REVIEWS

Bioactive Ingredients from Microalgae: Food and Feed Applications

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Abstract

Microalgae (green or blue-green ones) are among the most important organisms on the world, with a versatile and adaptive metabolism. They are able to synthesize bioactive molecules (mainly secondary metabolites such as unsaturated fatty acids, pigments, amino acids) with biomedical applications, enhancement of the nutritional value of food, animal feed/aquaculture, as well with impact on the environmental protection (as raw materials for biofuels). Last decade, by a targeted selection of wild microalgae strains, their cultivation in farms developed in parallel with the bioreactors'products. There are nowadays cultivated at industrial scale especially *Dunaliella salina p.*, *Spirulina platensis*, *Hematococcus pluvialis* or *Chlorella vulgaris* as valuable resources of polyunsaturated lipids and sterols, proteins, polysaccharides, carotenoid pigments, vitamins, minerals with antioxidant, antibacterial or antiviral effects. This review presents a systematic approach on the recent literature data collected the last years, underlying their morphologic and biochemical potential, the advanced technologies to use the bioactive components of different microalgae, new formulations which incorporate, stabilize and store their bioactivity and increase the bioavailability of their components in food and feed. Although their morphologic and biochemical potential is well described, there are presented new data on their bioactive components and formulations using emerging technologies for new application approaches which aims their use as ingredients in added value products for food, cosmetics and feed industry, to be exploited for commercial use. This review updated the last findings in these areas, underlined the reason for the scientific and technological advances, due to their huge potential, not only in environment, energy, but more and more as ingredients for food and feed/ aquaculture products, in the future.

Keywords: biotechnology, bioactive molecules, food and feed application, microalgae

Introduction

Consumers are more and more interested on the nutritional value and health benefits of food products containing natural ingredients with improved sensorial properties. The European Union regulations encourage producers to reduce as much as possible the use of food/feed synthetic ingredients and additives, finding new products with improved nutritional value, bioavailability and functionality. According to the recent studies, the green and blue-green microalgae are considered best sources for several applications, especially in food and feed industry (Plaza et al., 2008; Lee and Marino, 2010; Kovač et al., 2013; Enzing et al, 2014; Herrador, 2016).

This review aims to present recent data (less than 10 years) about the new findings of bioactive components from microalgae to produce new formulations which incorporate, stabilize and store their bioactivity and bioavailability in food and feed.

Microalgae characterization and potential uses

The generic term "algae" describes a diversity of micro- or macroscopic aquatic organisms that can survive and proliferate by photosynthesis, being versatile due to their simple vegetative and reproductive structures, comparatively to higher plants. They are found either in lakes, rivers, estuaries or oceans, soils and in symbiotic relationships with other organisms (e.g.lichens) and may be cultivated by different biotechnologies (Thajuddin and Dhanasekaran, 2016). It is estimated that aprox. 300.000 different algae species may exist, out of them 40.000 species being described. The term "microalgae" includes either prokaryotic cyanobacteria or eukaryotic species found in sea or fresh waters or soil (Richmond, 2004). The large microalgae group includes species from different phyla such as *Chlorophyta sp.* (green algae), *Cyanophyta sp.* (blue-green algae), *Rhodophyta sp.* (red algae), *Cryptophyta sp., Haptophyta sp., Pyrrophyta sp., Streptophyta sp., Heterokontophyta sp.*. Fig.1 shows the microscopic morphology of some representative microalgae.

Microalgae are able to grow either heterotrophically, mixotrophically, or cultivated under photoautotrophic conditions, being able to capture light, to use nitrogen and phosphorus as energy supply for their optimal growth and convert CO_2 to carbon rich biomass, contributing meanwhile to the global oxygen production. It is estimated that about 50 % of the global oxygen is produced by microalgae.

While green microalgae contains mainly chlorophylls and carotenoids as accessory pigments inserted in the photosynthesis complex, blue-green microalgae (cyanobacteria) are rich in phycocyanin, a pigment-protein complex from the light-harvesting phycobiliprotein family (Eriksen, 2008; Rastogi and Sinha, 2009).

The biotechnology and bioprocess engineering of microalgae developed considerably the last decade to obtain a variety of products (Harun et al., 2010). The exploitation of microalgae for bioenergy generation or for H_2, biofuels and CO_2- sequestration, is developing and reviewed recently (Skjånes et al., 2013). Considering their large biodiversity and new developments in genetic engineering, microalgae represents one of the most promising sources for new products and applications, as reported recently (https://algen.eu/node/95). Fig.2 shows the most important economic areas where microalgae proved to have applications, according to recent scientific and technological data.

Microalgae have positive effect on the human nutrition and health, due to their high protein and functional lipids (polyunsaturated fatty acids) (Cardozo et al., 2007; Kovač et al .,2013; Greque de Morais et al., 2015; Priyadarshani and Rath, 2012). Also, more than 40 different species of microalgae are used in fish and shellfish farming (aquaculture) (Sen Roy and Pal 2015), as natural food/feed ingredients, a good source of protein, pigments and PUFAs also for pets and farm animals (Yaakob et al., 2014). Microalgae extracts are used also for tissue regeneration, wrinkle reduction as ingredients to skin and hair care products (Suh et al., 2014; Wang 2015). For wastewater treatments the microalgae biomass may remove nitrate and phosphate ions and further used as a raw material in biogas plants. The components of microalgae wall and storage sugars can be used to produce bioethanol while their lipids can be used to produce biofuels (Skjånes et al., 2013).

Microalgae produce also carbohydrate hydrocolloid polymers that influence water storage and particle adherence in soils or have nitrogen-fixing ability (e.g. cyanobacteria) useful in soil fertilizing. Some microalgae may produce also bioactive compounds that make higher plants more resistant against plant diseases. Considering the general awareness about global warming, as a result of CO2 increase, microalgae are able to fix CO_2 and convert to valuable biomolecules (Herrador, 2016).

Although there are several companies producing microalgae biomass as well as different microalgae-based products, there is still a high demand for further research. Until now only *Spirulina sp.* and *Chlorella sp.* are approved for the direct food use, as well for feed. *Dunaliella sp.* is approved also for the production of pure β-carotene and *Haematococcus sp.* for astaxanthin, both carotenoids being highly reccomended as nutraceuticals or food additives (Spolaore et al., 2006; Santhosh et al., 2016). Other microalgae species like *Chlamydomonas sp.*, *Chlorococcum sp.*, *Scenedescmus sp.*, *Tetraselmis chuii, Nanochloropsis sp.*, *Nostoc sp.* proved to have good applications in aquaculture, in feed, fertilizers or cosmetic

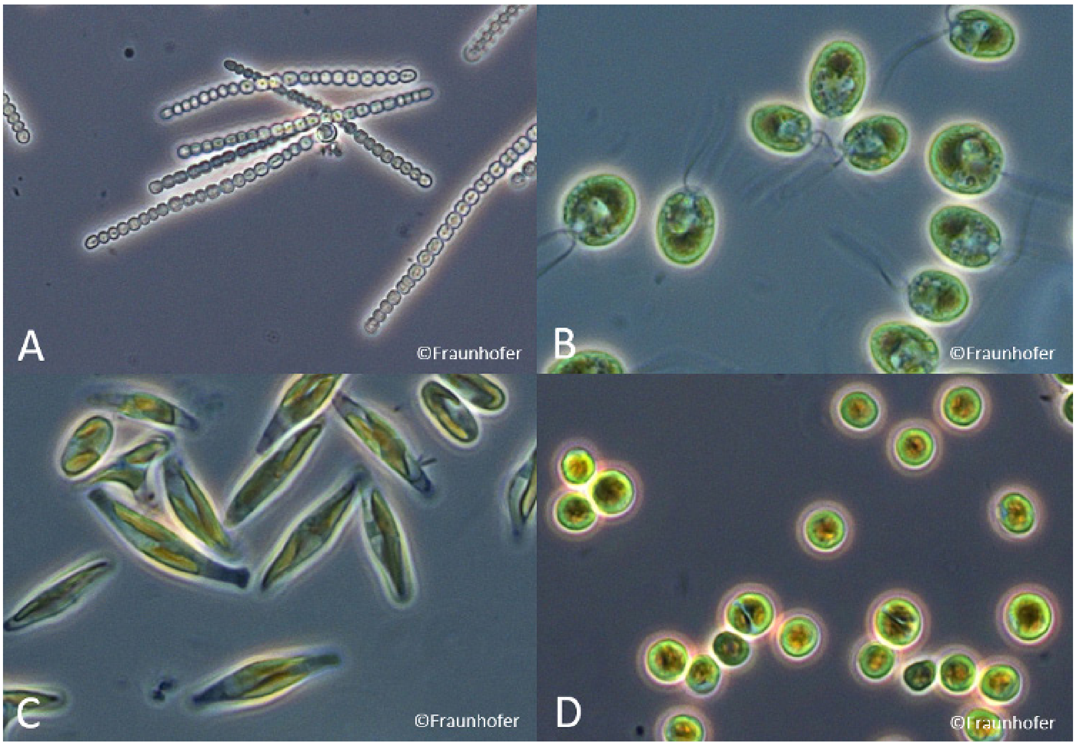

Figure 1. Microscopic photos (magnification 1000x) of different microalgae species. A: *Anabaena sp. (cyanobacteria)*, B: *Chlamydomonas reinhardti sp. (green microalgae)*, C: *Phaeodactylum tricornutu sp. (green microalgae)*, D: *Chlorella sorokiniana sp. (diatomee)* (from Derwenskus and Holdmann, 2016).

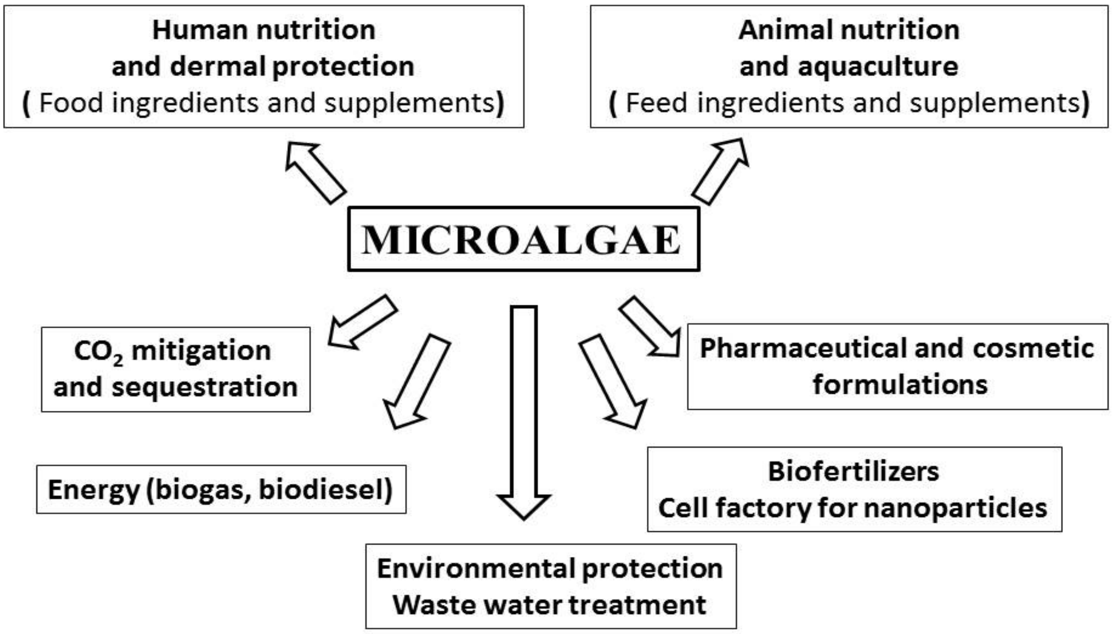

Figure 2. The diversity of microalgae applications: involvement in energy production, environmental protection and life sciences with extensive applications in food and feed industry.

Table 1. Bioactive components and main molecules found in microalgae.

Bioactive components	**Main molecules**
Storage lipids (TAGs)	Triacyl glycerides of fatty acids (mainly C16-C18), up to 70% of dry mass
Membrane lipids	Phospho- and glycolipids with unsaturated fatty acids like DHA(C22:6), EPA(C20:5), ARA(C20:4), GAL(C18:3) (aprox. 7% of dry mass)
Storage Carbohydrates	Alpha and beta-glucans, incl. inositol, up to 50% from dry mass
Proteins	All types of amino acids, up to 50% from dry mass
Macro- and oligominerals	Sodium, Potassium, Calcium, Magnesium, Iron, Zinc, Manganese salts
Secondary metabolites	Carotenoid and chlorphyll/phycocyanin pigments, phytosterols, vitamins (vitamin E, thiamine, cobalamine, biotin), antifungal, antiviral, antibacterial molecules. Each, 1-5% of dry mass

ingredients, although not yet considered as Generally Recognized as Safe (GRAS).

Due to the high diversity of microalgae species, and their growth conditions, their specific bioactive components and the emerging extraction procedures and formulations, their real potential is not yet fully valorized until now, as food and feed ingredients, their safety aspects being not yet well documented.

Microalgae bioactive components and their activity

Green or blue-green microalgae proved to have the capacity to synthesize essential biomolecules, from inorganic sources used as nutrients for their survival and secondary metabolites for the adaptation to the environment and defense against pathogens. The high biodiversity of microalgae is correlated with a large variety of specific bioactive compounds, many of them being still unknown.

Table 1 include the most important nutrients and secondary metabolites from microalgae, with examples of main molecules.

Details about the specific composition of many microalgae were reported recently (Kovač et al., 2013; Sen Roy and Pal, 2015, Greque de Morais et al., 2015).

The most common lipids in microalgae represents around 22% and include triacylglycerols (TAGs), phospholipids and glycolipids or phytosterols which contain fatty acids ranging from C12 to C24, often with mono- (MUFAs) and polyunsaturated (PUFAs) fatty acids C_{16} and C_{18}.. Storage lipids (TAGs), generally accumulate in lipid vesicles called oil bodies in the cytoplasm (Sayeda et al., 2014).

In microalgae, carbohydrates represent around 12-17%, e.g. alpha and beta glucans. In some green microalgae (such as *Chlorella sp*.), polysacharides or exopolysaccharides can be found in the cell surrounding the glycocalyx and as extracellular polymers, having a great prebiotic potential.

The eicosapentaenoic acid (EPA) has been used for the treatment of inflammatory and heart diseases, asthma, arthritis, migraine or headache and psoriasis. Similarly, docosahexaenoic acid (DHA) proved some health benefits in cancer treatment, AIDS (acquired immune deficiency syndrome) or heart disease. Both PUFAs prevent hypercholesterolemia, there have antioxidant, detoxifying capacity and proved to have good immune stimulatory effects (Sen Roy and Pal, 2015, Greque de Morais et al., 2015). Microalgae phytosterols (sitosterols, campesterol and stigmasterol) prevents as well the deposition of blood cholesterol and prevent the onset of cardiovascular disorders, being precursors of vitamin D_2 and cortisone (Luo et al., 2015).

It was reported that the biomass of Spirulina (*Arthrospira platensis)* promotes the growth of probiotic bacteria, such as *Lactobacillus casei*, *Streptococcus thermophilus*, *Bifidobacteria* and *Lactobacillus acidophilus* along with suppression of harmful bacteria such as *Proteus vulgaris, Bacillus subtilis* and *Bacillus pumulis*. Such prebiotic properties of microalgae are caused not only by the presence of polysacharides and lignin, but also by monosaccharides, enzymes, PUFAs, peptides, polyphenols and superior alcohols (Greque de Morais et al., 2015; Raposo et al., 2013, 2016).

Many microalgae are important sources of proteins (up to 60%) including all types of amino acids as well non-typical ones like microsporine-like (MAAS) (Waghmare et al., 2016). Certain species of microalgae such as *Chlorella vulgaris* have the ability to produce higher levels of starch, especially induced by nutrient limitation (Dragone et al 2011), its polysaccharides being responsible for the antiviral activity (Santoyo et al 2010). Lectin, a protein extracted from microalga BTM 11 was able to inhibit hepatitis C virus and to suppress the activity of *Staphylococcus aureus*, *E. Coli*, and *Salmonella typhii* (Cardozo et al., 2007).

Cell and growth media extracts of various unicellular algae (such as *Chlorella vulgaris, Chlamydomonas pyrenoidos* or *Tetraselmis chuii*) showed *in vitro* antibacterial properties against Gram-positive and Gram-negative bacteria, as well *in vitro* antifungal activities, while other studies showed the antiviral activity of extracts from *Chlorella vulgaris* against herpes virus*.* (Maligan et al., 2011; Priyadarshani and Rath, 2012).

Carotenoid pigments are a class of secondary metabolites with multiple beneficial effects (Socaciu, 2008). Natural β-carotene is extracted mainly, at industrial scale from *Dunaliella salina* and has superior properties comparing with the synthetic one. Other studies showed that β-carotene has beneficial effects in controlling cholesterol, reducing the risks of heart disease and macular degeneration, due to its strong antioxidant effect (Priyadarshani and Rath, 2012; Skjånes et al., 2013). The last years, astaxanthin obtained from *Hematoccocus pluvialis* has gained popularity and became a commercial product, due to its health benefits such as antioxidant activity, antidiabetic and anti-inflammatory properties (Papadaki et. al 2017). The high chlorophyll content of microalgae have chelating activity and helps the liver and ulcer recovery, increases blood haemoglobin and stimulates the cell growth (Harun et al., 2010; Jiang et al., 2004).

Other microalgae ingredients showed anti-tumor activity, e.g. borophycin, a cytotoxic molecule extracted from blue-green *Nostoc linckia* and *Nostoc spongiaeforme* , calcium-spirulan from *Spirulina sp.*, as well polysaccharides from *Porphyridium cruentum* (Rastogi and Sinha, 2009; Cardozo et al., 2007; Raposo et al., 2013)

Extracts of *Phaeodactylum tricornutum* and *Chlorella stigmatophora* showed anti-inflammatory properties along with some analgesic and antioxidant activities (Raposo et al., 2013), being active in treating gastrointestinal cancer or to keep a good balance of female health (Kim and Pangestuti, 2011).

Microalgae formulations used for food and feed applications

The simplest technology to store and use the microalgae extracts and bioactive molecules is the heat-assisted drying by advanced technologies like spray drying, or fluid bed drying. Freeze drying is also used, but the costs of formulations are too high to be upgraded at large scale. Spray-drying became the most common high temperature-assisted microencapsulation procedure used for the protection of microalgae oil containing unsaturated fatty acids. As a common carrier, sodium caseinate is used to prevent the degradation of labile components (Bao et al., 2011). Until now, spray dried powders are used as such in commercial products or mixed with excipients in capsules or in tablets, e.g. commercial products of *Chlorella* or *Spirulina* on the market (Henrikson, 2010; Priyadarshani and Rath, 2012).

Considering that bioactive components like carotenoids are labile to light and temperature, protective, innovative microencapsulation procedures were also developed to incorporate such molecules, in micro- and nanoemulsions, liposomes or cyclodextrins. Recently (Affandi et al., 2011) it was reported the incorporation, stabilisation, protection, solubilisation of asthaxanthin in nanoemulsions, improving its bioavailability up to 3 months storage. Biosurfactants like phospholipids from *Spirulina sp.* extracts were also used to produce nanoemulsions (Carvalho et al., 2015) stable for 30 days, having increased solubility and permeability though gastro-intestinal tract, as proved by *in vitro* studies (Hemalatha et al., 2013, 2015). Similarly, ß-carotene nanoemulsions showed good physical stability (Silva et al., 2011).

De Assis et al., (2014) reports the development and characterization of nanovesicles containing phenolic compounds extracted from *Spirulina Strain LEB-18* and *Chlorella pyrenoidosa*, using the film hydration methodology. Solid lipid nanoparticles proved to be good carriers to improve the physical and chemical properties of microalgae oil (Wang et al 2014) as functional ingredients for food products. *Chlorella vulgaris* cells were also used as carriers to incorporate curcumin increasing its stability (Jafari et al., 2016).

Microalgae-based ingredients for aquaculture, feed and food

The preferred protein ingredient of feed in aquaculture is still fishmeal, but nowadays microalgae are more and more used worldwide as an alternative protein-rich source. In fish feeding trials, many types of microalgae biomass have been found to be useful for increasing fish growth and to improve the bioavailability, physiological activity, stress response, starvation tolerance, disease resistance and carcass quality (Sen Roy and Pal, 2015). Beside the protein content, the flesh pigmentation of fish is increased by carotenoids (beta-carotene with provitamin A activity, astaxanthin, lutein), chlorophylls, phycocyanin being essential for improving the taste and antioxidant capacity of the fish flesh.

The commonly used microalgae in aquaculture are *Chlorella* sp., *Tetraselmis sp., Isochrysis sp., Pavlova sp., Phaeodactylum sp., Chaetoceros sp., Nannochloropsis sp., Skeletonema sp.* and *Thalassiosira* sp. (Priyadarshani and Rath, 2012)

Algae farming have gained a lot of interest and continued to develop due to the increased need for natural feed ingredients also for farm animals, due to their high concentrations of proteins and pigments, minerals, unsaturated fats. Several nutritional studies, recently reviwed, show the high nutritional capacity of microalgae in the diet of pigs, cows, sheep, chicken and other domestic animals (Yaakob et al., 2014).

The algal biomass proved to be also a promising source of food ingredients or supplements, in the search of functional ingredients to replace the synthetic ones. The most relevant microalgae used as raw materials for food applications are are *Chlorella vulgaris, Haematococcus pluvialis*, *Dunaliella salina* and *Spirulina maxima* (Plaza et al., 2008; Enzing et al., 2014; Lee and Marino, 2010; Priyadarshani and Rath, 2012). Table 2 summarise the most important biomolecules found in microalgae with increasing applications in food and feed industry, as reviewed recently.

Besides *Dunaliella salina,* also *Chlorella zofigiensis* and *Muriellopsis sp.* are considered suitable candidates for carotenoid production (Christaki et al., 2012, Kaur et al., 2009). Large scale carotenoid production from microalgae refers mainly to astaxanthin and β-carotene from *Haematococcus pluvialis* and *Dunaliella salina,* respectively. β-carotene and derivatives are used as a food colorants (E160) providing the yellow colour to margarine, to enhance the colour of the flesh of fish and the egg yolk, and to improve the health and fertility of grain-fed cattle (Priyadarshani and Rath, 2012).

Other types of colours are also used, to mention chlorophylls and phycobilins pigments. Major producers of phycobiliproteins are the *Porphyridium*, *Spirulina platensis, Spirulina fusiformis, Gleotrichia natans, Nostocsp, etc* (Kaur et al., 2009).

Mostly, the major producers of microalgae biomass are located in Asia and USA to mention Hainan Simai Pharmacy Co. (China) Earthrise Nutritionals (California, USA) Cyanotech Corp. (Hawaii, USA) Myanmar Spirulina factory (Myanmar) for *Spirulina sp. (Arthrosphira)*, Taiwan Chlorella Manufacturing Co. (Taiwan) and Klötze (Germany) (for *Chlorella sp.*), Cognis Nutrition and Health (Australia) (for *Dunaliella salina*) (Priyadarshani and Rath, 2012)

Many companies worldwide produce foods, feeds and fertilizers based on microalgae are Algaspring, Simris Alg, Bggy power, Metabolium, Ecoduna, Algomed, Neoalgae, Phytolutions, Olmix SA. (Herrador, 2016), but also pharmaceutical companies are increasingly using tablers and capsules of astaxantin and beta-carotene.

Table 2. Biomolecules from microalgae useful as food and feed/aquaculture supplements/ ingredients

Biomolecules	Use	Strains of microalgae
Astaxanthin	vitamin E (antioxidant activities) pigmentation / aquaculture	*Haematococcus* , *Chlorella zofingiensis, Chlorococcum* sp.,
Lutein	natural food colorant	*Chlorella* sp., *Scenedesmus* sp., *Muriellopsis* sp., Tetraselmis chuii
Beta-carotene	pigmentation /aquaculture pro-vitamin A (retinol)	*Dunaliella salina, Tetraselmis chuii, Nanochloropsis gaditana, Chlamydomonas sp., Chlorella sorokiniana*
Chlorophyll Phycocyanin	pigmentation/aquaculture food supplement	*Spirulina* sp.
Polyunsaturated fatty acids (PUFAs)	food supplement	*Nannochloropsis sp.*
Beta-1,3-glucan	immunostimulant	*Chlorella* sp.

Conclusion

The study of wild or cultivated cicroalgae (green or blue-green) and their bioactive components became the last decade more intense, diversified and economically feasible. The high metabolic versatility, their adaptation to different environmental conditions, and their capacity to synthesize valuable bioactive molecules is more and more documented. Although their morphologic and biochemical potential is well described, still new technologies and new applicative approaches for their use as ingredients in added value products for food, cosmetics and feed industry, exploited for commercial use is scarce. This review updated the last findings on microalgae characterization, on their bioactive components and their activities, formulation of new ingredients by emerging technologies. It is underlined the reason for using microalgae in diverse areas, considering their huge potential, not only in environment, energy, but more as ingredients for food and feed/ aquaculture products, in the future.

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