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A Comprehensive Overview on Microalgal-Fortified/Based Food and Beverages

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ABSTRACT

Microalgae produce a variety of compounds that can be used for aquaculture, nutraceutical purposes, and third-generation biofuel sectors. Moreover, they have been considered for the production of fortified food and beverages claiming to be healthier than other foods. Using microalgal oil or incorporating microalgae biomass or their metabolites in food and beverages provides health benefits due to properties such as anti-inflammatory, antioxidant, immune-enhancing, and to their role against various diseases such as cardiovascular metabolic, atherosclerosis, and hypertension. This review focuses on the worldwide research carried out about the incorporation of microalgae – either biomass or their high-value compounds – in food and beverages, and on the microalgal fortified/based food and beverages currently present worldwide on the market. The metabolites bioavailability aspect and the current legislation are considered. There is an ever-growing interest in this field but the volume of production is still limited. Various challenges, one of which is the cost of producing biomass, need to be overcome for a profitable market. More effort, involving different expertise, is needed to improve the microalgal production system, from cultivation to harvesting and biorefinery, to produce improved novel products.

KEYWORDS Microalgae; biomass; metabolites; food, beverages; fortification; bioavailability; legislation

Introduction

Microalgae are widespread, photosynthetic microorganisms able to grow rapidly using, similarly to plants, light, sugars, CO_2, N, P, and K. Some of them can also grow heterotrophically (organic carbon sources are used for metabolism) and mixothrophically (inorganic and organic carbon sources are concurrently used for metabolism). They include algae sensu stricto and cyanobacteria.[1] The former are eukaryotes and the most abundant are the diatoms (Bacillariophyceae), the green algae (Chlorophyceae), and the golden algae (Chrysophyceae). The latter – Cyanophyceae or blue-green algae – are prokaryotes and, in this review, they are referred to as microalgae. There are thousands of different species of microalgae and cyanobacteria. In particular, it has reported the existence of 50,000 species of which 30,000 have been studied and analyzed.[2] Diatoms dominate in phytoplankton life and with more than 100,000 species are considered the largest group of biomass producers.[3] Among the thousands of species of microalgae that are believed to exist, only few species are industrially cultivated to produce useful chemical compounds[3,4] such as lipids, proteins, carbohydrates, vitamins, and pigments as well.

Microalgae have been considered for nutraceutical compounds production[5,6] and also for third-generation biofuels[7] as both in monoculture[8,9] and in mixed culture cultivation.[10–12] Microalgal high-value biological active compounds – pigments, antioxidants, β-carotenes, polysaccharides, triglycerides, and vitamins – have been reviewed[13] and they are used in various sectors such as the food production.[2,14–16] Enzing et al.[3] reported three main ways to use microalgae in the food sector: whole dried microalgae as dietary supplement, high-value molecules extracted from microalgae, and dried microalgae biomass added to new food formulations. The use of microalgal biomass for human consumption is allowed for few species and it is related to food safety regulations.[14]

Microalgae commonly cultivated as additives for human foods are mainly chlorophyta and cyanobacteria.[17–19] In particular, *Chlorella* sp., *Haematococcus pluvialis*, *Dunaliella salina*, *Aphanizomenon flos-aquae*, *Arthrospira* sp. (formerly *Spirulina*), *Schizochytrium* sp.,[20,21] and *Scenedesmus* sp.[22] are used for human nutrition.

Proteins produced by microalgae have high nutritional value because of their composition of essential amino acids.[17,23] High content of proteins is reported for *Chlorella* (up to 58% of dry weight)[17] and *Arthrospira* (50–70% of dry weight)[24] (Tables 1–3).

In the food industry sector, polysaccharides are mainly derived from macroalgae. An exopolysaccharide produced by the microalga *Porphyridium cruentum* can substitute for carrageenans.[17]

Polyunsaturated fatty acids (PUFAs) such as γ-linolenic acid (GLA) and long chain PUFAs such as eicosapentaenoic (EPA), docosahexaenoic (DHA), and arachidonic (AA) acids have important role in human health, giving protection against cardiovascular, coronary and heart diseases, atherosclerosis, metabolic diseases, rheumatism, skin diseases, and cancer.[22,25,26] *Chlorella minutissima* produces high content (45% w/w) of EPA.[27] *Arthrospira* is a good source of GLA (8–31.7%).[28] Hoseini et al.[24] have reported values of 10–20% and 49% of GLA for *Arthrospira maxima* and *Arthrospira platensis*, respectively. *Nannochloropsis* sp., *Isochrysis* sp., *Nitzschia* sp., *Phaeodactylum* sp., and *P. cruentum* (up to 27% of fatty acids) produce EPA (Table 1), *Porphyridium* sp. produce AA (up to 36% of fatty acids), while *Crypthecodinium* sp. (40–50% of fatty acids), *Ulkenia* sp., and *Schizochytrium* sp. produce DHA (Table 1).[5,14,20,29–32] *Pavlova lutheri* produces EPA and DHA (Table 1).[33] Patents for producing DHA by *Crypthecodinium cohnii* are reported.[34] Also, other PUFAs than DHA from microalgae are used in the production of infant formula and functional food.

Microalgae also produce chlorophylls, carotenoids (β-carotene, astaxanthin, lutein, and zeaxanthin), and phycobiliproteins (phycocyanin, phycoerythrin). These compounds are pigments that can be used both as food colorants, thus replacing various synthetic dyes, and as food supplement for important properties such as anti-inflammatory, antioxidant, hypolipidemic, enhancing the immune system function, and inhibiting tumor progression.[35]

Astaxanthin is produced by *H. pluvialis* (up to 0.2–2.0% on dry weight basis)[20,36–38] and by *Euglena* cf. *sanguinea* (80% of the carotenoid pool),[18] lutein by *Muriellopsis* sp. (4–6 mg/g),[39,40] and β-carotene is produced by *D. salina* (Table 2).[20,22,38,41] Pangestuti and Kim[42] have reported phycobiliproteins from marine algae; phycocyanin is mainly produced by *A. platensis* (Table 2)[24,43,44] and *Synechococcus* sp. (80–100 mg/g)[45] while phycoerythrin by *P. cruentum* (32.7% dry weight)[46] and *Rhodosorus marinus* (8.0% dry weight).[47] Phycocyanin is mainly reported to be used as food colorant and as supplement for health food.[48]

Moreover, microalgae – first *Arthrospira* sp. and *Chlorella* sp. among others – are a source of

vitamins such as A, B1, B2, B6, B12, C, E, folic acid, and pantothenic acid (Table 3).[14,49] Vitamins produced in more concentration are B3 (range 7.8–23.8 mg/100 g dry weight for *Isochrysis galbana* and *Chlorella*, respectively), C (range 10.0–19.1 mg/100 g dry weight for *Chlorella stigmatophora* and *Tetraselmis suecica*, respectively), and E (range 0.00045–66.9 mg/100 g dry weight for *Schizochytrium* sp. and *C. stigmatophora*, respectively).

Nowadays, there is an ever-growing interest in the application of microalgae for the development of healthier, innovative, and attractive food products. Nevertheless, the production and distribution of new formulates are still limited[50] even if since the year 2000, the microalgae cultivation for food and feed production had a fivefold increase.[51]

Important aspects to consider in the development of novel microalgal-based food/beverage formulations are the cost-effectiveness of the sector, the biological availability of functional compounds from microalgae and their stability throughout the life of the products,[52] and the consumers' perception and acceptance of the microalgal-based products. This paper aims to contribute, as an overview, to the knowledge on the sector of novel food/beverage formulates from microalgae as (1) biomass incorporated to foods and (2) metabolites extracted from microalgae and incorporated in foods. Moreover, challenges to face up in order to improve this novel sector are discussed.

Applications to the food sector

At present, the technology of production, recovery, and transformation of microalgae utilizes low volumes with associated high cost. For this reason, the extraction of high-value compounds to be used as supplements and nutraceuticals for human nutrition is considered the most encouraging sector compared to the bulk production of carbohydrates and proteins, which needs higher volume of production and, therefore, cost-effective scale-up process in order to reduce the costs. Moreover, the high-value compounds from microalgae are more effective for food applications compared to the synthetic alternatives. This is determined by their chemical conformation, which makes them more effective for specific applications, such as in infant formula and dietary supplements.[20] There are also many examples of the use of whole microalgae biomass incorporated in food, and this confirms the active industry research in order to reduce the cost and, at the same time, increase the volume of production. The microalgal concentration of valuable compounds (Tables 1–3),[13,20,53–79] often higher compared to other sources, can influence positively human health and express the real potential use of microalgae in the food sector.

The regulation about the use of microalgae as food/supplement varies among countries (Table 4). In the European Union (EU), until now, novel foods (food not consumed to a significant degree in the EU prior to 15 May 1997) and food ingredients are regulated by the Regulation (EC) No. 258/97.[80] *Chlorella pyrenoidosa*, *Chlorella vulgaris*, and *Heterochlorella luteoviridis*, having been on the market and consumed before 15 May 1997, are not subject to the Novel Food Regulation.[81] Under Regulation (EC) No. 258/97, the oil from *Schizochytrium* sp. rich in DHA and EPA has been allowed to be placed on the market in Europe as a novel food ingredient.[82–84]

Also, astaxanthin from *H. pluvialis* was approved as novel food ingredients in 2006, *Tetraselmis chuii* was approved as novel food to be used as sauce, special salt, and condiment under Regulation No. 258/97 in 2004, and the oil from *Ulkenia* sp. was approved in 2009 as a novel food ingredient in bakery products, cereal bars, and nonalcoholic beverages including milk-based beverages.[85] Current applications for food/food ingredient concern the microalga *Nannochloropsis gaditana* and the EPA-rich oil derived from the microalga *Phaeodactylum tricornutum*. In order to simplify the current authorization procedures taking into account recent developments in Union law and technological progress, a new regulation on novel food was established with effective validity at the end of 2017.[86] According to this regulation, a novel food falls into different categories among which is a “food consisting of, isolated from or produced from microorganisms, fungi or algae.”

In the United States, the Food and Drug Administration (FDA) classified some microalgae as generally recognized as safe.[87–94] Examples are *Euglena gracilis* (for conventional food and beverage products such as baked, cereal, dairy and milky products, processed fruits, fruit juices, soft candy, and soups), *Arthrospira* sp. (for breakfast/power/energy bars, smoothies, and condiment for salads and pasta), DHA oil from *Schizochytrium* sp. (for soy protein bars, processed vegetable drinks, hard and soft candy, nondairy and powdered cream substitutes, jams and jellies, milk and flavored milk, and soy milk), DHA oil from *Ulkenia* sp. (for addition in a vast variety of foods), D. salina powder (for bread, rolls, cookies, crackers, cheese, mayonnaise, tofu, and fermented soybean products), *Auxenochlorella protothecoides* (formerly *Chlorella*) (algal flour for baked goods, beverages and beverage bases, dairy product analogs, egg products, fats and oils, sauces, processed vegetables, vegetable juices, and soups), and *C. vulgaris* (for power bars, sport beverages, energy drinks, and low fat soy milk).

In Australia, the Food Standards Australia New Zealand approved DHA-rich oil from *Schizochytrium* sp. and *Ulkenia* sp. as novel food ingredients.[95]

In Japan, the Ministry of Health, Labour and Welfare defined the specifications and standards for food additives where are reported the approved food additives.[88] *Chlorella* powder is generally provided as food and food additives – color, flavor, and taste enhancer. Also, in the list of food additives appear the phycocyanin from *Arthrospira* sp., carotene from *Dunaliella* sp., and the microalga *Haematococcus* as colorant.

Microalgae can be used both as biomass and, after extraction processes from the biomass, as pigments and oils in a variety of food and beverages (Figure 1). Applications of microalgae in different food and beverage categories are considered below.

Microalgae added to dairy and probiotic products

The incorporation of microalgal biomass to these products aims to increase their nutritional properties also via the stimulation of the lactic microflora typical of these products. Cheeses and cheese analogs, also called processed/imitation cheeses, are products in which the milk components are partially or wholly replaced by non-milk-based components, improved for their functional role by the addition of dried microalgal biomass.[96] Positive effects of the addition of *Chlorella* biomass (0–2%) into Appellenzer cheese have been reported on lactic acid bacteria (LAB) viability: a biomass concentration of 0.5% positively influences the quality and sensory properties.[97] The use of *Arthrospira* – also decolored, odorless, and tasteless biomass – is used to produce cheese, tofu and yoghurt.[98] The incorporation of *Chlorella* biomass (0.5%) during cheese production improved physical–sensory properties.[99,100] Recently, Shalaby and Yasin[101] reported the formulation of innovative croissant stuffed with cheese obtained with *Chlorella* spp.; the 2–3% of the microalgae improved croissants taste, texture, and quality.

Microalgae, especially *C. vulgaris* and *A. platensis,* in coculture with LAB are used in yoghurt and probiotic fermented milks production. Various authors have reported the stimulation role that microalgae have on the growth of probiotic bacteria both in the products and in the human gut.[102–104] Stimulation of the growth of LAB in fermented milk incorporated with microalgae has been reported.[16,105–108] Adding *C. vulgaris* and *A. platensis* at different concentrations to yoghurt determined higher viability of two probiotic LAB strains of *Lactobacillus acidophilus* LA-5 and *Bifidobacterium* lactis BB-12 than the product free of microalgae both at the end of fermentation and throughout the storage.[109] On the other hand, the same authors suggest that using high concentration of microalgae confers unpleasant color and graininess to the product. Therefore, using low concentration of microalgae gives better sensory characteristics. Supplementing fermented milk with microalgae could give a way to sensory defects that can be avoid by adding flavors, texture improvers, and colors.[110] Varga et al.[111] have reported that *A. platensis* added to fermented milk has positive effect on *Bifidobacterium* sp. showing also antifungal effect on spoilage yeasts and molds. Novel yoghurt products, frozen with low fat, high protein, supplemented with *Arthrospira*, and papaya pulp, have been reported.[112] In particular, the best sensory characteristics were scored by the formulation with 6% of *Arthrospira* and 10% of papaya. Functional drinkable skimmed milk yoghurts added with *Chlorella* have been reported.[113] The authors tested both microalgal powder (0.25%) and microalgal liquid extract (2.5–10%); in both cases, the storage of the yoghurt at 4°C maintained the quality in terms of pH, titratable acidity, and viable count of LAB. Even so, the yoghurt with no addition showed higher sensory acceptability than the other formulates. Recently, authors reviewed the use of *C. vulgaris* and *A. platensis* to produce functional dairy products.[110,114] One of the requirements of probiotic fermented milks is the high number of viable LAB; therefore, any supplementation must positively affect this aspect other than the sensory properties of the final product. The microalgae were added in the range of 0.25–1%; generally, this supplementation increased probiotic bacteria viability but only the product with low concentration of microalgal biomass gained good sensory attributes. Due to this, despite having health benefits, consumers may not accept the product due to its strange color and its graininess. Possible solutions could be the addition of colorants to homogenize the discolorations and the addition of texture improvers to solve the off-texture problems. In addition, the incorporation of microalgae determines a price increase of the final product; so, the possible solution is a more costeffective production of the microalgae.

The effective release of the compounds from cells and how it is affected by the food production and processing techniques are aspects to consider for compound availability both for human and lactic microflora.

Microalgae added to bakeries, pasta, dressing, and beverages

Growing interest in the market for new food products with microalgae and the variety of foods that can be fortified with microalgae – pasta, biscuits, bread, noodles, snack foods, candy or gums, drink mixes, soft drinks, soups, and sauces – has motivated other researches in the field.[49,98]

The development of soups, noodles, bread and rolls, biscuits, ice cream, soy sauce, and powdered green tea based on *Chloroidium ellipsoideum* (formerly *Chlorella ellipsoidea*) has been reported.[2] The addition of *A. platensis* (10%) to conventional bread enhanced the nutritional quality and the shelf life of the bread without negative impact on sensory evaluation. In particular, the microalgal-enriched bread resulted in higher proteins and volatile compounds content. The sensory assessment was satisfactory even perceiving algal taste.[115] Adding *Chlorella* powder to white bread determined an improvement of its quality characteristics in terms of volume and color while the texture was not significantly affected. The bread with 0.2% of *Chlorella* powder scored high sensory evaluation.[116] Novel cookies and pasta enriched with *Nannochloropsis oculata* were characterized by good concentration of PUFAs (e.g., cookies enriched with 3% of biomass had a value of DHA + EPA of 298 mg/100 g). Regarding the sensory evaluation, biomass incorporation up to 2% and 3% was positively evaluated for cookies and pasta, respectively.[117] Bakery *Arthrospira* products with 2% of microalga such as rice pasta, sfogliatine, and grissini are produced in Italy (Microlife Nutrition).

Novel biscuits rich in functional compounds were developed with the addition of *C. vulgaris*,[118,119] *H. pluvialis*, and *I. galbana*.[17,120] Industrial production of traditional Iranian biscuits supplemented with *A. platensis* was reported; the biscuits fortified with 1.0–1.5% of microalga exhibited improved and desirable functional and sensory characteristics.[121] *Arthrospira* powder was also used as ingredient for an orangeflavored chewable wafer[98] and used by the industry Microlife Nutrition (Italy) to produce chocolate bars and biscuits (Table 5). *Arthrospira* sp. was used at the concentration of 2.6% to produce snacks with improved nutritional content (increase of 22.6% in proteins, 28.1% in lipids, and 46.4% in minerals) without significantly affecting physical parameters and the sensory quality with an acceptability index of 82%. Also, these snacks showed physical and microbiological stability during one year of storage.[122] *A. platensis* as extract or as phycocyanin can be incorporated in sour milk and green tea.[123] Kim and Chung[124] have evaluated the addition of *Chlorella* powder (0–4%) to make a yellow layer cake; the novel cake exhibited improved quality from textural, sensory, and functional point of view.

A. protothecoides is the component of AlgaVia® Lipid-Rich Whole Algae (algal flour) and AlgaVia® Protein-Rich Whole Algae (algal protein) (Corbion, The Netherlands). These food ingredients deliver lipids rich in polyunsaturated fats, proteins plus fibers, and micronutrients, respectively, and they are used to make various types of food (Table 5). AlgaVia® Lipid-Rich Whole Algae is commercially available throughout North America (US, Canada, Mexico), the EU, the United Kingdom, Australia/New Zealand, and Brazil while AlgaVia® Protein-Rich Whole Algae is not commercially available in Brazil.

Fradique et al.[125] have reported the enrichment of fresh spaghetti with biomass of *C. vulgaris* and *A. maxima*. The supplementation (0.5–2%) with these microalgae resulted in an improvement of cooking and textural properties; the addition of microalgae resulted in an increase in the raw pasta firmness and its color remained relatively stable after cooking. These microalgae pastas had higher acceptance scores for colors, similar to pastas produced with vegetables, and good cooking and textural properties.

Different Italian companies produce pasta with *Arthrospira* (Table 5) such as tagliolini, rice fusilli, corn penne (2% of microalga) (Microlife Nutrition), corn flour conchiglie (10% of microalga) (Nutracentis), wheat fusilli, kamut penne, and spelt spaghetti (3% of microalga) (La finestra sul cielo). In addition, French companies produce novel pasta such as tagliatelle duo curry and Spirulina (Cornand) and Tagliatelles with *Spirulina* (GlobeXplore) (Table 5).

A total of 328 new packaged food, drink, and pet food products with *Chlorella* were launched recently in Japan, United States, South Korea, Canada, Germany, and other countries.[126]

The incorporation of microalgal biomass is an interesting and promising alternative to the production of conventional oil-in-water emulsions such as mayonnaises and salad dressings. Main advantages of the microalgal use are natural color, source of biologically active compounds, and antioxidant effect. The possibility to use microalgal biomass as coloring agent in oil-in-water emulsions has been reported.[127] In these kinds of food, the trend is to replace the egg yolk lipoproteins with pea proteins in order to reduce the fat content. Studies on the interaction between pea proteins and microalgal biomass have been carried out to conclude that the presence of the biomass does not destabilize the product texture; on the contrary, it can present a synergistic effect improving the texture and sensory properties.[128] Green and orange *C. vulgaris* biomass and *H. pluvialis* biomass are reported to be suitable for the oil-in-water vegetable food emulsions since they give stable and attractive color, good texture, and antioxidant effect.[129] *A. maxima* and *Diacronema vlkianum* were used to produce novel vegetable gel desserts rich in PUFAs and improved for texture.[123] Batista et al.[130] have studied the effect of the addition of *H. pluvialis* and *A. maxima* on the viscoelastic behavior of gel vegetarian food in order to set the best conditions for these kinds of foods.

Chlorella powder was used to improve the quality of some traditional Korean foods: Mul-kimchi, a drinkable broth made with fermented vegetables, added with 0.05% of *Cholrella* exhibited increased LAB population, good sensory characteristics, and improved shelf life during refrigerated storage[131]; Sulgidduk, a steamed rice cake, supplemented with 0.2% and 0.5% of *Chlorella* exhibited the best characteristics of quality.[132]

Microalgae of Chlorophyceae family including *Chlorella*, *Scenedesmus*, and *Chlorococcum* can be used together with *Saccharomyces cerevisiae* to make an alcoholic beverage with pleasant and wine-like taste.[133]

Microalgal biomass incorporation is an appealing alternative to the conventional production. Generally, this fortification is accepted if the improvement of the healthiness characteristics corresponds to an equal or improved level of sensory characteristics compared to the conventional products. According to the product considered, the expectations are different (the absence of graininess for yoghurt, homogeneity in texture for pasta and mayonnaises); therefore, the viability of these novel food and beverage products depends on a balance between nutritional and sensory requirements.

Different from the previous sections, reporting the incorporation of microalgal biomass on food, the two following sections report the use of microalgal pigments and oils extracted from the biomass.

Pigments from microalgae added to miscellaneous products

One of the characteristics of microalgae is their color due to the presence of various types of pigments. Other than chlorophylls, which are the primary photosynthetic pigments, they can have carotenoids and phycobiliproteins (Figure 2). These natural pigments are important in the food sector since they can be used both to give color and for their antioxidant properties.

Among microalgal pigments, phycocyanin, mainly produced by *A. platensis*, is used in the food industry especially in dairy and milky products (fermented milk, milk shakes, and ice creams), jellies, coated soft candies, soft drinks, health drink, sour milk, green tea, confectionaries (blue smarties), desserts, and sweet cake decorations.[16,44,134] The blue pigment phycocyanin of the red microalga *Porphyridium aerugineum* is used mainly in drinks such as Pepsi® and Bacardi Breezer®[44] due to its stability to pH changing and to light exposure (Table 5). Phycocyanin derived from *Arthrospira* is used to produce Linablue® as natural blue food colorant (DIC Corporation). This product can be used for beverages, frozen desserts, macarons, and candy-coated chocolates (Table 5). Sekar and Chandramohan[44] have reported three patents concerning the use of phycobiliproteins in drink/beverage compositions: CN 1127611,[135] CN 1096178,[136] and WO 03099039.[137]

Table 5 reports the use of β-carotene and astaxanthin, their health and technological aspects, and the producer companies.

Oil from microalgae added to different food

The main source of long chain PUFAs, especially ω-3, is fish oil; however, the use of this oil as food additive gives problems since it confers fishy smell and taste and poor oxidative stability. A good alternative is the use of oil from various species of microalgae. Also, even though microalgal oil can undergo lipid oxidation, compared to fish oil, it is richer in vitamins and also in other substances such as carotenoids and phenolics that can protect it from oxidation.[138]

Valencia et al.[139] have reported significant enrichment of ω-3 PUFAs in a new formulation of dry fermented sausages made incorporating *Schizochytrium* sp. oil partially substituting pork backfat. Healthier dry fermented sausage formulations were obtained by the incorporation of mixed linseed and algae oils.[140]

Oil emulsion from microalgae was used to enrich strawberry flavored yoghurt,[141] ice cream,[142] and milk.[143] The yoghurt and ice cream supplemented with algae oil emulsion to provide 500 mg ω-3 fatty acids per 272 g serving and 300 mg ω-3 fatty acids per 65 g serving, respectively, scored moderate acceptability by trained panel due to distinguishable strong fishy flavor.

According to the Commission Implementing Decision (EU) 2015/545/CE and 2015/546/CE,[82,83] DHA and EPA-rich oil from *Schizochytrium* sp. can be used in dairy products except milk-based drinks (200 mg/100 g or for cheese products 600 mg/100 g), dairy analogs except drinks (200 mg/100 g or for analogs to cheese products 600 mg/100 g), spreadable fat and dressings (600 mg/100 g), breakfast cereals (500 mg/100 g), food supplements (250 or 450 mg DHA per day for normal population and pregnant women, respectively), foods for energy restricted diets for weight reduction (250 mg per meal replacement), and other foods for particular nutritional uses (200 mg/100 g), bakery products (breads and rolls), and sweet biscuits (200 mg/100 g), cereal bars (500mg/100 g), cooking fats (360 mg/100 g), non-alcoholic beverages (80 mg/100 ml), infant formula and follow-on formula (in accordance with Directive 2006/141/EC), processed cereal-based foods, and baby foods for infants and young children (200 mg/100 g). The DHA-rich microalgae oils from *Schizochytrium* sp. and *Ulkenia* sp. can be used as food ingredients in a variety of foods and in infant formula.[144,145]

C. cohnii cells containing DHA-rich oil can be used in baby foods and infant formula; many of the latter containing Martek Corporation's (USA) DHA-rich oil are available worldwide –United States, United Kingdom, Mexico, China, and Canada[32] (Table 5). A microalga, originally found in the sap of a chestnut tree in Germany, is cultivated and used by the company Corbion (The Netherlands) to extract oil to produce the AlgaeWise® Ultra Omega-9Algae Oil that can be used as culinary oil – also for frying – and spray coatings and to make margarines and spreads, sauces and dressings, and bakery. This microalga is also used to produce the AlgaeWise® Algae Butter structuring hard-stock (Corbion) whose application is in the bakery industry, spreads, and confectionery (Table 5).

To affirm effectively the usefulness of microalgal fortified products, knowledge about the fate of the microalgal compounds after the food ingestion is fundamental. The following section gives the state-of-art of the microalgae and their valuable compounds bioavailability.

Bioavailability of functional compounds from microalgae

The term bioavailability was defined by the FDA as "the rate and extent to which the active ingredient is absorbed and becomes available at the site of action."[146] After food ingestion, the bioavailability involves the gastrointestinal digestibility and solubility of the food elements, the adsorption by intestinal endothelium, the distribution by circulatory system, and the utilization by the target site. Also, bioavailability is divided as bioaccessibility, which examines compounds of a whole food released into the gastrointestinal tract to identify those elements effectively accessible for adsorption, and bioactivity, which refers to the intestinal adsorption of food elements, the transport to the target site, the elements biotransformation at the target site, and the physiological response.[147] Bioavailability, bioaccessibility, and bioactivity are assessed by different *in vitro* and *in vivo* studies that need to be harmonized to obtain univocal data. Considering protein digestibility, for example, a harmonizing attempt is the INFOGEST, a static in vitro digestion model.[148,149] Moreover, the data on bioavailability often refer to seaweeds and algal supplements while the knowledge about the bioavailability of compounds from microalgae, also as biomass incorporated into a food matrix, is still limited. Important aspects to consider in view of compounds release and availability, the real assimilation during the digestion process and their interaction with the gut microbiome,[52] are the effect of the food processing techniques on the algal compounds and the interaction among them and the food matrix.

Becker[150] reported that *Scenedesmus obliquus*, *Spirulina* sp., and *Chlorella* sp., exhibited protein digestibility coefficient values of 88.0%, 77.6%, and 76.6%, respectively, in comparison with casein and eggs that have higher coefficients. The protein digestibility of *S. obliquus*, *C. vulgaris*, *Nannochloropsis granulata* increased by disrupting cells with different methods[151–154] or pH-shift process for *N. oculata*[155] while the protein digestibility of *A. platensis* was greater for the fresh biomass compared to the treated one. Little differences in protein digestibility were reported for *Nostoc commune* treated and untreated biomass.[156] Also, the presence in the microalgal cell of high level of polysaccharides and polyphenols causes a reduction of protein digestibility.[157,158]

Concerning the bioavailability of PUFAs, it is important to consider their presences in the different classes of lipids (polar and neutral), their positions in the triacylglycerols,[159] and their bonds with phospholipids or glycolipids.[160] In nutrient-starved cells of *Nannochloropsis*, the neutral lipids (as storage molecules function) can contain 90% or more of triacylglycerols-rich in C16 series of fatty acids, which have limited value as food ingredient. During starvation, the mechanisms of degradation of polar lipids (as structural molecules function) and of inter-conversion and repackaging of PUFAs such as EPA could have a role in distributing the ω-3 fatty acids in the different classes of lipids with possible modification of bioavailability in food products.[161] The position of fatty acids in the triacylglycerols affects the nutritional properties, the adsorption, and the organism metabolism. In fact, the triacylglycerols with PUFAs in sn-2 position have more nutritional properties and they are better adsorbed compared to triacylglycerols with PUFAs casually rearranged.[159] It has been reported that PUFAs bound to phospholipids are more bioavailable than those bound to triacylglycerols.[162] Kagan et al.[160] studied the appearance of

EPA and DHA in human plasma after the ingestion of breakfast followed by oil from *N. oculata* (rich in EPA conjugated to phospholipids and glycolipids) and krill oil (rich in EPA and DHA conjugated to phospholipids). The fatty acids appeared in plasma 3 h after breakfast and the concentration of EPA was higher with the ingestion of microalga oil; this could be attributable to the bond with glycolipids as a more effective system to deliver EPA to human. Using in vitro digestion models, Cavonius et al.[155] have reported that intact cells of *Nannochloropsis* were not accessible and that cell disruption coupled with pH-shift processing with protein solubilization increased the accessibility of lipids. Meal supplementation with *D. vlkianum* (single-dose of 101 mg/kg EPA + DHA, human equivalent dose of 24.2 g of microalga) in rats increased the fatty acids content in erythrocytes, liver, heart and adipose tissues, and in serum.[163] Clinical trials testing DHA supplements from *C. cohnii* and *Schizochytrium* along with a snack bar fortified with supplement from *Schizochytrium* highlighted that all DHA forms were equally bioavailable with a dose–response relationship. In addition, the similar plasma DHA values between capsules and fortified snack bars suggest that consuming DHA supplement with food increases its bioavailability.[164] Another study providing algal-DHA oil with orange juice concluded that potentially food may favor the DHA adsorption.[165] The type of food matrix appears to have a role in the ω-3 bioavailability; in particular, nanoemulsion technology using yoghurt as a food vehicle caused a rapid increase in DHA levels.[166]

Fabregas and Herrero[77] reported that the concentration of vitamins (E, B1, and β-carotene)

from *T. suecica*, *I. galbana*, *Dunaliella tertiolecta*, and *C. stigmatophora* exceeded the concentration present in food recognized as source of these vitamins. For fat-soluble vitamins, it was reported that the bioavailability increases consuming lipid-rich foodstuffs.[167] Also, the vitamins bioavailability increases by microalgal biomass disruption.[168]

The efficiency of carotenoid absorption is affected by food matrix, type of processing, other food components, and nutritional and physiological status.[169] The bioaccessibility of *N. oculata* and *Chaetoceros calcitrans* was significantly higher in dried extract form than the powdered form.[168] Gille et al.[170] reported different roles of biomass treatment on carotenoids bioaccessibility from two microalgae species. *Chlamydomonas reinhardtii* is a good carotenoid source without processing, while processing methods are necessary for *C. vulgaris*. β-carotene in *Spirulina* is considered more digestible than the vitamin in leafy green vegetables given its unicellular state.[171] Despite the volume of production of *Spirulina* worldwide, the same authors reported that the study on vitamin A bioavailability in humans is limited and carried out using *Spirulina* supplements.

The limitation of bioavailability of astaxanthin from *Haematococcus* is due to the physical barrier of cysts rich in astaxanthin. Mechanical disruption and autoclaving of the astaxanthin-rich algal biomass were effective to increase the pigment bioavailability.[172] The bioavailability of astaxanthin from *Haematococcus* was related to the timing of ingestion; so, the astaxanthin bioavailability was higher after meal than before meal.[173]

In summary, to produce a microalgal-based product that effectively has beneficial properties, various aspects along the production process have to be taken into account. First, the cultivation with optimal nutrients concentration or in starving condition affects the type of fatty acids present in lipids and it appears to modulate the PUFAs position in the triacylglycerols with an impact on bioavailability. On the other hand, the starvation reduces the proteins content; therefore, the cultivation techniques should guarantee a balance between protein and lipid production. Second, it is important to know the structure of the chemical compounds present in microalgae since this affects the release of the different compounds making them available. Connected to this aspect is the need to treat the biomass by different methods to aid the bioavailability of the valuable intracellular compounds. Also important is the food matrix incorporating the microalgae since often the presence of lipids in the food increases the bioavailability of valuable compounds such as vitamins.

Challenges and future perspectives

The microalgal-based food and supplement/nutraceuticals global market is a sector with a great potential of expansion. Currently, microalgae are used both as dried whole algae and to extract high-value compounds to be used as ingredients, supplements, and colorants. Compared to the market based on other sources, the microalgae volume of production and market size for food and nutraceuticals are small with important sign of increase since the beginning of the century.[51] The large-scale commercial production of microalgae for protein/carbohydrates impacting on food-feed security can still be considered a less developed industry. Dried whole algae *Arthrospira* and *Chlorella* for dietary supplements have the largest volume of production volumes worldwide (5000 and 2000 t of dry matter/year, respectively) with an estimated value of production of about $40 millions/year each.[3] In addition, the market of high-value molecules from microalgae such as astaxanthin, ω-3 fatty acids, and β-carotene has larger market potential in spite of smaller volume of production. As example, the production from microalgae of polyunsaturated fatty acids such as DHA and EPA is only 240 t/year with market value estimated to be higher than $300 million/year.[3] The total production cost for microalgae-derived products becomes higher due to the extraction techniques; for example, the total cost for microalgae astaxanthin is €465.58/kg.[3] These values highlight that microalgal biomass is an expensive material compared to other types of biomass (wheat straw 0.03 €/g).[174] For larger size plants (100–200 ha), the total cost (capital, labor, other variables such as utilities and fertilizers) of microalgal biomass production, expressed as kg dry weight, is estimated as 0.68 and 1.8€ for open ponds and flat photobioreactors (PBRs), respectively. Considering the lower size of the real production plants, the resulting cost is higher than the above estimation.[175,176] Recently, Ruiz et al.[177] reported differences in costs according to the plants location; for a plant of 100 ha in South Spain, the total cost (capital and operational costs) of microalgal biomass production is estimated 5.2 and 3.4 €/kg for open ponds and flat PBRs, respectively, while for The Netherlands, these values are 11.0 and 6.0 €/kg. The biorefinery cost (capital and operational costs) for food-feed sector is 0.9 and 1.4 €/kg for South Spain and The Netherlands, respectively.

The current volume of market for D. salina is about 3000 t/year (for carotene), for *H. pluvialis* is about 700 t (for astaxanthin), for *Shizochytrium* and *C. cohnii* is 20 and 500 t/year, respectively (for DHA).[178] The volume of the microalgae market is expected to increase reaching US$44.6 billion by 2023 with expanding at a compound annual growth rate of more than 5.2% from 2016 to 2023. Also, prevision of expansion is reported for the global nutraceutical market[178]; so, it is foreseeable an increase in demand for microalgal biomass and valuable compounds.

In order to expand the industry of food products or ingredients based on microalgae, different challenges need to be overcome. First, it is important to use a cultivation system highly efficient and able to minimize contamination risks; this is a closed system in comparison to an open system subjected to the weather variability and contamination risks. However, in large-scale cultivation with closed systems, temperature and oxygen control are important to avoid culture collapsing. Temperature control can be realized by water spraying on the PBRs or by heat exchangers while oxygen control by using efficient degassers.[177] An efficient use of the light can be obtained by correct design of the PBRs, the use of light-emitting diodes, or embedding light guides to PBRs.[179,180] Moreover, the material of the PBRs has to prevent the microalgae adhesion to avoid biofouling problems.[181] Another aspect to consider for an advantageous cultivation is the integration in the microalgae plants of the systems to reuse CO2 waste and waste streams rich in phosphorous and nitrogen. In order to have a cost-effective scale-up, researches on PBRs are needed. A way to improve the production of microalgae and reduce costs is growing the microalgae in heterotrophic or mixotrophic cultivation. In particular, in mixotrophic process, microalgae synthesize compounds through both autotrophic and heterotrophic pathways requiring less light energy and organic compounds and, at the same time, recycling wastewater.[182] Another factor to consider in order to reduce the cost of production is the location of the plants including the microalgae ocean cultivation.[182] With the aim to monitor with the smallest possible delay the process variables during the microalgae cultivation, new monitoring methods such as ion-specific field effect transistor sensor for physicochemical parameters, biomass concentration by image analysis, lipid concentration by infrared spectrophotometry, and dielectric scattering are proposed.[183]

The biorefinery step involves costs for disrupting the microalgal biomass and for extracting valuable compounds as well. Alternative methods of the current methods to disrupt cells such as hydrodynamic cavitation, electrical fields, electromagnetic fields, and acoustic cavitation were reported.[177] Usually, microalgae are produced for one specific aim, for example, to extract a valuable pigment. A potentially more efficient approach could be a cascade biorefinery system that allows the use of all the possible microalgal compounds.[167]

Another aspect is the selection of robust microalgal strains with increased tolerance to photoinhibition, photosaturation, oxygen saturation, capture and conversion of CO_2, and producing high concentration of compounds useful for the food/nutraceutical sector. Omics technologies are also required to study pathways involved in the valuable compounds production and to understand the role of nutrient depletion and other stress on their production.[184] On the other hand, a possibility is the development and use of genetic-modified microalgae, but this requires consideration of both the regulations about the use of modified organisms and the consumers' acceptance.

Defining the best food formulation and the best way of food ingestion (raw or cooked food) appears of great importance to enhance the bioavailability of the microalgal functional compounds. This presupposes collaboration among phycologists, food engineers, and clinicians to rigorously verify the compounds bioavailability.

The consumer acceptance of formulated new food plays an important role on their success on the market. The acceptance or rejection of novel food depends on different factors[185] such as sociodemographic factors, safety and healthiness perception of novel food, sensory appeal (taste, texture, color, and odor), and consumer's lifestyle. To increase the microalgae-based food/beverages market, it is necessary that consumers trust the novel formulates; therefore, a key factor is the increase of consumers' knowledge about the microalgae used, the possible risks related to their use, the nutritional and health benefits, and the technology of production.

Conclusion

Considering the consumer's demand for novel foods both healthier and sensorial attractive, the modern food industry is oriented to design and produce new food formulations using natural compounds. In this scenario, microalgae have acquired great interest since they confer to the product improved nutritional values, functional properties other than attractiveness.

In the recent years, many investigations on the possible use of different microalgae –biomass and metabolites – in a variety of foods have been carried out. In addition, various microalgae-based products are available on the market all over the world even if the volume of production of these foods remains limited.

To expand the sector of the microalgal fortified/based food and beverages, future needs concern: microbiologists for discovering new microalgae species and their properties, engineers for developing more cost-effective cultivation and biorefinery systems, food scientists for improving sensory appeal, and clinicians for improving compounds availability.

The literature available on this field and the food and beverages currently available on the market give the idea of the great interest for this sector and stimulate the different areas of expertise in studying the microalgae, reducing cost of production, and designing new functional foods and beverages.

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Figure caption

Fig. 1. Possible use of microalgae in foods and beverages.

Fig. 2. Chemical structures of main pigments produced by microalgae. In phycocyanin, the CH=CH2 group indicated by the asterisk is replaced by CH_3–CH_2.

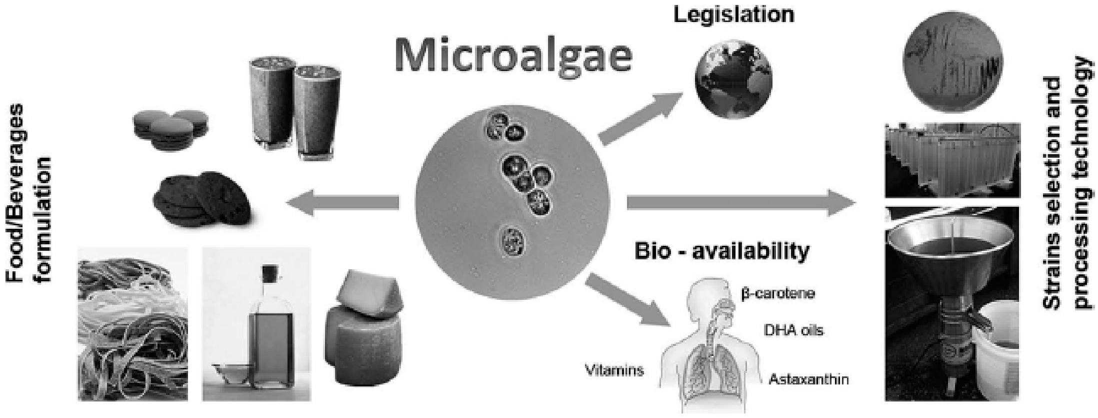
Microalgae
Legislation
Food/Beverages formulation
Strains selection and processing technology
Bio - availability
β-carotene
DHA oils
Vitamins
Astaxanthin

Fig. 1

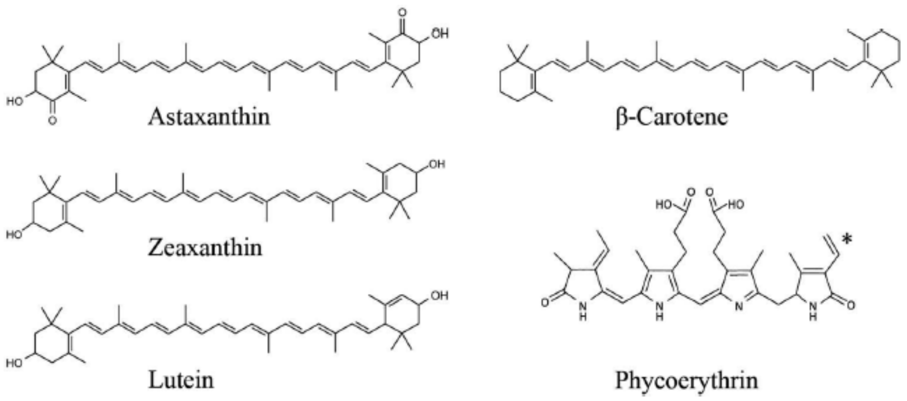

Fig. 2

Table 1. Chemical profile of different microalgae – proteins, carbohydrates, lipids, essential amino acids, and ω-3 fatty acids.

Microalga	**Proteins (% dry matter)[a]**	**Carbohydrates (% dry matter)[a]**	**Lipids (% dry matter)[a]**	**Essential amino acids (g/100 g protein)[b]**									**ω-3 fatty acids (mg/g dry weight)[c]**	
				Ile	Leu	Val	Lys	Met	Cys	Trp	Thr	His	EPA	DHA
Chlorella vulgaris[a]/*Chlorella* sp.[b/c]	51-58	12-17	14-22	4.4	9.2	6.1	8.9	2.2	0.4	-	4.7	2.4	0.4[*]	-
Dunaliella salina	57	32	6	4.2	11.0	5.8	7.0	2.3	1.2	0.7	5.4	1.8	-	-
Scenedesmus obliquus[a,b]/*Scenedesmus* sp.[c]	50-56	10-17	12-14	3.6	7.3	6.0	5.6	1.5	0.6	0.3	5.1	2.1	0.2[*]	-
Arthrospira maxima	60-71	13-16	6-7	6.7	9.8	7.1	4.8	2.5	0.9	0.3	6.2	2.2	-	2.2[*]
Nannochloropsis sp.	47.7	11.2	32.2	4.7	9.4	6.0	6.8	8.4	0.1	-	4.9	2.6	36.8	-
Nitzschia laevis[c]/*Nitzschia closterium*[a,b]	36.2	21.3	35.4	4.8	7.2	5.1	5.6	2.2	0.6	1.7	4.9	2.4	20-40	-
Isochrysis galbana	40.3	19.6	31.1	5.1	9.2	6.1	3.1	2.5	1.0	2.5	4.6	1.7	0.8	15.5
Pavlova sp.[a,c]/*Pavlova lutheri*[b]	24-29	6-9	9-14	4.8	7.7	5.8	6.2	3.1	0.81	3.0	4.5	2.5	18.0	13.2
Phaeodactylum tricornutum	30	8.4	14	4.6	7.0	5.1	6.4	2.7	-	2.6	4.8	1.5	28.4	0.2
Tetraselmis sp.[a,c]/*Tetraselmis chuii*[b]	52	15	16-45	3.4	7.3	4.8	5.6	2.4	-	2.3	4.0	1.6	4.8	0.2
Ulkenia sp.	na	na	na	na	na	na	na	na	na	na	na	na	-	50
Schizochytrium sp.	12.5	38.9	38.9	0.2[†]	0.8[†]	0.3[†]	0.4[†]	< 0.1[†]	< 0.1[†]	< 0.1[†]	0.5[†]	0.1[†]	-	11-24
Crypthecodinium cohnii	12-15	40-50	12-15	na	na	na	na	na	na	na	na	na	-	20-60

[a]References 20, 53–57

[b]Referneces 58–60

[c]References 59, 65–69

[*]Expressed as % total fatty acids

[†]Expressed as % dry weight

na, not available

Table 2. Chemical profile of different microalgae – pigments.

Microalga	Pigments (% dry weight)					
	β-carotene	Astaxanthin	Zeaxanthin	Lutein	Allophycocianin/ C-phycocianin	B-phycoerythrin /R-phycocianin
Dunaliella salina	2.7-12.0	0.2	1.1	0.7	np	np
Haematococcus pluvialis	0.04-0.1	4.0-10.0	na	0.8	np	np
Chlorella zofingiensis	na	1.5->4.0	na	0.4	np	np
Auxenochlorella prototheicoides	na	0.08	na	4.6	np	np
Arthrospira platensis	5×10^{-3}	7×10^{-4}	1.3×10^{-4}	6×10^{-6}	9.5/9.6	na
Porphyridium cruentum	na	na	na	na	na	32.7/11.9

References 13,70

na, not available
np, not present

Table 3. Chemical profile of different microalgae – vitamins.

Microalga	**Vitamins (mg/100 g dry weight)**										
	A	B1	B2	B3	B5	B6	B9	B12	C	E	H
Chlorella	30.8	1.7	4.3	23.8	1.1	1.4	0.094	0.0001	10.4	1.5	-
Isochrysis galbana	127 500*	1.4	3.0	7.8	0.1	0.2	0.3	0.1	11.9	5.8	0.01
Schizochytrium sp.	0.0336	4.4	2.9	14.0	3.5	1.4	0.1	0.0549	-	0.00045	0.3
Arthrospira	0.34	2.4	3.7	12.8	-	0.4	0.094	-	10.1	5.0	-
Tetraselmis suecica	493 750*	3.2	1.9	8.9	3.8	0.3	0.3	0.1	19.1	42.2	0.1
Chlorella stigmatophora	82 300*	1.5	2.0	8.3	2.1	0.2	0.3	0.1	10.0	66.9	0.1
Dunaliella tertiolecta	137 500*	2.9	3.1	7.9	1.3	0.2	0.5	0.1	16.3	11.6	0.1

References 77–79

*Expressed as IU/Kg dry weight (1 IU= 0.6 µg of β-carotene)

Table 4. Microalgae and compounds currently approved as either food or food additives in various countries.

Species/Product	**European Union**	**United States**	**Australia and New Zealand**	**Japan**
Tetraselmis chuii	Regulation (EC) N° 258/97 (80)			
Ulkenia sp.	Decision 2009/777/EC (85)			
Arthrospira sp. (formerly *Spirulina*)		GRAS[1] Notice N° 000101 (2002) (87)		
Phycocyanin from *Arthrospira* sp. (formerly *Spirulina*)				Specifications and Standards for Food Additives (95)
DHA rich oil from *Schizochytrium* sp.	Commission Implementing Decision (EU) 2015/545 (83) Commission Implementing Decision (EU) 2015/546 (83) Commission Implementing Decision 2014/463/EU (84)	GRAS Notice N° 000137 (2003) (88)	Schedule 25 (2017) (94)	
DHA rich oil from *Ulkenia* sp.		GRAS Notice N° 000160 (2004) (89)	Schedule 25 (2017) (94)	
Dunaliella salina		GRAS Notice N° 000330 (2010) (90)		
Auxenochlorella protothecoides		GRAS Notice N° 000351 (2010) (91)		
Chlorella vulgaris		GRAS Notice N° 000396 (2011) (92)		
Chlorella sp.				Specifications and Standards for Food Additives (95)
Euglena gracilis		GRAS Notice N° 697 (2017) (93)		
Carotene from *Dunaliella* sp.				Specifications

Haematococcus algae colour	and Standards for Food Additives (95)

[1]Generally Recognised As Safe

Table 5. Examples of microalgal food and beverages present on the worldwide market.

Microalgae/ Micro-algal compounds	Genus/Specie	Product/ percentage of incorporation	Company	Health aspects	Technological aspects	References
Dried biomass - AlgaVia® Lipid-Rich Whole Algae	*Auxenochlorella protothecoides*	Sauce (3%) Dressing (1%) Bakery (4%)	Corbion (The Netherlands)	Reduced saturated fat, rich in fibre, proteins, micronutrients, monounsaturated fatty acids	Enhance taste and texture, emulsification, water binding	http://algavia.com/wp-content/uploads/2017/09/AlgaVia-Lipid-Rich.pdf
Extracted oil - AlgaWise®	Not available	Food oil	Corbion (The Netherlands)	Monounstaurated fats and fatty acids (93%),saturated fat (4%), ω-9s	High heat cooking	http://algawise.com/
		Algae butter		Reduced saturated fat, no *trans* fat	Sharp melting properties	
Dried biomass	*Arthrospira* (formerly *Spirulina*)	Chocolate bars biscuits, pasta (2%)	Microlife Nutrition (It)	Rich in iron, β-carotene, A,B, K, proteins		https://microlife.bio/collections/alimentazione

		Pasta (2-10%)	Nutracentis (It), La finestra sul cielo (It), Cornand (Fr), GlobeXplore (Fr)			http://www.nutracentis.com/, http://lafinestrasulcielo.it/it/prodotti/pasta-e-condimenti/pasta-speciale/pasta-alla-spirulina/1/, http://www.cornand.fr/FR/les-pates-haute-couture/pates-laminees.htm, https://www.algues.fr/fr/boutique/tagliatellines-spiruline
Phycocyanin	*Phorphyridium aerugineum*	Pepsi®, Bacardi Breezer® (na)	PepsiCo. (USA), Bacardi & Co Ltd (Bermuda)		Colour stability	(45)
	Arthrospira (formerly *Spirulina*)	Linablue® (0.01-0.1%)	DIC Corporation (Japan)		Colour/antioxidant	http://www.dic-global.com/us/en/products/health_foods/
EPA/DHA	*Chrypthecodinium*	Food ingredient	Martek/DSM (USA/NL)	Anti-inflammatory, Protection against cardiovascular disease, atherosclerosis	Avoiding fish oil taste	(3)
	Nannochloropsis		Blue Biotech (Germany)			
	Odontella		InnovaIG (Fr)			
	Schizochytrium		Xiamen Huison Biotech Co. (China)			
	Not available		Cellana (Hawaii)			http://cellana.com

β-carotene	*Dunaliella*	Food additive, colourant	Cognis Australia BASF (Au/DE) Tianjin Lantai Laboratory (China) Nature Beta Technologies (Israel) Pro Algen (India)	Anti-inflammatory Antioxidant	Natural colour	(3)
Astaxanthin	*Haematococcus*	Food ingredient, additive	Algatech (India) Blue Biotech (Germany) Fuji Chemicals (Japan) Mera Pharma (USA) BioReal (Sweden)	Anti-inflammatory Antioxidant		(3)

na, not available