyanobacteria (43.1-63.0 g/ 100 g) and Cryptophyta (47.5-47.7 g/ 100 g), which use protein as an integral part of their cell wall. Overall, species with high growth rates may have higher protein levels when compared to slower growing species, except for Bacillariophyta – due to the weight of their siliceous frustule [5].

The amino acid composition of proteins extracted from several microalgae is shown in **Table 4**. Essential amino acids are those that cannot by synthesized *de novo* by humans and must be supplied by the diet. Essential amino acids for humans are phenylalanine, valine, threonine, tryptophan, methionine, leucine, isoleucine, lysine, and histidine. The content of these amino acids in whole egg or wheat-derived proteins has been reported to be comparable to that of microalgae-derived proteins [7]. Other studies suggested that

303 microalgae-derived proteins compete favourably, in terms of quantity and quality, with
304 conventional proteins derived from plant or animal sources [16]. Despite the nutritional
305 quality of microalgae-derived proteins, the propagation and utilisation of microalgae
306 proteins is limited because of high production costs as well as technical difficulties to
307 incorporate microalgae into palatable foods. One of the main obstacles for the
308 application of microalgae protein in food systems is the dark green colour of the
309 biomass. However, the intense green colour of the microalgae biomass can be removed
310 (or partially removed) if proteins are further purified by precipitation and separation,
311 facilitating their incorporation into foods as novel ingredients [51, 52]. Indeed, several
312 products formulated using microalgae-derived proteins are currently commercially
313 available (Table 1). As mentioned previously, protein extraction from microalgae
314 biomass is not an easy task and the optimization and development of novel extraction
315 techniques would facilitate the incorporation of protein concentrates or protein isolates
316 derived from microalgae into novel foods.
317 Microalgae-derived proteins are valuable in the food industry not only for their high
318 nutritional quality, but also for their techno-functional properties. High protein
319 solubility is of key importance for successful functional application of foods. Proteins
320 isolated from *Tetraselmis* sp. showed 100% solubility in the pH range 6.0 to 8.5 [51].
321 Other important functional properties of proteins include their interactions with water
322 and oil, important for the food industry because of their effects on flavour and texture
323 [53]. High water-holding capacity (WHC) values help to maintain freshness and mouth
324 feel and are highly desired in foods such as sausages, custards, or baked products [54].
325 The oil-holding capacity (OHC) is also of great importance from an industrial point of
326 view since it reflects the emulsifying capacity, a key property for proteins used in
327 products such as sausages or mayonnaise [52]. The WHC of *Porphyridium cruentum*,

328 *Phaeodactylum tricornutum*, and *Nannochloropis* spp. was calculated as 8.1, 4.5, and
329 4.0 g/g of biomass, suggesting potential applications in the manufacture of beverages,
330 soups, and baked products [55]. WHC and OHC of a protein concentrate generated from
331 *Chlorella pyrenoidosa* were recently calculated as 3.09 ± 0.01 and 2.02 ± 0.04 g/mL,
332 respectively [52]. These values compared well with those obtained for proteins from
333 other food sources such as common beans [54]. Emulsifying and foaming properties are
334 also of interest for food processors. Ursu, Marcati, Sayd, Sante-Lhoutellier, Djelveh and
335 Michaud [56] observed lower emulsifying properties for proteins extracted from *C.*
336 *vulgaris* at pH 12 when compared to those extracted a pH 7. In that study, the
337 emulsifying capacities of both proteins compared favourably to those of commercial
338 proteins. In a more recent study, Waghmare, Salve, LeBlanc and Arya [52] reported the
339 foaming capacity and stability of proteins concentrated from *Chlorella pyrenoidosa* as
340 95.00 ± 1.14 and 97.45 ± 0.46%, respectively. Overall, the nutritional and functional
341 properties of microalgae-derived proteins are comparable to those of other plant- or
342 animal-derived proteins. Microalgae-derived proteins can be industrially used as, for
343 example, egg replacers (Table 1). After removal of pigments, microalgae protein-rich
344 extracts can be used for the development of novel foodstuffs with high organoleptic and
345 nutritional quality.

346 **5.1.1 Bioactive peptides**

347 Bioactive peptides are specific sequences of amino acids that have biological activity
348 once released from their parent protein and ingested. The use of bioactive peptides as
349 functional food ingredients is a rapidly developing area of food innovation mainly
350 because of their demonstrated biological activities relevant to the management of
351 human health [16]. One of the main challenges for the commercialization of purified
352 bioactive peptides as food ingredients is the high costs associated with their production

353 [2]. Therefore, protein hydrolysates containing bioactive peptides and generated by
354 microbial fermentation or enzymatic hydrolysis of food-derived proteins are more likely
355 to be industry relevant. In order to reduce costs, bioactive peptides have been generated
356 from low-value food sources including animal [57] and plant [58] processing co-
357 products. Algae are excellent sources of bioactive peptides with both *in vitro* and *in vivo*
358 bioactivities. Although most of the peptides identified and reported over the last five
359 years were generated by enzymatic hydrolysis of macroalgae, some microalgae-derived
360 peptides have also been identified (Table 5). The most common bioactivity reported for
361 peptides derived from microalgae is the ability to inhibit angiotensin-I-converting
362 enzyme (ACE-I; EC 3.4.15.1), which is associated with antihypertensive effects.
363 Although, to the best of our knowledge, microalgae-derived peptide products are yet to
364 be marketed, several functional foods with bioactive peptides derived from other food
365 sources are currently being commercialized, mainly in Japan. These include the
366 antihypertensive product Calpis (Calpis Food Industry Co., Ltd., Tokyo, Japan), which
367 contains the ACE-I inhibitory peptides IPP and VPP [2].

368 **5.2 Pigments**

369 Foods are coloured mainly by four major groups of pigments, chlorophylls, carotenoids,
370 anthocyanins, and betanin. These are responsible for the green, yellow/orange/red,
371 red/blue, and red colour of foods [59]. Despite the enormous efforts being made by food
372 processors and food scientists to find novel natural sources for pigments and colorants
373 and to increase recovery yields, only few natural food colour additives have reached the
374 market. Main reasons include their lower stability, higher production costs, and limited
375 range of hues [59]. Natural food pigments are actively being studied not only because of
376 the colour they impart to foods but also for their potential health-promoting properties.
377 For example, enrichment of tomato juice with anthocyanins obtained from strawberry

processing co-products resulted in higher colour retention during storage as well as higher antioxidant activity, higher anthocyanin content, and total phenolic content [60]. Incorporation of natural pigments into food formulations would add value to the product and potentially promote health while allowing to differentiate from competition.

In algae and higher plants, carotenoids play multiple and essential roles in photosynthesis including light harvesting, maintaining structure and function of photosynthetic complexes, quenching chlorophyll triplet states, and scavenging reactive oxygen species [24]. These compounds are lipophilic compounds with isoprenoid structures that have a wide range of applications in the food, healthcare, and nutraceuticals industries. In fact, the global carotenoid market was estimated to be 1.2 billion USD in 2016 and is expected to increase to over 1.5 billion USD by 2021 [61]. Main carotenoids from microalgae with commercial interest include carotenes such as β-carotene and the xanthophylls astaxanthin and lutein.

β-Carotene is probably the most industry relevant carotenoid, mainly because the natural form of β-carotene has an active form of pro-vitamin A, an additive to vitamin supplements and health food products, which can be easily absorbed and has a stronger effect when compared to its synthetic counterpart [62]. The most important process for natural production of β-carotene is the culture of *Dunaliela* species (mainly *D. salina*), which can accumulate up to 12% of β-carotene on a dry weight basis depending on the cultivation conditions [24]. Industrial production of β-carotene from microalgae started in the 1980s in Israel, Australia, and the United States and now also occurs in India and China [63]. In addition, *Haematococcus pluvialis* is one of the most important sources of astaxanthin (3,3'-dihydroxy-β,β'-carotene-4,4'-dione), which is produced from β-carotene and serves as a precursor of vitamin A [50]. Over 300 tons of *Haematococcus* biomass (mainly *H. pluvialis*) are produced annually as a source of astaxanthin [64].

Other species capable to accumulate carotenoids, including β-carotene, lutein, astaxanthin, canthaxanthin, violaxanthin, neoxanthin, and lycopenes, among others [65], include *Botryococcus braunii*, *Clamydomonas nivalis*, *Chlorella* sp., *Cloromonas nivalis*, *Chlorococcum* sp., *Scenecesmus* sp., *Euglena* sp., and *Dunaliella* sp., among others [66]. Chlorophylls are green and liposoluble compounds that contain a porphyrin ring in their structure, which are responsible for converting solar energy into chemical energy [50]. Other microalgae-derived pigments with industrial interest include phycobilins or phycobiliproteins, which are brilliant-coloured and water soluble antennae-protein pigments organized in supramolecular complexes called phycobilisomes and function as accessory pigments for photosynthetic light collection [50]. Main classes include allophycocyanin, phycocianin, phycoerythrin, and phycoerythocyanin, which are responsible for the blue/green, blue, purple, and orange colour of certain microalgae, respectively [50].

5.4 Other valuable compounds

Marine microorganisms have been proved to be rich sources of biologically active compounds. The range of bioactivities reported from extracts of microalgae over the last decades includes antioxidant, antimicrobial, antiviral, anticarcinogenic, antihypertensive, and other bioactivities [63]. Despite the large amount of scientific evidence supporting the health-promoting potential of microalgae, the vast majority of the currently approved (or in clinical trials) drugs from marine microorganisms come from other microorganisms rather than microalgae [67]. Plant polyphenols generated a large amount of scientific research due to their *in vitro* and *in vivo* antioxidant capacities, especially since the publication of the "French paradox" phenomenon, which unveiled a low incidence of coronary heart disease in France caused by the regular consumption of wine and despite consuming a diet rich in saturated fats. Currently,

algae-derived polyphenols are one of the current trends in functional foods for the prevention of cardiovascular diseases and diabetes [68]. Several foods have been enriched in polyphenols after incorporation of microalgal biomass into their recipes [69, 70]. Microalgae are also rich in lipids. The main components and applications of the algae lipid fraction have been thoroughly reviewed in several review papers and book chapters [50, 71-73].

6. Future perspectives and conclusions

The composition and characteristics of the cell walls of microalgae depend on several factors which include microalgal specie, growth conditions and phase, or the presence of stress factors. Microalgal cell walls require strong pre-treatments which include high pressures, temperatures, or pH variations in order to facilitate the release of intracellular bioactive compounds. Most of the emergent methods studied for improving the recovery of bioactive compounds still require further research to reach industrial levels. Therefore, in order to develop large-scale and energy-efficient production of bioactive compounds from microalgae, further knowledge on microalgal composition and disruption methods is still needed. However, when used for food purposes, the utilisation of the whole biomass could facilitate the consumption of microalgae-derived bioactive compounds. Indeed, the production of microalgae for food applications is a reality and the number of food containing microalgae formulated and studied or launched into the market has significantly increased during the past 4-5 years [4]. This trend is likely to continue to grow as algal-derived polyphenols are an emerging trend in the development of functional foods for the preventions of a number of diseases [68] and astaxanthin has been suggested as one of the three main ingredients to watch during 2019 because of its huge potential [4].

In conclusion, dried microalgae biomass as well as microalgae-derived compounds such as proteins and pigments can be used to develop a huge number of innovative food products. Strain selection and cultivation, harvesting, drying, and cell disruption strategies are key aspects that need to be considered when isolating valuable compounds from microalgae. The development of environmentally friendly, food-grade, and economical approaches for microalgal cultivation, harvesting, and drying will require

458 further investigation but will certainly facilitate the utilisation of this resource in the
459 food industry.

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Conflict of interests

The author declares no conflict of interests

Figure legends

Figure 1. Microscope images of several microalgae species

A. *Scenedesmus* species obtained using a scanning electron microscope; B. *Chlorella minutissima* obtained using a laser scanning microscope; C. *Phaeodactylum tricornutum* obtained using the laser scanning microscope; and D. *Tetraselmis suecica* obtained using a confocal microscope. Figure reprinted from Gerardo, Van Den Hende, Vervaeren, Coward and Skill [74] with permission from Elsevier.

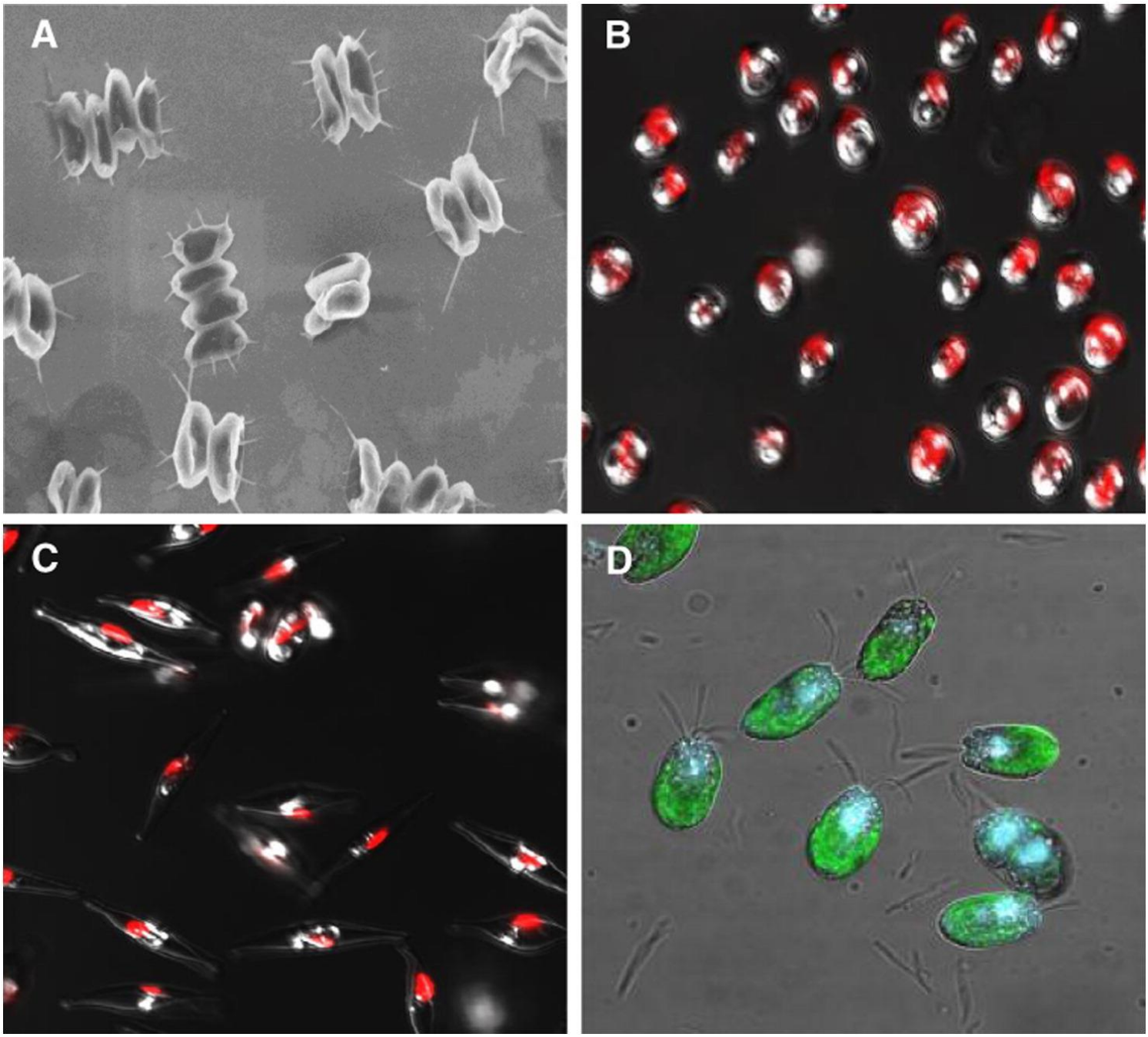
Figure 2. Open and closed photobioreactors

Photobioreactors shown are located in Almeria, Spain. Figures were kindly provided by Professor Francisco Gabriel Acién-Fernández (Department of Chemical Engineering, University of Almería).

Figure 3. Bead mill

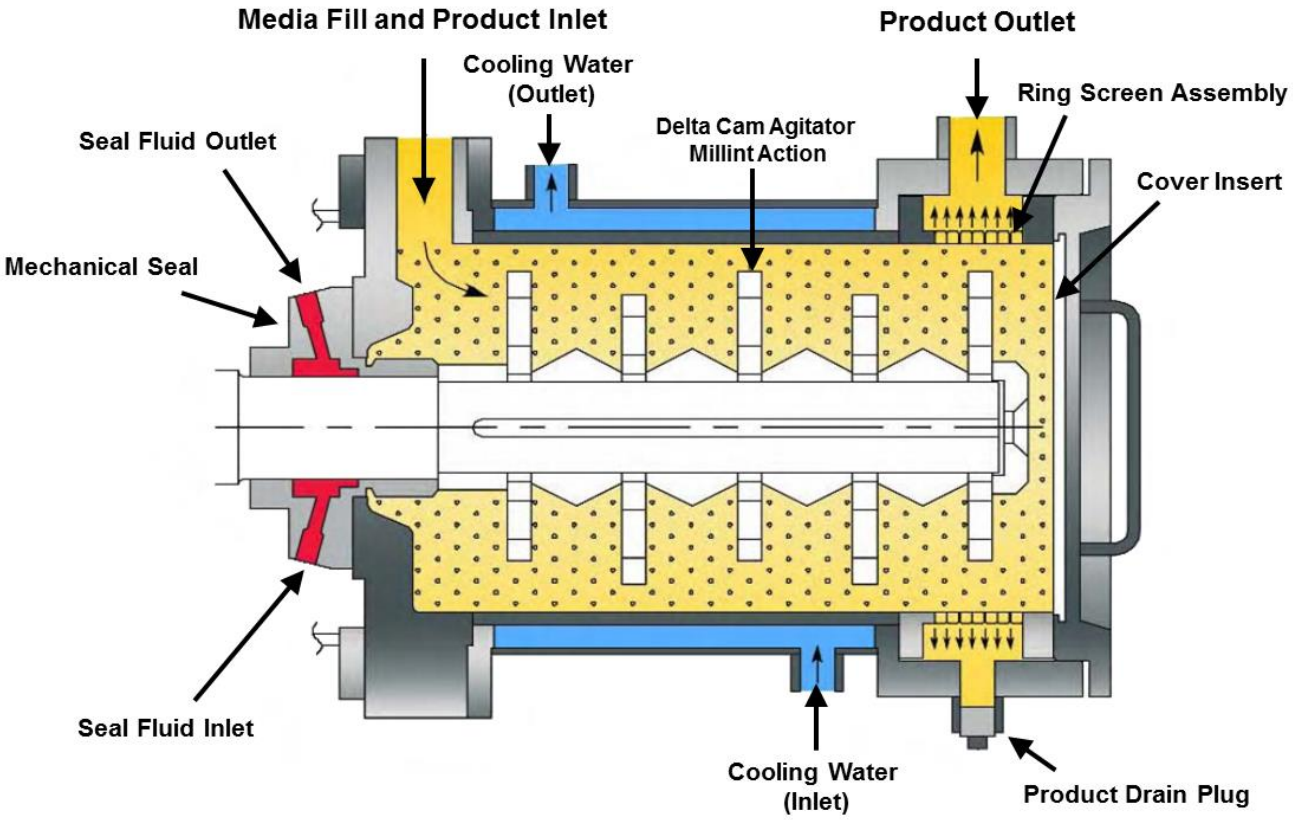
Figure reprinted from Günerken, D'Hondt, Eppink, Garcia-Gonzalez, Elst and Wijffels [11] with permission from Elsevier.

482 **Figure 1.**



484 **Figure 2.**

Figure 3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Product Outlet
Cooling Water
(Outlet)
Ring Screen Assembly
Seal Fluid Outlet
Delta Cam Agitator
Millint Action
Cover Insert
Mechanical Seal
Seal Fluid Inlet
Cooling Water
(Inlet)
Product Drain Plug

488 **Table 1. Foods and beverages containing microalgae and/or microalgae-derived compounds commercialize in 2018**

Product	**Brand**	**Company**	**Country of commercialization**	**Product description**
Microalgae biomass				
Freeze-dried *Tetraselmis chui*	Plancton Marino Veta la Palma	Fitoplancton Marino SL, Spain	Spain	Natural product which can be used in sauces, special salts, and condiments[1]
Dried *Tetraselmis*, *Spirulina*, and *Dunaliella* species	Algaefeed	Algalimento, Spain	Spain and other EU countries	Dried microalgae cultivated in open raceway ponds with applications in aquaculture, food production, cosmetics, and as sources for pigments and antioxidants.
Organic *Spirulina* tablets	Sanatur	Sanatur, Germany	Germany	Dietary supplement featuring the EU Green Leaf, Bio, Naturland and Vegan V-Label European Vegetarian Union logos
Organic *Spirulina* microalgae tablets	Vitatrend	IQ Pharma, Germany	Germany	Dietary supplement featuring the EU Green Leaf and Naturland logos
Beverages				
Smoothie	Natura	Naturawerk, Germany	Germany	Smoothie powder with chlorella, spinach, lucuma, and baobab powders. Features the EU Green Leaf logo and the Vegan Neuform Quality
Smoothie	Be Raw!	Purella Food, Poland	Poland	Pasteurized pressed juice with added *Chlorella*
Wheat grass cocktail	Rabenhorst	Haus Rabenhorst, Germany	Italy and Germany	Organic functional juice drink containing young wheatgrass, green tea, agava juice and spirulina
Omega 3 yogurt	Priégola Simbi	Ganadería Priégola,	Spain	Enriched in DHA and omega 3 fatty acids obtained

drink		Spain		from *Schizochytrium* sp.[2]
Sauces and spreads				
Peanut spread with *Spirulina*	Better & Different	Better & Different, Israel	Israel	Kosher certified low in sodium and suitable for vegans
Vegan sauce with smoked pepper	The Good Spoon	Good Spoon Foods, France	France	Free from eggs and instead made with microalgae
Original vegan supernaise sauce	The Good Spoon	Good Spoon Foods, France	France	Contains microalgae "flour" as an egg replacer. Features the V-label European Vegetarian Union
Low fat spread	St Hubert DHA Cérébral & Vision	St Hubert, France	France	Low fat spread enriched in DHA extracted from *Schizochytrium* sp.[2]
Baked goods				
Sea salt algae crackers	Helga	Evasis Edibles, Germany	Austria	Green, crispy, and spicy snack containing chlorella and linseed. Features the EU Green Leaf logo
Bio matcha and *Spirulina* biscuits	Próvida	Próvida Produtos Naturais, Portugal	Portugal	Protein- and fibre-rich biscuits featuring the EU Green Leaf logo
Jeju green tea Castella cake	Paris Baguette	Paris Croissant, South Korea	South Korea	Premium product made with fragrant Jeju green tea and containing *Chlorella*
Oat and rice cakes with *Spirulina*	Gullón Vitalday	Galletas Gúllon, Spain	Spain	-
Snacks				
Super green superfood bar	OHi	OHi Foods, USA	USA	Paleo and vegan certified product free from gluten, GMO, grain, and soy and containing organic *Spirulina*

Spirulina truffles	Of the Earth Superfoods	Alara Wholefoods, UK	UK	Hand-made spirulina and lemon truffles rich in chlorophyll and phytochemicals
Smoked seaweed & sea salt organic puffs	Honest Fields	SC Honest Fields Europe, Romania	Romania	Corn-based snack containing *Chlorella* suitable for vegans and vegetarians bearing the EU Green Leaf logo
Cereal bars	All Seasons Health	All Seasons Health, UK	UK	Organic snack bars containing spirulina, chlorella, and blue-green algae
Baby roll	Pei Tien	Pei Tien, Taiwan	Taiwan	Algae-flavoured rice roll suitable for babies from eight months onward
Green tea and *Spirulina* candies	Cesare Carraro	Incap, Italy	Italy	Claimed to provide total well-being
Other foods				
Rice seasoning	Sengran	Woorichan, South Korea	South Korea	Sengran spinach, chlorella, spinach, cabbage, green tea, green laver, and tofu green rice seasoning
Food supplement	4+ Nutrition	International Sport Nutrition, Italy	Italy	Instant powdered food supplement suitable for athletes performing intense physical exercises containing chlorella
Egg replacer	Follow Your Heart Vegan Egg	Earth Island, USA	France and UK	Egg replacer made with whole microalgae flower and proteins
DHA-enriched cooking oil	Taisun	Taisun Enterprise, Taiwan	Taiwan	Cooking oil blend made from olive and mustard oil enriched in DHA extracted from microalgae
Baby food	Hero Baby Pedialac	Hero, Spain	Spain	Milk for premature babies from birth, containing DHA extracted from microalgae
Baby food	Tony Baby	Norbel Baby,	Taiwan	Baby food containing DHA and AA extracted

		Taiwan		from microalgae

Abbreviations: AA, arachidonic acid; DHA, docosahexaenoic acid; EU, European Union; GMO: genetically modified organisms. Data accessed on March 2019 from MINTEL, available at http://www.mintel.com/

[1] Accepted as "novel food" by EFSA in 2013 in accordance Regulation (EC) No 258/97 [17].

2 Accepted as "novel food" by EFSA in 2015 in accordance Regulation (EC) No 258/97 [75]

493 **Table 2. General composition of microalgae biomass.**

Genus	Species	Growth phase	Protein (g/ 100 g)	Lipid (g/ 100 g)	Carbohydrate (g/ 100 g)	Ash (g/ 100 g)
Phylum - *Bacillariophyta*						
Nitzschia	*paleacea*	Exponantial	30.2	23.4	17.1	24.0
Nitzschia	*paleacea*	Early or late	30.3	27.1	16.1	23.6
Phaeodactylum	*tricornutum*	Exponantial	38.3	17.1	19.9	13.9
Phaeodactylum	*tricornutum*	Early or late	44.5	17.3	25.7	11.0
Minidiscus	*trioculatus*	Exponantial	21.9	19.6	19.3	34.0
Minidiscus	*trioculatus*	Early or late	43.0	27.3	22.3	22.6
Phylum – *Chlorophyta*						
Chlorella	*vulgaris*	Exponantial	39.6	15.9	8.1	6.3
Chlorella	*vulgaris*	Early or late	20.3	41.7	51.0	N.A.
Dunaliella	*salina*	Exponantial	38.0	18.8	16.8	11.7
Dunaliella	*salina*	Early or late	9.9	9.3	42.8	11.2
Dunaliella	*tertiolecta*	Exponantial	42.1	12.1	25.1	12
Dunaliella	*tertiolecta*	Early or late	40.0	2.9	30.3	N.A.
Tetraselmis	*suecica*	Exponantial	45.6	7.9	9.2	18.5
Tetraselmis	*suecica*	Early or late	25.2	9.8	36.6	15.2
Botryosphaerella	*sedutica*	Exponantial	11.1	14.5	7.1	N.A.
Botryosphaerella	*sedutica*	Early or late	4.4	19.9	7.3	N.A.

Phylum – *Cryptophyta*						
Rhodomonas	*salina*	Exponantial	47.5	18.3	16.0	10.0
Cryptomonas	sp.	Exponantial	47.6	21.1	4.1	16.0
Hillea	sp.	Exponantial	47.7	11.8	20.8	N.A.
Phylum – *Cyanobacteria*						
Arthrospira	*platensis*	Exponantial	63.0	6.6	15.3	8.4
Arthrospira	*platensis*	Early or late	50.3	18.1	7.2	N.A.
Nostoc	*paludosum*	Exponantial	43.1	6.6	25.6	5.5
Nostoc	*paludosum*	Early or late	50.3	8.3	23.4	N.A.
Phylum – *Haptophyta*						
Diacronema	*lutheri*	*Exponantial*	32.6	11.3	16.4	6.4
Diacronema	*lutheri*	*Early or late*	26.5	23.4	21.8	N.A.
Diacronema	*vlkianum*	*Exponantial*	24.4	31.9	29.9	4.9
Diacronema	*vlkianum*	*Early or late*	29.1	38.2	22.8	2.3
Isochrysis	*galbana*	*Exponantial*	34.5	20.8	14.0	14.8
Isochrysis	*galbana*	*Early or late*	32.6	25.2	12.8	12.2
Pseudoisochrysis	*paradoxa*	*Exponantial*	43.0	26.5	10.1	11.7
Pseudoisochrysis	*paradoxa*	*Early or late*	27.8	32.3	26.5	9.1
Phylum – *Ochrophyta*						
Nannochloropsis	*oculata*	*Exponantial*	42.4	23.6	10.5	10.1

Nannochloropsis	*oculata*	*Early or late*	21.2	33.7	13.7	N.A.

Abbreviations: N.A., data not available.

Data expressed on a dry weight basis. Values represent the average of data reported in different studies, obtained from a microalgae composition database reported by Finkel, Follows, Liefer, Brown, Benner and Irwin [5], who summarized over 1560 observations published from 1961 to 2015.

498 **Table 3. Processing strategies for increased recoveries of valuable compounds from microalgae**

Technology	Microorganism	Product studied	Conditions studied	Main outcomes	Reference
BM	*Tetraselmis suecica*	Proteins	A 100 g/L suspension was bead milled with 0.4 mm beads at a filling percentage of 65%. Temperature was kept constant at 25 ºC	Soluble protein yield was 22.5% (at room temperature and with no addition of chemicals – pH 6.5). Extract contained 50.4% protein and showed excellent functionality.	[31]
BM	*C. sorokiniana*	Starch, proteins and chlorophyll	Beads with diameters ranging from 0.2 to 1.3 mm at speeds ranging from 8 to 14 m/s. Temperature was maintained below 20 ºC	Authors developed a kinetic model, including cell size distribution, for *C. sorokiniana* disruption in continuous bead miller. Optimum conditions were glass beads of 0.4 mm at impeller velocity of 14 m/s.	[76]
BM	*C. vulgaris, N. oleoabundans* and *T. suecica*	Proteins and carbohydrates	Beads with diameters ranging from 0.3 to 1.0 mm at a constant filling volume of 65%. Temperature was kept constant at 25 ºC	Bead mill was operated close to a minimum for *C. vulgaris* and *N. oleoabundans* at 0.3-0.4 mm beads. *T. suecica* appeared to be significantly weaker. Protein release was achieved in early stages of disintegration.	[77]
BM	*C. vulgaris*	Proteins	Biomass concentrations ranged between 25 and 145 g/kg and agitator speeds between 6 and 12 m/s. Beads with 1.0 mm diameter at a constant filling volume of 65% were used. Temperature	Over 97% of cell disintegration was achieved. An optimum rate of disintegration and protein release was observed at 9-10 m/s, regardless of biomass concentration. Proteins were released at 85% lower energy input than for cell disintegration.	[30]

			was kept below 35 ºC		
BM, PEFs, HPH, and ET	*N. gaditana*	Proteins	PEFs Pulses: Monopolar square wave pulses (5 ms) every 5 s EFS: 30 kV/cm Concentration: 15-60 g/L. HPH 300-1500 bar at 9 L/h. ET Alcalase® at 5% (v/w) at pH 8.0 and 50 ºC for 4 h. BM A 100 g/L suspension was bead milled for 1 h with 0.5 mm beads at a filling percentage of 65% and 8 m/s.	No significant increase in the protein recovery yields after PEF processing, except for a 10% increase after extraction of 60 g/L using 10 pulses (energy input calculated as 10.4 kWh/kg). HPH resulted in a 5-fold higher release of protein when compared to PEFs processing (energy input calculated as 10.4 kWh/kg). ET: 3-fold higher protein release when compared to PEFs (0.34 kWh/kg). BM: Results were comparable to those obtained for HPH. Stopping the process after 3.5 min would be enough to release 95% of the maximum amount of released protein (0.43 kWh/kg).	[35]
PEFs	*Auxenochlorella protothecoides*	Proteins and carbohydrates	Energy: 52-211 kJ/kg EFS: 23-43 kV/cm Biomass concentration: 36-167 g/kg	Increased release of soluble intracellular matter into the suspension.	[9]
PEFs	*Ankistrodesmus falcatus*	Lipids	Biomass concentration: 1.9 g/L	Increased cell disruption (90% of the cells being lysed) and lipid recoveries. PEFs combined with organic solvents resulted in	[40]

			EFS: 45 kV/cm	decreased contact times.	
PEFs	*C. vulgaris* and *Neochloris oleoabundans*	Proteins	Batch PEF EFS: 7.5-30 kV/cm Pulse duration: 1-40 square wave pulses (0.05-5.00 ms) every 3 s. Continuous PEF Pulse duration: square wave monopolar pulses with a pulse duration of 2 µs. EFS: 20 kV/cm	Highest yield of protein, 13%, was obtained with *N. oleabundans* cultivated in seawater in a batch mode. Conditions studied were not efficient in liberating protein and the required energy input for PEFs was higher than the mechanical benchmark.	[78]
PEFs combined with temperature	*C. vulgaris*	Carbohydrates and proteins	PEFs EFS: 20 kV/cm Pulses: 5 µs length and 50-200 kHz frequency Temperature: 25 to 65 ºC	Combined PEF-temperature treatment does not sufficiently disintegrate cells to release carbohydrates and proteins at yields comparable to the benchmark bead milling	[42]
PEFs and HPH	*C. vulgaris*	Proteins and carbohydrates	PEFs EFS: 10-30 kV/cm Energy: 20-100 kJ/kg Pulses: 5 µs length HPH Diameter: 100 µm Pressure drop:150 MPa	PEFs promoted the selective release of small water soluble compounds (36.0 and 5.2% w/w of total carbohydrates and proteins respectively) while HPH caused the instantaneous and complete release of all intracellular material	[33]

			Volumetric flow: 155 mL/min Number of passes: 1-10		
HVED and HPH	*Parachlorella kessleri*	Carbohydrates, proteins, and pigments	HVED Application of 1-800 pulses at a pulse repetition rate of 1 Hz. Time of electrical treatment varied from 1 to 8 ms. HPH Pressure: 400-1200 bar Volumetric flow: 10 L/h Number of passes: 1-10	HVED was effective for the extraction of ionic cell components and carbohydrates (421 mg/L) and ineffective for extraction of pigments and proteins (15% of total protein). Protein extraction was 4.9-fold higher after HPH (at 1200 bar).	[79]
HPH	*Nannochloropsis oculata*	Intracellular compounds	Pressure exerted on the homogenising valve: 75/10, 875, 125/18, 125, and 230/33, 350 MPa/psi Number of passes: 1-6	HPH conditions must be calculated based on target cell components and cell properties (age of the culture). Soluble protein and sugar yields were 22.7-50.4 mg/g and 55.0-62.5 mg/g, respectively.	[34]
PEFs and US	*Nannochloropsis* spp.	Proteins, carbohydrates, pigments, and polyphenols	PEFs EFS: 20 kV/cm Energy: 160 J per pulse. Pulses: 0.01-6.00 ms with a 2 s pause between pulses. US	PEFs allowed a selective extraction of water-soluble proteins which were different to those extracted from US treated suspensions. US processing was more effective than PEFs in extracting pigments (chlorophylls and carotenoids), proteins, and polyphenols.	[80]

			Treatment during 0-600 s (200 W, 24 kHz)		
US	*Nannochloropsis* spp.	Polyphenols and chlrophylls	Power: 100-400 W Frequency: 24 kHz Processing time: 0-30 min	Optimal conditions were 400 W, 5 min, binary mixtures of water-DMOS and water-ethanol at 25-30%, and microalgae concentration of 10%. Different solvents and binary mixtures were studied. Recovery efficiency decreased as DMSO > ethanol > water.	[81]
US, soaking, and MAE	*C. closterium* and *Dunaliella tertiolecta*	Pigments	US Duration: 3-15 min Power: 4.3-12.2 W MAE Duration: 3-15 min Power: 25-100 W Pressure: vacuum or atmospheric pressure	Freeze-drying can weaken the cell membrane and facilitate solvent solubilisation of pigments (in these species). MAE is very efficient when a mechanical resistance such as a frustule in diatoms limit solvent efficacy and has weak utility for extracting pigments from species lacking frustule and a thick outer exopolysaccharide envelope.	[82]
MAE	*P. purpureum*	Pigments	Temperature: 40-120 ºC Duration: 10 s to 5 min Agitation: 600 rpm	MAE allowed a 180- to 1080-fold reduction of the extraction time. Maximum phycoerythin yield was obtained after 10 s at 40 ºC. Flash irradiation for 10 s at 100 ºC was the optimum process to extract phycocyanin and allophycocyanin	[83]

Abbreviations: PEFs, pulsed electric fields; HPH, high pressure homogenization; ET, enzymatic treatment; BM, bead milling; US, ultrasounds; TPC: total phenolic content; EFS: electric field strength; MAE: microwave-assisted extraction

502 **Table 4. Amino acid profile of proteins obtained from different microalgae.**

Amino acid	*Chlorella pyrenoidosa* (g/100 g of protein)	*Tetraselmis* sp. (g/100 g of protein)	*Amphidinium carterae* (g/100 g of protein)	*Dunaliella tertiolecta* (g/100 g of protein)	*Isochrysis galbana* (g/100 g of protein)	*Skeletonema costatum* (g/100 g of protein)	*Chlorella vulgaris* (g/100 g of protein)	*Chlorella vulgaris* (g/100 g of protein)	*Tetraselmus chuii* (g/100 g of protein	*Scenedesmus almeriensis* (g/100 g of protein)
Alanine (Ala, A)	5.08 ± 0.19	9.39 ± 0.03	7.3 ± 0.2	7.1 ± 0.2	7.4 ± 0.3	6.7 ± 0.1	10.7	11.47 ± 0.15	2.79 ± 0.17	1.88
Arginine (Arg, R)	5.91 ± 0.07	5.01 ± 0.03	6.5 ± 0.2	5.6 ± 0.6	5.8 ± 0.9	4.1 ± 0.0	7.4	6.00 ± 0.28	2.66 ± 0.09	5.63
Aspartic acid (Asp, D)	8.12 ± 0.16	11.18 ± 0.14	9.1 ± 0.2	12.3 ± 0.5	12.6 ± 0.7	13.4 ± 0.1	8.6	10.14 ± 0.18	3.71 ± 0.25	1.41
Cysteine (Cys, C)	2.82 ± 0.06	1.91 ± 0.05	0.2 ± 0.0	0.7 ± 0.2	0.6 ± 0.0	0.3 ± 0.0	1.3	1.92 ± 0.09 **	N.R.	N.D.
Glutamic acid (Glu, E)	7.87 ± 0.23	13.46 ± 0.32	13.6 ± 0.3	12.8 ± 0.4	12.1 ± 0.2	13.5 ± 0.0	10.3	14.35 ± 0.06	4.67 ± 0.12	7.19
Glycine (Gly, G)	9.73 ± 0.42	5.58 ± 0.08	5.1 ± 0.2	5.8 ± 0.1	5.8 ± 0.2	6.2 ± 0.1	7.0	5.26 ± 0.36	2.25 ± 0.14	10.94
Histidine (His, H) *	1.64 ± 0.01	1.82 ± 0.03	3.0 ± 0.4	2.1 ± 1.2	2.0 ± 0.2	1.6 ± 0.1	1.6	2.18 ± 0.25	0.65 ± 0.13	7.03
Isoleucine	6.20 ± 0.14	4.34 ±	4.0 ± 0.1	4.3 ± 0.1	5.1 ± 0.2	5.7 ± 0.0	3.4	4.49 ±	1.57 ± 0.11	4.53

(Ile, I) *		0.17						0.60		
Leucine (Leu, L) *	3.44 ± 0.06	8.02 ± 0.93	8.4 ± 0.1	8.3 ± 0.1	9.3 ± 0.3	8.3 ± 0.1	8.2	9.80 ± 0.31	3.08 ± 0.09	2.19
Lysine (Lys, K) *	8.14 ± 0.37	5.74 ± 0.06	7.1 ± 0.3	5.5 ± 0.3	5.4 ± 0.4	4.6 ± 1.2	5.4	7.10 ± 0.24	2.03 ± 0.15	5.47
Methionine (Met, M) *	3.30 ± 0.02	2.88 ± 0.02	1.9 ± 0.2	2.8 ± 0.3	2.6 ± 0.1	2.6 ± 0.1	2.6	1.92 ± 0.09 **	0.87 ± 0.12	1.09
Phenylalanine (Phe, F) *	3.83 ± 0.11	4.91 ± 0.05	5.4 ± 0.1	5.6 ± 0.2	5.9 ± 0.1	6.1 ± 0.1	6.0	7.84 ± 0.24 ***	1.95 ± 0.07	2.50
Proline (Pro, P)	N.D.	4.58 ± 0.13	4.2 ± 0.3	4.9 ± 0.2	4.1 ± 0.5	3.7 ± 0.0	5.0	5.16 ± 0.24	N.R.	5.78
Serine (Ser, S)	2.79 ± 0.03	4.87 ± 0.02	5.5 ± 0.3	3.6 ± 0.1	4.1 ± 0.4	4.7 ± 0.1	4.4	3.30 ± 0.17	1.63 ± 0.09	16.25
Threonine (Thr, T) *	3.45 ± 0.04	6.34 ± 0.10	5.1 ± 0.1	4.6 ± 0.4	5.1 ± 0.4	5.2 ± 0.1	5.5	4.56 ± 0.38	1.81 ± 0.13	3.13
Tryptophan (Trp, W) *	N.R.	1.60 ± 0.01	N.R.	N.R.	N.R.	N.R.	0.2	1.15 ± 0.05	0.61 ± 0.01	<0.16
Tyrosine (Tyr, Y)	1.22 ± 0.01	4.38 ± 0.36	3.8 ± 0.3	3.2 ± 0.1	3.4 ± 0.2	3.2 ± 0.1	4.4	7.84 ± 0.24 ***	1.38 ± 0.15	3.44
Valine (Val, V) *	5.17 ± 0.05	6.04 ± 0.06	6.2 ± 0.1	5.7 ± 0.8	6.4 ± 0.2	5.9 ± 0.0	6.7	7.86 ± 0.26	2.27 ± 0.12	4.69

Reference	[52]	[51]	[84]	[84]	[84]	[84]	[56]	[85]	[17]	[86]

* Essential amino acids; ** Methionine + Cysteine; *** Phenylalanine + Tyrosine. Abbreviations: N.R., not reported;

504 **Table 5. Microalgae-derived bioactive peptides and associated bioactivities reported during the period 2013-2018**

Peptide sequence	Source	Generation	Purification and characterization	Observed bioactivity	Additional information	Reference
VLPVP	*Clamydomonas rinhardtii*	*In vitro* gastrointestinal digestion (pepsin, trypsin, and chymotrypsin)	HPLC-DAD	ACE-I inhibitory and antihypertensive	This study developed a transplastomic strain that accumulates antihypertensive peptides. Oral administration of this strain (30 mg/kg of body weight) recuded blood pressure in SHRs.	[87]
YMGLDLK	*Isochrysis galbana*	Trypsin at an E:S ratio of 5.4:100.0 (w/w) at 55.6 ºC	LC-MALDI-TOF/TOF	ACE-I inhibitory	ACE-I IC_{50} was 36.1 µM	[88]
LLAPPER	*Pavlova lutheri*	*In vitro* gastrointestinal digestion (pepsin, trypsin, and chymotrypsin)	IEC followed by LC-ESI-Q-TOF-MS	MMP-9 inhibitory	mRNA and protein expression levels of MMP-9 were reduced in the presence of LLAPPER. Inhibition of MMP-	[89]

					9 was caused by inactivation of NF-κB by reducing IκB-α degradation and phosphorylation	
MGRY	*Pavlova lutheri*	Fermentation by yeast *Hansenula polymorpha* (1%, v/v) at 37 ºC for 12 days	IEC followed by LC-ESI-Q-TOF-MS	Antioxidant and antiproliferative	*In vitro* DPPH, hydroxyl radicals, and hydrogen peroxide IC_{50} values were 0.285, 0.068, and 0.988 mM, respectively. The peptide also demonstrate inhibitory properties agains melanogenesis via melanin content and tyrosinase inhibition in B16F10 melanoma cells	[90]

Abbreviations: ACE-I, angiotensin-I-converting enzyme; DAD, diode array detector; E, enzyme; ESI, electrospray ionization; IEC, ion exchange chromatography; LC, liquid chromatography; MMP-9, matrix metalloproteinase-9; MS, mass spectrometry; NF-κB, nuclear factor κB; S, substrate; TOF, time of flight.

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