Functional Ingredients from Microalgae

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Abstract

A wide variety of natural sources are under investigation to evaluate their possible use for new functional ingredients formulation. Some records attested the traditional and ancient use of wild harvested microalgae as human food but their cultivation for different purposes started about 40 years ago. The most popular species are *Arthrospira* (traditional named, *Spirulina*), *Chlorella* spp., *Dunaliella* spp. and *Haematococcus* spp. Microalgae provide a bewildering array of opportunities to develop healthier food products using innovative approaches and a number of different strategies.

Respect to other natural sources of bioactive ingredients, microalgae have many advantages such as their huge biodiversity, the possibility to grow in arid land and with limited fresh water consumption and the flexibility of their metabolism, that could be adapted to produce specific molecules. All these factors led to very sustainable productions making microalgae eligible as one of the most promising food for the future, particularly as source of proteins, lipids and phytochemicals. In this work, a revision of the knowledge about the use of microalgae as food and as a source of functional ingredients has been performed. The most interesting results in the field were presented and commented, focusing on the different species of microalgae and to the activity of the nutritionally-relevant compounds. A summary of the health effects obtained together with pro and cons in the adoption of this natural source as functional food ingredients in terms was also proposed.

KEYWORDS: Microalgae, food sources, bioactive compounds, functional ingredients;

Introduction

There is increased consumers awareness that healthy diet is fundamental to prevent chronic diseases (cardiovascular problems, osteoporosis and cancer among others). Moreover, the social need to reduce the prescription of medications due to the increasing cost of healthcare, as well as the steady enhancement in life expectancy, also promoting the interests of companies and governmental agencies towards a large use of functional ingredients.[1] A food ingredient is considered "functional" if, besides its nutritious capacity, it has a scientifically proven benefit for one or more functions of the human organism, improving the state of health or well-being or reducing the risk of disease.[2]

Functional food concept was developed in Japan in the early 80s [3]; later on in the United States, the Food and Drug Administration (FDA) released statements about the relationship between the dietary intake of some foods or nutrients and the prevention of several diseases. [2] The European Commission under the IV Framework Program promoted the project FUFOSE (Functional Food Science in Europe) to get scientific support to a regulatory action about health claims in Europe. [4] The successive release of the present health claim regulation including the procedure for their acceptance by European Food Safety Authority (EFSA) further increased the interest of the food companies about new natural sources for functional ingredients [5] also including some algae and, even more interestingly, microalgae. [6]

In some countries (Germany, France, Japan, USA, China, Thailand), food companies have already started to market functional foods containing microalgae and cyanobacteria. [7] Food safety regulations for human consumption are the main constraint for the biotechnological exploitation of microalgal resources, however successful cases already exists. In 2002 the use of the marine diatom *Odontella aurita* by Innovalg (France) as a novel food was approved, following EC Regulation 258/97. Currently some microalgae-related health claims were evaluated by EFSA: among them the most interesting regarded *Chlorella pyrenoidosa* for antoxidative activity and *Spirulina* to improve glucose management. [8] A series of claims regarding eye health, oxidative balance, cardiovascular system and connective tissue and joints for *H. pluvialis* astaxanthin were recently rejected, however they will be likely resubmitted soon. [9]

In this work, a revision involving research for functional food ingredients from microalgae is presented. The most interesting results in this field are presented and commented focusing on the main cultivated species of microalgae and the activity of the compounds obtained.

Microalgae biology

Microalgae are a huge group of photosynthetic microorganisms from freshwater, brackish and marine systems, typically unicellular and eukaryotic. Some of the most significant groups of algae are green algae (Chlorophyceae), red algae (Rhodophyceae), diatoms (Bacillariophyceae), and brown algae (Phaeophyceae). Although cyanobacteria (blue green algae) are classified to the domain of Bacteria, being photosynthetic prokaryotes, often they are considered as “microalgae”. [10]

Eukaryotic microalgae can be either autotrophic or heterotrophic. Autotrophic microalgae require only inorganic compounds such as CO_2, N, S, P and light as an energy source for their growth and development. They convert captured solar energy into biomass (photosynthesis) with an efficiency that generally exceed those of terrestrial plants (3 % reported for marine microalgae against 0.2–2 % for terrestrial plants). [11] Some photosynthetic microalgae are mixotrophic, meaning they are able simultaneously to perform photosynthesis and to catabolize exogenous organic nutrients, but some species are not truly mixotrophs, but have the ability of switching between phototrophic and heterotrophic metabolisms, depending on environmental conditions. [12, 13]

With these simple growth requirements, microalgae can sustainably generate lipids, proteins, and carbohydrates at a large scale, offering promising environmentally friendly alternatives to the current consumer products.

Microalgae active compounds, such as carotenoids, phycobilins, fatty acids, polysaccharides, vitamins and peptides, can be used in feed, food, nutraceutical, cosmetics and pharmaceutical industries. [14]

The chemical composition of microalgae showed to be greatly variable also in agreement with some environmental factors, such as water temperature, salinity, light, nutrients availability and also to the production technologies. In outdoor cultivation most of the environmental parameters vary according to the season stimulating or inhibiting the biosynthesis of several nutrients; while in close photobioreactor systems the cultivation occur in well controlled conditions, but it is usually more expensive. [15, 16]

Microalgae cultivation for food production

Commercial large-scale production of microalgae started in the early 1960s in Japan with the culture of *Chlorella* used as a food additive, followed by the cyanobacterium *Arthrospira*. Only after 1980 large-scale algae production facilities were established in Asia, India, USA, Israel and Australia. [17] Commercial microalgae farms for value-added products are usually conducted in open ponds under autotrophic conditions in location having all the year relatively warm temperature or in fermenters under heterotrophic conditions.

Microalgae showed some important advantages respect to conventional land plants: they have much higher biomass productivities (around 10–50 times higher) and CO_2 fixation rate, moreover arid or low quality agricultural land is required for their cultivation. [18, 19] Although microalgae cultivation is carried out in aquatic environment, they use less water than terrestrial crops, so the freshwater consumption is strongly reduced. Furthermore, microalgae may be cultivated in brackish and sea water avoiding herbicide or pesticide application, and reducing the needs of external nutrients (NH_4, NO_3 and P). [20, 21]

Currently the microalgae biomass production is still in a developing phase and a lot of work is necessary to enhance the productivity and to reduce the production cost.

The most challenging problems for the microalgae production industry include capital and operating cost, difficulties in controlling the culture conditions, contamination of bacteria or unwanted algae, unstable light supply and weather. Several strategies have been proposed to cope with these difficulties. First of all it is important to select a good microalgae/cyanobacteria strain that are rich in the target products, can tolerate temperature changes, high salinity and/or alkalinity. These strains can easily become predominant in the culture environment, thus greatly reducing contamination problems.

Identifying preferable culture conditions for improving the production as well as designing efficient and cost-effective microalgae cultivation systems are also critical points. [22] In particular, the enrichment of different components (such as lipids, proteins or pigments) in microalgae biomass requires different cultivation conditions and operational strategies. Under stress conditions microalgae can change their metabolic pattern and strategies, in order to face the difficulties. [23]

In this way microalgae are induced to synthesize and produce various secondary metabolites, modifying also the quantity of representative primary metabolites (fat, carbohydrate and protein).

Microalgae are very useful for the production of secondary metabolites some of them have particular interest because they constitute high-value products with several applications. [24]

However under stress conditions the decrease or the arrest of growth rates and consequently the decrease of the total production and productivity was observed. In some cases it was possible that the productivity of an accumulated compound cannot reach the productivity under regular conditions because of the decrease in the growth rates. [25] This negative effect might be reduced applying a microalgae cultivation in multiple-stage process, in which in each stage optimum or appropriate conditions are adopted. [24] The topic of the optimization of a desirable compound under stress conditions is of particular significance and more research is needed.

Main potential applications

Microalgae market is largely to be explored yet, although the use of microalgae as a food source or supplement has occurred for centuries. [26] Nowadays, the utilization of high-value compounds derived from microalgae is restricted to only a few species of microalgae as summarized in **Table 1**. The freshwater green algae *Chlorella* and *Scenedesmus* and especially the cyanobacteria *Arthrospira platensis* and *maxima* are preferred for the use in human food, animal and fish feed, partially because of their high protein content (50–60% of dry biomass) and nutritive value. [7] Cyanobacteria, but also some green microalgae such as *Chlorella* and *Dunaliella*, showed an interesting polysaccharide fractions and are used as dietary supplements or pharmaceuticals. [27] A few species of diatoms and dinoflagellata are a good source of long chain polyunsaturated fatty acids (LC-PUFAs). [28, 29]

Among the microalgae pigments, carotenoids and phycobiliproteins showed to be the most important pigments from a commercial food perspective. [30,31]

Microalgae food Ingredients

Lipids

Fatty acids from microalgae are a reliable option to partly substitute the currently used vegetable oils. In many cases the percentages of linoleic (C18:2) and alpha/gamma-linolenic acids (C18:3) were higher than rape seed, soy or sunflower oils, while in other case microalgal oils with high palmitic acid (C16:0) useful for their food structuring properties could be obtained. [32]

The main point of interest about microalgal oil is the possibility to obtain very high concentrations of long chain polyunsaturated fatty acids (PUFAs) as eicosapentaenoic acid (EPA, 20:5, ω-3) and docosahexaenoic acid (DHA, 22:6, ω-3) which are the most interesting as functional ingredients.

The consumption of EPA and DHA supplements has been shown to prevent cardiovascular diseases and inflammation, [33] to improve brain function and development of nervous system in infants. [34-38]

The main source of EPA and DHA for human nutrition comes now from marine fish such as mackerel, cod, salmon and mullet.[39, 40] However, fish oil is not suitable for vegetarians and the fish smell is often a problem for the use of fish oil as food ingredient. Moreover, fish stocks are more and more limited [41,42] and the presence of some chemical contaminants such as mercury pushed companies to search for alternative sources.[43, 44]

Alternative EPA and DHA sources can be bacteria, fungi and plants that are all currently studied for commercial production. Unfortunately, fungi require an organic carbon source and usually showed slow growth rate, [45] and plants, beside the need arable land, should be genetically modified to produce long chain PUFAs.[46]

Instead, microalgae are the primary source of EPA and DHA in the marine food chain and usually their growth rate is high under a variety of autotrophic, mixotrophic and heterotrophic culture conditions.[47] The ω-3 fatty acid content of numerous microalgae strains have been studied (**Table 2**).[48-61] Strains from the genera *Phaeodactylum*, *Nannochloropsis*, *Thraustochytrium, Schizochytrium* [62] and *Koliella antartica* [63] have demonstrated high accumulation of EPA and/or DHA. *Phaeodactylum tricornutum* [53] and *Nannochloropsis sp*. showed an EPA content of up to 39% of total fatty acids.[64] Up to now FDA only approved docosahexaenoic acid (DHA) additive for infant formula: the DHA oil is produced from *Crypthecodinium cohni* or *Schizochytrium sp.* by Martek Biosciences.[65]

Carbohydrates from microalgae

Algae showed a relatively high photoconversion efficiency therefore they could accumulate high concentration of carbohydrates (more than 50% dry weight),[66] having relevant biological functions in algae cells, mainly as storage, protection and structural molecules.[67] The use of microalgae as a sustainable source of some carbohydrates is an opportunity which should be further explored. The composition of storage carbohydrates is closely linked to the species; cyanobacteria synthesize glycogen (α-1,4 linked glucan), red algae floridean starch (hybrid of starch and glycogen) and green algae amylopectin-like polysaccharides (starch). [68-70]

Sugars such as arabinose, xylose, mannose, galactose and glucose could be found together with less common sugars such as rhamnose, fucose and uronic acids. [71, 66]

Several microalgal species, such as *Porphyridium cruentum* (40–57 %), *Spirogyra sp*. (33–64 %), etc., naturally presented a high carbohydrate content, [72] and as mentioned for lipid, microalgae carbohydrate content can be modulated by cultivation and environmental factors, as nutrient starvation/limitation, salt stress, light intensity and temperature. The type of carbon source and metabolism process (i.e. autotrophic, heterotrophic and mixotrophic) is a major factor influencing the sugar content.

As summarized in **Table 3** microalgae polysaccharides, in particular those containing sulfate esters (sulphated exopolysaccharides), showed interesting applications. [73-85] Fucoidan, carrageenans and agarans were gaining wide attention due to their pharmacological abilities with potential medical applications. [74-83]

Microalgal proteins

Already during the 1950s some species of microalgae were proposed as innovative source of proteins. [86] This interest was related both to the high percentage of proteins in the microalgal biomasses and to the favorable amino acid profile as shown in **Table 4**. [87-90]

Many of the biological activities found for microalgae as antioxidant, [91, 92] antihypertensive, [93] immune-modulatory, [94] anticancer, [95] hepato-protective [96, 97] and anticoagulant, [98] are associated both with the whole proteins and with protein hydrolysates or peptides, that can be obtained with different enzymatic and fermentation processes.

Three species were most commonly used for protein production: *Chlorella* about 55% protein content, *Spirulina* (*Arthrospira*) about 65% and *Dunaliella*, about 57%. [99] The functional properties of defatted microalgae biomass, including *Porphyridium cruentum, Nannochloropsis spp*. and

Phaeodactylum tricornutum have been comparatively studied with soybean flour. [100] *Nannochloropsis spp*. and *P. tricornutum* showed higher compositions of hydrophobic and hydrophilic amino acids than soybean flour. [101]

A special attention was dedicated to *Spirulina* which has been one of the most investigated microalgae specie because of the good qualities and quantities of protein (60%–70% of dry weight). *Spirulina* proteins are rich of essential amino acids and they showed a good digestibility. [17] So it has been used for a long time as protein supplement and to manufacture healthy foods. ONU General Assembly (Second Committee, Agenda item 52) initiated a revised draft resolution about the use of *Spirulina* to combat hunger and malnutrition and to achieve sustainable development" which was submitted by: Burundi, Cameroon, Dominican Republic, Nicaragua and Paraguay. As a follow-up on this resolution, FAO was requested to prepare a draft position on *Spirulina,* that was presented in 2008. FAO underlined that "*Spirulina appears to have considerable potential for development, especially as a small-scale crop for nutritional enhancement, livelihood development and environmental mitigation*" presenting also other numerous advantages. [17]

Also in the case of *Spirulina* its nutritional quality was very much dependent by the species of microalgae, by the season of harvesting and by the accurateness of the down-stream process. [102]

Phycobiliproteins are a peculiar microalgae protein group; they are photosynthetic accessory pigments, including phycoerythrin, phycocyanin, allophycocyanin and phycoerythrocyanin. *Arthrospira spp,. Synechococcus spp*. (blue-green algae) [103] and *Porphyridium cruentum* (red algae) are the most interesting algae that are presently used to extract phycobiliproteins. [101] These particular groups of proteins have been used as natural colorants in foods such as chewing gums, dairy products, ice creams and candies [104]. They have been marketed in a variety of nutraceutical products such as tablets, capsules, [100]etc. showing a variety of functional activities, such as antioxidant, neuroprotective, anti-inflammatory, hepatoprotective, hypocholesterolemic and anticancer. [105]

Microalgae bioactive peptides may be produced through solvent extraction, enzymatic hydrolysis, and microbial fermentation of the biomass. Food and pharmaceutical industries preferred enzymatic hydrolysis method because of the lack of residual organic solvents or potential toxic compounds in the products. Some bioactive peptides have demonstrated multifunctional activities based on their structure and other factors including hydrophobicity and charge or microelement binding properties. [106, 107]

Micronutrients

Vitamins

Thanks to their autotrophic and unicellular nature, microalgae biomass can be a valuable source of all essential vitamins (A, B1, B2, B6, B12, C, E, nicotinate, biotin, folic acid and pantothenic acid). In terms of vitamin content they are comparable to bakery yeast and meat and they are superior to vegetable commodities, such as soybeans and cereals. [90]

Microalgae vitamin content is correlated with the genotype, the growth phase, the nutritional status of the alga and the light intensity. Moreover, post harvesting treatments as drying processes could have a considerable effect on vitamin content, [108, 109] especially on the heat unstable vitamins as B1, B2, C, and nicotinic acid.

The presence of Vit B12 in Chlorophyceae or Rhodophyceae is rather surprising, since it was accepted that these algae were not able to synthesize this vitamin. This vitamin probably derives from bacteria closely associated or grown together with the algae (phycosphere). [90]

Carotenoids

Over a hundred different carotenoids have been identified from microalgae [110, 111,] but, as emphasized by several authors who reviewed pigments of specific taxonomic groups [111- 116], algal accessory pigments and in particular carotenoid composition was highly variable within taxonomic groups. The chemical-physical stability of algal carotenoids was related to the natural species distribution: carotenoids from thermophilic algae were less temperature sensitive [117] thus making them more attractive for commercial applications.

The intrinsic antioxidant activity of carotenoids constitutes the basis for their protective action against oxidative stress; however, not all biological activities claimed for carotenoids relate to their ability to inactivate free radicals and reactive oxygen species. According to Prasanna et al. [118], specific groups of carotenoids had activities against specific types of cancer and were also able to stimulate the immune-system, therefore potentially utilized in more than 60 life-threatening diseases— as various form of coronary heart diseases, premature ageing and arthritis [119].

The main carotenoids produced by microalgae are β -carotene from *Dunaliella salina* and astaxanthin from *Haematococcus pluvialis*. *Dunaliella* had the highest content of 9-*cis* β-carotene among all natural sources studied [120-123] and β-carotene rich *Dunaliella* powder has been marketed in many countries since the 1980s. Microalgae natural β-carotene is preferred by the health market and consumers, because it is a mixture of *trans* and *cis* isomers better adsorbed by living organisms than the all-*trans* form obtained via chemical synthesis. [124] β -carotene is routinely used in soft-drinks, cheeses and butter or margarines. [125] Also ε- and α carotenes are produced by some Cyanobacteria, while common algal xanthophylls include astaxanthin, fucoxanthin, and zeaxanthin, which presented commercial value [114].

Carotenoids are important natural dyes at low concentration: canthaxanthin, astaxanthin and lutein from *Chlorella* have been widely used as pigments in particular added to salmon, trout and poultry feed to intensify the reddish color of meat and yolk. [31, 126, 127]

Numerous benefits have been claimed for astaxanthin: it enhanced eye health, improved muscle strength and endurance and it protected the skin from premature ageing, inflammation and UV-A damage. Many positive features such as growth, vision, reproduction, immune function, and regeneration were reported also in animal nutrition [128-131] therefore FDA approved astaxanthin as a feed additive for use in the aquaculture industry in 1987, and in 1999 astaxanthin was further approved for use as a dietary supplement .[127] Astaxanthin natural sources are: microalgae, yeast, shrimp, krill and plankton. Among the natural sources of astaxanthin, crustacean exoskeletons and yeast *Xanthophyllomyces dendrorhous (Phaffia rhodozyma)* are not utilized because the former is in limited quantity and showed a low astaxanthin content, while the latter had an astaxanthin content (4-25 g kg^{-1}) much lower than that found in microalgae. [132]

The ketocarotenoid astaxanthin can be found in the microalgae *Haematococcus pluvialis*, *Chlorella zofingiensis* and *Chlorococcum sp.*. Maximal levels of astaxanthin in *C. zofingiensis* was about 0,3–0,6 % dry weight [133, 134], that was lower than those reported in *H. pluvialis* (4–5% of cell dry weight) [135], but the fast growth exhibited by this strain and the high cell population achievable in culture can compensate for the lower concentration of bioactive compound, making *C. zofingiensis* as an attractive possible candidate for the mass production of astaxanthin.

H. pluvialis is a freshwater green alga that can synthesize and accumulate astaxanthin under oxidative stress and it is the one that accumulates it to the highest levels, so it is now cultivated at large scale by several companies using distinct approaches, due to the difficult to synchronize the culture cellular phases and to applied different cultivation stages.

The *H. pluvialis* astaxanthin presented a yield between ~70 – 94% using different extraction methods.[136, 137] Up to now, no efficient and cheaper method has been achieved due to its thick cell wall hampering solvent extraction of astaxanthin.

The world leader in microalgae technology Cyanotech Corporation, produced BioAstin® Natural Astaxanthin and Hawaiian Spirulina Pacifica®. These products are FDA approved and Generally Recognized as Safe (GRAS) for use in food products.

In addition, Roche corporation has begun a large-scale production of synthetic astaxanthin, which consists of a mixture 1:2:1 of isomers (3S, 3S'), (3R, 3S'), and (3R, 3R) respectively, since 1990.[138]

Microalgae Health effects

Extensive studies have been devoted to the evaluation of microalgae health benefits on an array of conditions including hypercholesterolemia, hyperglycerolemia, cardiovascular diseases, inflammatory diseases, cancer and viral infections. A number of known healthy phytochemicals present in microalgae and already investigated from other vegetables source have been studied, however data on microalgae biomass are scarce and underline the importance to carry out extensive studies. For example EFSA rejected two health claim requests regarding *Chlorella pyrenoidosa* for antoxidative activity and *Spirulina* to improve glucose management because of lack of data regarding human clinical studies. In this review the main studies of microalgae bioactive metabolites, whole biomasses and crude extracts performed on culture tissues, animals and humans are listed in **Table 1S**[139-157, 75, 80, 85, 95]**, 2S** [158-201, 85] and **3S** [202-220, 80, 190], respectively, which are provided as supplementary material. From these data **Table 5** was constructed: here the main findings related to the health benefits were grouped according to the main health outcome and the relevance of the available in vitro, animal and human studies were highlighted.

In vitro experiments (**Table 1S**) were carried out using various cell lines; they consistently demonstrate the healthy effect of various microalgae species; the species most studied were *Chlorella* and *Arthrospira,* showing the abilities to modulate several biochemical pathways related to anticancer, antioxidant, antimicrobial, anti-inflammatory and immunomodulatory activities. In many cases, particularly about anticancer and antimicrobial activities also convincing evidence have been obtained on animals (see **Table 2S and Table 5**). Animals studies, besides the activities of tests in vitro, showed other important health effects of *Chlorella* and *Arthrospira* as hepatoprotective, antihyperglycemic and antihyperinsulinemic.

Few human studies have been performed on microalgae as a whole biomass (see **Table 3S**). Most of them suffered from limited sample size and some also from poor experimental design. The research outcomes were on anti-inflammatory, antioxidant activity (anti-aging) and lipid management. Data were promising however it is important to underline that many further evidence should be provided to confirm the healthy activity on humans claimed for the microalgae already on the market. In addition, it is necessary to standardize the dose of microalgae and the modality of use and the preparation of extracts or bioactive compounds from microalgae biomass.

Critical points in large scale use of microalgae as food ingredients

Extraction of the desired components

The food industry demand and the increase in microalgae applications in different sectors are supporting the research efforts aimed at solving the problems in microalgae production and food use, and at developing cost-effective processes. Despite the high content of functional ingredients in microalgal biomasses, above highlighted, there are still some bottlenecks to solve to achieve profitable large scale production.

Many microalgae species showed a thick polysaccharide/cellulosic cell wall representing about 10% of the algal dry matter. The intact cell wall posed serious problem in down-stream process as well as in the use as food/feed, since they are difficult to digest for humans and other non-ruminants. Literature data and our own experience [131] pointed out the need to develop for each strain/species effective treatments to disrupt the cell wall and make microalgae intracellular constituents accessible for digestive enzymes or for ingredients/extracts production. New developments based on enzymatic treatments, ultrasound or microwave-assisted processes, high pressure homogenisers should be optimised. [221]

In cosmetics usually hydrosoluble and/or lyposoluble extracts from microalgae are usually adopted. Unfortunately, the yield of these extracts is very low determining a tremendous increase of the production costs, if no effective solutions for the byproducts are found. [222-223]

Now it is important to underline the algae-based biorefinery concept: the efficient use of algae biomass through its fractionation, results in several isolated products from the biomass, to apply in different market sectors. The integration of the emerging biorefinery concept with other industries can provide huge environmental and economic advantages. Energy, water, land and materials input could be reduced and optimized. New developments are expected, including the logistics and life cycle assessment, in order to assure the environmental and economical sustainability and viability of the technology.[221]

Techno functionality of the microalgae ingredients

The feasibility of incorporating microalgal biomass in conventional or innovative food preparations is conditioned by processing type, by the nature of the food matrix (*e.g*. emulsion, gel, aerated dough systems) and to the interactions with other food components (*e.g.* proteins, polysaccharides, lipids, sugars, salts). Besides coloring and nutritional purposes, introducing microalgal ingredients in food systems, can cause significant changes in food physical properties. [224]

From the sensory standpoint the major obstacles are represented by the powder like consistency of the dried biomass, its dark green color and its slightly fishy smell, which limit the incorporation of the algal material into conventional foods.

Many example combining whole algal biomass or extracts with known foods by applying various methods such as heating, baking, mixing was reported. The addition of microalgae into bread or noodle can be done at limited percentage, as dough consistence and taste became unpalatable and after cooking noodles changed into an unattractive brownish color. Incorporation of algae into ravioli-like food items masked the coloring effect, but anyway changed the taste considerably. Pasta could represent an interesting vehicle to enrich with microalgae a staple food in many country, even though a change in color during cooking may occur and the shelf-life can be reduced. Many efforts in food design research are in progress to meet incorporation of microalgae biomass in food, preserving the microalgae functional activities, the rheological properties and the shelf life of final products.

Consumer acceptance and safety issue

In the developing countries, where a great demand of protein for nutritional reasons exist, additional problems arise because of socio-ethnological barriers and very conservative restrictions against unknown food ingredients. [87]

At the moment the main commercial success of microalgal biomass can be observed in the healthy food market as pills of microalgae powder, that are sold as panacea against almost all the diseases.

It is worth to remember that before a novel ingredient can be introduced to the market as food ingredient for human consumption, the approval by regulatory authorities is required and a safety dossier must be provided. Food ingredients derived from microalgae such as oils and proteins are unique due to the non-traditional nature of the source organism used for their production. To ensure the consumer safety of these ingredients some essential elements of safety assessments need to be considered.[32] Chemical and physical characterization of the products is important as safety considerations, that often revolves around its individual components. The most critical points of microalgae safety for human consumption are: naturally occurring toxins, contamination by heavy metals and hazardous levels of pathogenic microorganisms. To ensure the production of a safe microalgae product an hazard analysis of the process must be done to define the critical control points that must be monitored. Standard guidelines or protocols of cultivation, harvesting and down streaming, provided by international regulatory organizations (eg. EFSA and FDA) could be useful to assure the quality and safety of productions in terms of both nutritional values and contamination levels.

Cost-effective production processes

While isolation and characterization of microalgae have been performed for many years, their massive cultivation still remains an underdeveloped research area needing a lot of R&D efforts towards cost-effective technologies. [225, 55] The selection, isolation and study of organisms, which may possess unique mechanism for efficient production of functional ingredients, should continue; simultaneously the development of innovative large-scale culture systems - through a deep knowledge of algal strains physiology - leading to high and sustainable growth rates should be developed. [55]

Some of the issues needing greater attention are: [55, 225, 226]

- Stability of such strains, identification of new strains, able to grow faster at high cell density;
- Increasing the growth rate of biomass and its nutrient content;

- Reduction of photo-oxidation susceptibility which damages cells;
- Identification of factors including biochemical triggers and environmental that enhances the biomolecules content.
- Biomass production with higher yield through the use of genetic engineering to increase the photosynthetic efficiency or to produce higher yields of active bio-molecules;

It is important to underline that the genetic engineering in food industry is not well accepted by consumers, in particular in Western country that prefers the consumption of "natural" and organic products.

Tailored production technologies to obtain food and feed ingredients

Massive microalgae biomass productions can be obtained using open (raceways and ponds) or closed systems (photobioreactors). Open ponds and raceways are generally low-technology systems and at the moment they account for about 99% of total world production.

Photobioreactors allow cultivations in well controlled conditions particularly for high added-value applications lowering contamination risks of foreign organisms and a better utilization of light giving high productivity.

Unfortunately, capital and management costs using photobioreactors showed to be more than ten times of the open systems. Culture systems must be designed in relation to the cultivated microalgae species and location with special attention to culture mixing, optimization of irradiance and gas exchange. [225] At the moment open systems seem to be the only way to obtain microalgae biomass at relatively cheaper cost, suitable for food applications. Many effort must be done to achieve the massive utilization of photobioreactors especially in terms of investment and management costs.

Harvesting and drying of microalgae are two bottlenecks in microalgae productions. For some species like *Spirulina* harvesting is quite simple with net filtration systems but the majority of cultivated microalgae require continuous centrifuges with high energy consumption. Dehydration in small cultivation plants is obtained by solar or by oven drying but big plants commonly use spraydrying technologies. Development of economical, quick and efficient processes for harvesting and de-watering of biomass, depending on the end use, is another area of interest for R&D. [55, 225-228]

Concluding remarks and future prospects

Microalgae can be a consistent source of large numbers of natural compounds with high value, including pigments, PUFAs, carbohydrates, proteins and others, which have a wide range of applications as functional ingredients. Microalgae as bioreactors have several advantages over bacteria, yeast, plants, and other systems for active biological molecules production, including sustainability, safety, alternative culture methods and scalability.

On the other hand, there are still a large number of bottlenecks that need to be solved before that eukaryotic microalgae and cyanobacteria can be shifted from a niche market to the large use as food commodities. For all microalgae derived ingredients, serious R&D efforts and further consumer understanding as well as market campaigns to promote their advantages and acceptability are required.

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Table 1 Functional ingredients from microalgae: microalgae species, technology production systems and commercial products

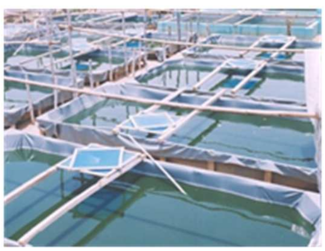
Microalgae production systems	Functional ingredients	Microalgae Species	Commercial products
Ponds and Raceways   	Proteins Phycobiliproteins Carotenoids PUFA	*Arthrospira maxima* *Arthrospira platensis* *Chlorella spp.* *Dunaliella salina* *Dunaliella bardawil*	   Nutraceutical products: tablets, capsules, energetic drinks.  Natural dyes to human foods
Photobioreactors 	Astaxanthin	*Haematococcus pluvialis*	 High antioxidant nutraceutical products Colorants to salmon, trout and poultry feed
Fermenters 	Lipids PUFAs	*Crypthecodinium cohnii* *Schizochytrium sp.* *Nitzschia laevis*	 Nutritional supplements. Additive for infant formula Vegetarian products

Table 2 Comparison of EPA and DHA fatty acid contents as percentage from total lipids in examples of fish and microalgae

Organism	Amount of long chain omega-3 (%)	Type of omega-3 fatty acid	Reference
Fish			
Merluccius productus	34.99	EPA + DHA	
Theragra chalcogramma	41.35	EPA + DHA	
Hypomesus pretiosus	33.61	EPA + DHA	
Sebastes pinniger	29.8	EPA + DHA	
Oncorhynchus gorbusha	27.5	EPA + DHA	Huynh and Kitts [42]
Mallotus villosus	17.8	EPA + DHA	
Sardinops sagax	44.08	EPA + DHA	
Clupea harengus pallasi	17.32	EPA + DHA	
Microalgae			
Nannochloropsis oceanica	23.4	EPA	Patil et al [43]
Nannochloropsis salina	~28	EPA	Van Wagenen et al [44]
Pinguiococcus pyrenoidos	22.03	EPA + DHA	Sang et al [45]
Thraustochytrium sp	45.1	EPA + DHA	Scott et al [46]
Chlorella minutissima	39.9	EPA	Yongmanitchai and Ward [47]
Dunaliella salina	21.4	EPA	Bhosale et al [48]
Pavlova viridis	36.0	EPA + DHA	Hu et al [49]
Pavlova lutheri	41.5	EPA + DHA	Guihéneuf et al [50]
Isocrysis galbana	~28.0	EPA + DHA	Yago et al [51]
Schizochytrium sp.	32.5	DHA	Wu et al [52]
Crypthecodinium cohnii	31.1	DHA	Swaaf et al [53].
Aurantiochytrium sp.	40	DHA	Hong et al [54].
Phaeodactylum tricornutu	25.8	EPA	Reis et al [55]

Table 3 Proposed biological activity of microalgae polysaccharides

Microalgae	Polysaccharide extracts	Biological activity	Main sugar component	Reference
C. vulgaris	Crude Polysaccharide	Antioxidant		Mohamed [76]
S. quadricauda	Crude Polysaccharide	Antioxidant		Mohamed [76]
Porphyridium sp.	Crude Polysaccharide	Antioxidant	Xylose, galactose	Tannin-Spitz et al [77] Geresh and Arad [78] Arad [79]
Porphyridium sp.	Sulphated polysaccharide	Anti-inflammatory	Xylose, galactose	Matsui et al. [80] Geresh and Arad [78] Arad [79]
H. lacustris	Water-soluble polysaccharide	Immuno stimulating		Park et al. [81]
G. impudium KG-03	Sulphated polysaccharide	Antiviral	Galactose	Kim et al. [75] Lee [82]
R. reticulate	Extracellular polysaccharide	Antioxidant	Xylose, galactose	Chen et al. [83] Geresh and Arad [78] Dubinsky [84]
C. stigmatophora	Crude Polysaccharide	Anti-inflammatory /immunomodulating	Glucose, xylose	Guzman et al [85]
P. tricornutum	Crude Polysaccharide	Anti-inflammatory /immunomodulating	Glucose, mannose	Guzman et al [85]

Table 4 Protein content (g kg^{-1})and essential amino acid profile (% on total protein content) of different algae compared with conventional protein sources and the WHO/FAO reference pattern. [87, 90]

Source	Protein content g kg^{-1}	Leu	Val	Lys	Phe	Met	Try	Thr	His
		% on total protein content							
WHO/FAO		7.0	5.0	5.5	6.0	3.5	1.0		
Egg	132	8.8	7.2	5.3	5.8	3.2	1.7	5.0	2.4
Soybean	370	7.7	5.3	6.4	5.0	1.3	1.4	4.0	2.6
Chlorella vulgaris	510-580	8.8	5.5	8.4	5.0	2.2	2.1	4.8	2.0
Dunaliella bardawil	350-480	11.0	5.8	7.0	5.8	2.3	0.7	5.4	1.8
Scenedesmus obliquus	500-560	7.3	6.0	5.6	4.8	1.5	0.3	5.1	2.1
Arthrospira maxima	600-710	8.0	6.5	4.6	4.9	1.4	1.4	4.6	1.8
Arthrospira platensis	600-710	9.8	7.1	4.8	5.3	2.5	0.3	6.2	2.2
Aphanizomenon sp	600	5.2	3.2	3.5	2.5	0.7	0.7	3.3	0.9

Table 5 Summary of the evidence about the health effects investigated for microalgae biomass, crude extracts and metabolites by human, animal and in vitro studies. The details are given in supplementary material (table 1S, 2S and 3S).

Health effect	Microalgae	In Vitro evidence	Animal evidence	Human evidence	Ref.
Anticancer	*Arthrospira platensis, Chaetoseros sp., Chaetoseros calcitrans, Chlorella sp, Chlorella vulgaris, Chlorella ellipsoidea, Cocconeis scutellum, Dunaliella salina, Odontella aurita, Isochrisys galbana, Gymnodinium sp., H.pluvialis, Microcystis aeruginosa, Oscillatoria neglecta, Dunaliella bardawil.*	++	++	-	95, 142-154, 176, 182, 189, 191
Glucose management	*Arthrospira versicolor, Parachlorella beijerinckii*	-	+	-	181, 196
Hepatoprotective	*Chlorella vulgaris, Arthrospira platensis*	-	+	-	167, 179, 183
Lipid management	*Crypthecodinium cohnii, Schizochytrium sp, Dunaliella bardawil, Porphyridium sp, Arthrospira maxima, Nannochloropsis oculata, Ulkenia*	-	++	++	160,169, 185- 187, 190, 201-212, 219 220
Antimicrobial	*Chlorella sp, Cyanothece spp., Cyanospira capsulata, Scenedesmus quadricauda, Arthrospira sp., Arthrospira platensis, Chlorococcum sp., Nostoc commune*	++	++	-	146, 150, 157, 159 178, 184
Immunomodulation	*Aphanizomenon flos-aquae, Chlorella stigmatophora, Phaeodactylum tricornutum, Arthrospira sp.*	+	++	-	140, 85, 75, 158, 173
Antiviral	*Ankistrodesmus convolutus, Gyrodinium impudium, Porphyridium sp., Synechococcus elongatus*	+	-	-	139, 156, 75
Antifibrosis	*Navicula incerta*	+	-	-	155
Antioxidant	*Arthrospira platensis, Arthrospira maxima, Botryococcus braunii, Dunaliella bardawil, Dunaliella salina, Haematococcus pluvialis, Chlorella sp, Chlorella vulgaris.*	++	++	++	141, 160-165 168, 170, 172, 174, 175, 177, 179, 180 192-195, 213-218
Anti-inflammatory	*Chlorella stigmatophora, Phaeodactylum, tricornutum, Porphyridium sp., Arthrospira maxima, Chlorella stigmatophora, Dunaliella bardawil*	++	++	+	85, 171 163, 166
Detoxification	*Parachlorella beijerinckii*	-	++	-	198-200

++ More than 3 studies; + Between 1 and 3 studies; - No studies