Journal of Engineering Science *Vol. XXVII, no. 1 (2020), pp. 75 - 98*
Fascicle *Food Engineering* *ISSN 2587-3474*
Topic *Biotechnologies, Food Chemistry and Food Safety* *eISSN 2587-3482*

DOI: 10.5281/zenodo.3713372
CZU 551.464.7:664

MICROALGAE – NON-TRADITIONAL SOURCES OF NUTRITIENTS AND PIGMENTS FOR FUNCTIONAL FOODS

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Received: 12.18.2019
Accepted: 02.28.2020

Abstract. The aim of this review is to draw the attention of researchers and technological engineers from the Moldovan food industry towards the potential of microalgae as a non-traditional source of nutrients and biological active substances, such as proteins, essential amino acids, carotenoids, vitamins, polyunsaturated fatty acids ω_3, phytosterols, polysaccharides, phenolic acids, microelements, etc., which can be used to increase the nutritional and functional value of conventional foods. The study synthesizes information regarding the profile of biologically active substances obtained from various microalgae species, analyses the nutritional value of microalgae biomass and their field of application. This review focuses on pigments contained in microalgae (carotenoids, chlorophylls and phycobiliproteins), deals with their biological activity and health benefits. It draws attention to the results of the recent research, which proves that microalgae pigments exhibit pronounced antioxidant properties, protect cells from the radiation, capture free radicals and reduce the oxidative stress in the body, prevent cancer, inflammation and cardiovascular diseases, modulate the immune system, prevent the macular degeneration, etc. Review describes in more detail the carotenoids class and elucidates the qualitative and quantitative content of carotenoids in some microalgae. It discusses the areas of use of the pigments accumulated in microalgae and their further application as natural food additives and dyes.

Keywords: *bioactive compounds, carotenoids, chlorophylls, health benefits, food additives, phycobiliproteins, phytonutrients, xanthophylls.*

Introduction

One of the food industry objectives is elaboration and implementation in production of functional foods, which could bring health benefits to population. Functional foods are obtained by adding lacking functional ingredients or phytonutrients to conventional products, consumed on a daily basis.

Functional ingredients, which are not synthesized by the human body, are essential and must be supplied with food.

Besides the traditional sources of phytonutrients, (vegetables, fruits, berries, plants, etc.), microalgae are in the spotlight due to their high nutritional value and the ability to synthesize biologically active substances with a varied structure: carotenoids, proteins, essential amino acids, vitamins, polyunsaturated fatty acids ω_3, polyphenols, phytosterols, polysaccharides, sulfur compounds, microelements, etc. [1 - 8]. Research has proven the health benefits of microalgal metabolites, for example the pigments from microalgae – carotenoids, chlorophylls and phycobiliproteins exhibit pronounced antioxidant properties, protect cells from the ultraviolet radiation, capture free radicals and reduce the oxidative stress in the body, prevent cancer, inflammation and cardiovascular diseases, modulate the immune system, prevent the macular degeneration and are widely applied in the food, cosmetic and pharmaceutical industries, etc. [9 - 13]. Carotenoids with a varied structure, in much higher concentrations compared to traditional sources, can be obtained from microalgae [3]. Most of the carotenoids suggested by the pharmaceutical industry are chemically synthesized, but due to the side-effects associated with medicine administration, public interest in recent times has focused on natural products with health-promoting effects as alternatives to conventional drugs [12]. Furthermore, pigments from microalgae are natural colorants, vitamin precursors, harmless to the human body and can substitute adverse, synthetic additives and noxious dyes used by the food industry [1, 4, 11].

Nevertheless, despite being one of the richest sources in phytonutrients, with a high nutritional value and health benefits, the microalgae's potential is not fully explored. Consequently, the modern food industry's current objectives are to use the microalgae as a renewable source of bioactive substances and natural colorants, obtain and establish the chemical structure of the new compounds, study the biological activity and the technological requirements regarding the optimization and implementation of phytonutrients in the production of functional foods.

Microalgae – organisms with a promising potential

The term "Microalgae" includes cyanobacteria and eukaryotic microorganisms, which are microscopic aquatic organisms, and similar to terrestrial plants, use the solar energy and carbon dioxide for photosynthesis and produce a wide variety of complex substances and biologically active compounds. There are microalgae that have their habitat in the soil. Of the 200.000 estimated species for microalgae, up to 50.000 have been described so far [1 - 4].

The aquatic microalgae, some of the oldest terrestrial organisms, are adapted to survive in adverse environmental conditions – wastewater, waters with varied salinity, high or low temperatures, *UV* radiation, environments with varied nutrients' availability, etc., producing chemicals of various structures [3 - 6]. They are characterized by the ability to grow and multiply rapidly, within a short life cycle, also being easy to manipulate. Microalgae's efficiency in fixing the carbon dioxide (CO_2) in the atmosphere is 10 to 50 times higher than that of terrestrial plants [14]. Microalgae are also responsible for the circuit in nature of elements such as sulfur, phosphorus, carbon, nitrogen and microelements. They consume nutrients from wastewater, including chemicals and heavy metals. Over 99,9% of algal biomass is mainly made from: *C*, *N*, *S*, *P*, *O*, and *H*, sodium (*Na*), potassium (*K*), calcium (*Ca*), magnesium (*Mg*), iron (*Fe*), chlorine (*Cl*), silicon (*Si*), and other traces of elements. Thus, the cultivation of microalgae reduces the level of pollutants in the air and in the aquatic environment [15].

Being one of the most important trophic links in the aquatic ecosystems, microalgae are a renewable, valuable source of biologically active substances, highly cultivated in recent years and used in the food, pharmaceutical, cosmetic, medical, agricultural, zootechnical, poultry, fish industries, etc., for their high content of lipids, fatty acids, essential acids, steroids, carotenoids, pigments, polyphenols, vitamins, oligosaccharides, polysaccharides, amino acids, proteins, halogenated compounds, sulfur compounds, microelements, etc. [1 - 11]. Most microalgae accumulate metabolites in biomass; some, known for their high lipid content, are being researched as an ecological, renewable source of fuel – biodiesel [5 - 6].

Microalgae – a source of functional ingredients

Nowadays, there is a growing demand for functional foods, beneficial for the human body. The functional foods are obtained by adding the functional ingredients or micronutrients lacking in the daily consumed conventional food [16]. Functional ingredients such as essential fatty acids (*ω-3*, *ω-6*), phytosterols, prebiotics and probiotics, carotenoids, polyphenols, vitamins, etc., are not synthetized in the human body, being essential, they must be supplied with food.

A healthy lifestyle implies a balanced diet that includes phytonutrients delivered both from traditional sources (vegetables, fruits, berries, plants, etc.) as well as from non-traditional sources such as microalgae. The Chinese have been consuming the *Nostoc* microalgae species for more than 2000 years [3], lately; species such as *Spirulina* and *Chlorella* have been introduced as functional foods in Japan, Taiwan and Mexico [6 - 10]. Currently, the most consumed microalgae belong to the species *Spirulina sp., Chlorella sp., Dunaliella terticola, D. salina, Odontella aurita* and *Aphanizomenon flos-aquae,* due to their high nutritional value and high protein content [17].

Microalgae biomass is regarded as a superior source of phytonutrients and antioxidants, not only because of the greater productivity of microalgae, compared to conventional terrestrial sources but also because of the content of bioactive substances in the cell, estimated as follows: 8–14% pigments, 12–30% carbohydrates, 4–20% lipids, 40–70% proteins and significant amounts of vitamins *A*, *C*, B_1, B_2, B_{12}, *E*, *K*, and *D* [7].

Table 1 lists the biologically active substances that are obtained today from microalgae [6-9, 17-19].

Table 1

Major bioactive compounds extracted from microalgae

Microalgae	Bioactive compounds
Botryococcus braunii	Linear alkadienes (C_{25}, C_{27}, C_{29} and C_{31}), alkatrienes (C_{29})
Chlorella ellipsoidea	Zeaxanthin, violaxanthin
Chlorella minutissima, Nanochloropsis, Nitzschia, Phaeodactylum and Odontella aurita	Eicosapentaenoic acid (*EPA*)
Chlorella protothecoides	Lutein, zeaxanthin, canthaxanthin
Chlorella pyrenoidosa	Lutein, sulfated polysaccharides
Chlorella sp.	Carotenoids, sulfated polysaccharides, sterols, polyunsaturated fatty acids (*PUFA*s)
Chlorella vulgaris	Astaxanthin, canthaxanthin, peptide, oleic acid

Continuation Table 1

Chlorella zofingiensis	Astaxanthin
Crypthecodinium cohnii, Schizochytrium spp.	Docosahexaenoic acid (*DHA*)
Dunaliella salina	Trans-*β*-carotene, cis-*β*-carotene, *β*-carotene, oleic acid, linoleic acid, palmitic acid
Dunaliella spp.	Diacylglycerols
Haematococcus pluvialis	Astaxanthin, lutein, zeaxanthin, canthaxanthin, *β*-carotene, oleic acid
Isochrysis galbana, Phaedactylum tricomutum	Lipids, fatty acids
Nostoc linckia, Nostoc spongiaeforme	Borophycin
Nostoc sp.	Cryptophycin
Porphyridium sp.	Phycobiliproteins
Spirulina fusiformis	Diacylglycerols
Spirulina platensis	Phycocyanin, C-phycocyanin, phenolic acids, tocopherols, vitamin *E*, neophytadiene, phytol, *PUFAs*, oleic acid, linolenic acid, palmitoleic acid
Spirulina sp.and Porphyridium cruentum	Polysaccharides

Microalgae are used in the food industry for the high protein content, similar to that from traditional sources, but having a higher quality than the proteins from oat, rice, soy and legumes, thus lower compared to meat, milk and fish proteins [6].

According to bibliographic sources, microalgae produce 4-15 tons of protein per hectare annually, whereas legumes yield 1-2 tons/Ha per year, and soy – 0.6-1.2 tons/Ha per year [9]. Moreover, microalgae are an excellent source of ω_3 polyunsaturated fatty acids in their most active form – *α*-linolenic essential acids (*ALA*), eicosapentaenoic (*EPA*) and docosahexaenoic (*DHA*) [10, 19], minerals and vitamins [2, 13, 20].

Algae dried biomass is easily digested by the human body. In vitro investigations through the enzymatic method with application of pepsin and pancreatin, have shown that *Arthrospira platensis (spirulina*), *Chlorella sorokiniana IAM-C212* and *Chlorella vulgaris* had the highest digestibility, while *Tetraselmis suecica, Phaeodactylum tricornutum*, and *Porphyridium purpureum* were the least digestible, likely because of the presence of robust cell walls or of exopolysaccharides that might had limited the action of digestive enzymes [3].

Nowadays, *Chlorella* and *Spirulina* species are consumed as functional foods in dry form, tablets or encapsulated, etc. *Spirulina* has been called a “superfood” due to the high content of nutrients, of which up to 70% of the dry mass being protein [20 - 23].

Spirulina is also used for its antioxidant, immunostimulatory and cholesterol-lowering properties; while the sulfated polysaccharides contained in biomass act as antiviral agents.

Spirulina has been shown to contain 6.7 times more protein than tofu, 1.8 times more calcium than milk, 51 times more iron than spinach and 31 times more carotenoids than carrot [20, 21].

Table 2 shows the mass parts of proteins, lipids and carbohydrates in some of the cultivated microalgae species [9, 22, 23].

Table 2

Composition of microalgae species in percentage of dry biomass matter

Microalgae sp.	Composition, % of dry matter		
	Protein	**Lipids**	**Carbohydrates**
Chlorella vulgaris	51-58	14-22	12-17
Chlorella pyrenoidosa	57	2	26
Chlamydomonas rheinhardii	48	21	17
Dunaliella salina	57	6	32
Dunaliella bioculata	49	8	4
Haematococcus pluvialis	48	15	27
Isochrysis galbana	50-56	12-14	10-17
Euglena gracilis	39-61	22-38	14-18
Pophyridium cruentum	28-39	9-14	40-57
Prymnesium parvum	28-45	22-38	25-33
Spirulina maxima	60-71	6-7	13-16
Spirulina platensis	46-63	4-9	8-14
Scenedesmus obliquus	50-56	12-14	10-17
Synechococcus spp.	63	11	15
Tetraselmis maculata	52	3	15

Chlorella, in addition to valuable nutrients, contains *β*-1,3-glucans, which stimulate the immunity, decrease the blood triglyceride concentration, capture free radicals and eliminate the toxins [23].

The concentration of provitamin *A*, vitamins *E*, B_1 and folic acid in microalgae is higher compared to the traditional sources: *Dunaliella tertiolecta* synthesizes vitamins B_{12} (cobalamin), B_2 (riboflavin), vitamin *E* (tocopherol) and provitamin *A* (*β*- carotene). *Tetraselmis suecica* is an excellent source of vitamins B_1 (thiamin), B_3 (nicotinic acid), B_5 (pantothenic acid), B_6 (pyridoxine) and vitamin *C* (ascorbic acid) [24].

Scientific research confirms that *Chlorella* species contain vitamins B_7 (biotin) and B_{12} in high concentrations [16]. Table 3 analyses the vitamins profile of *Spirulina* and *Chlorella* algae species [25].

Table 3

Vitamin profile of two species of microalgae: *Spirulina* and *Chlorella*

(in mg kg-1 unless otherwise stated)

Vitamins	***Spirulina***	***Chlorella***	**Vitamins**	***Spirulina***	***Chlorella***
Provitamin A	2330000 IU kg^{-1}	55500 IU kg^{-1}	Vitamin B_6	8	17
β-carotene	1400	1808	Vitamin B_{12}	3.2	1259
Vitamin E	100	<10 IU kg^{-1}	Inositol	640	1650
Thiamin B_1	35	15	Folic acid	0.1	296
Riboflavin B_2	40	48	Biotin	0.05	1916
Niacin B_3	140	238	Pantothenic acid	1	13

According to the 1997 Regulation, the EU included some microalgae in the list of foods authorized in the EU market. The list included *Aphanizomenon flos-aquae* from

Klamath Lake, *Arthrospira platensis, Chlorella luteoviridis, C. pyrenoidosa, and C. vulgaris. Odontella aurita, Tetraselmis chuii* and astaxanthin from *Haematococcus pluvialis* were successively approved as food or food ingredients [26].

In order to increase the nutritional and functional value of the conventional foods, microalgae and microalgae phytonutrients are used to enrich pasta, bakery products, snack foods, confectionery, sweets, beverages, dairy products, etc. For example, the dry biomass of *A. platensis (Spirulina*) can be incorporated in pasta flour up to 20% and up to 8.36% in biscuits; dry biomass of *Dunaliella* (without β-carotene and glycerol) up to 10% in bread flour; astaxanthin can be incorporated in cake flour up to 15%; *Chlorella sp*. Dry biomass can be added to yogurt in a proportion of up to 10% [9]. Microalgae are also and excellent source of nutrition for fish and aquatic organisms, for animals, cattle, swine, poultry, etc. [8, 10].

Pigments from microalgae

As mentioned above, microalgae are an excellent source of ecological and renewable pigments. They determine the microalgae's specific colour: e.g. the green colour is due to chlorophyll, the red and blue colours are due to phycobiliproteins, the yellow, orange and red colours – carotenoids synthesized by microalgae.

Microalgae are also classified by colour, e. g. *Chlorophyceae* (green color), *Rhodophyceae* (red color), *Cyanophyceae* (blue green), and *Pheophyceae* (brown). In most algae the predominant pigments are: chlorophyll *a, b* and *c, β*-carotene, xanthophylls, phycocyanin and phycoerythrin. Currently, pigments with predominant content are obtained from certain microalgae species, for example *β*-carotene is obtained from *Dunaliella salina* [27 - 29], astaxanthin is obtained from *Hematococcus pluvialis* and *Chlorella* species [30]; fucoxanthin is obtained from *Muriellopsis* and *Isochrysis aff. Galbana* [31], zeaxanthin from *Dunaliella salina* [28], phycobiliproteins- phycoerythrin and phycocyanin from *Porphyridium* and *Anabaena* species [32 - 34].

Pigments from microalgae - carotenoids, chlorophylls and phycobiliproteins are biologically active compounds, vitamin precursors in human diet and animal feed, and can be also used as additives and natural dyes in the food, cosmetic and pharmaceutical industries, etc. [35].

Different methods of obtaining pigments from microalgae biomass have been developed. As mentioned above, pigments in the plant cells are found not only in the form of free but also glycosylated, esterified with fatty acids or as protein complexes.

For a better extraction it is necessary to lysate the microalgae cell walls and to release the contained bioactive substances. Cell lysis and pigment extraction can be accomplished by several methods, which can be further combined: a) mechanical grinding; b) milling; c) freeze-thaw; d) ultrasonic assisted extraction; e) microwave extraction; f) supercritical fluids extraction; g) pulse electric field extraction; h) enzyme assisted extraction; i) organic solvent extraction.

These methods have advantages and disadvantages, described in the bibliographical sources [36].

Supercritical fluids extraction (CO_2 and ethanol) and the use of non-toxic and non-flammable, recyclable solvents are increasingly applied for the full use of the microalgae biomass, which, besides the pigments, contains various valuable biologically active compounds.

Carotenoids, structure and classification

Carotenoids, also called tetraterpenoids, are the most widespread class of photosynthetic pigments synthesized by plants, algae, fungi and cyanobacteria. Some fungi and insects (aphids, mites) generate carotenoids through other mechanisms [37, 38]. Animals do not synthesize carotenoids in their bodies; they are delivered with food and stored in the adipose tissues. It is well known the vital importance of carotenoids obtained from food for the development and normal functioning of the human body.

Up to 1100 carotenoid pigments are known today; they are responsible for the yellow, orange and red colors in plants, flowers, algae, fruits, vegetables, berries, etc., [36-38]. The carotenoids contained in the body tissues can be in the form of free, glycosylated, esterified with fatty acids or as protein complexes [36].

Most carotenoids are tetraterpenoids composed of 8 molecules of isoprene (2-methyl-buta-1,3-diene), $(C_5H_8)_8$ and contain 40 carbon atoms in the molecule. The simplest carotene is lycopene with a hydrocarbon chain (consisting only of a carbon and a hydrogen), which contains double conjugates bonds Figure 1.

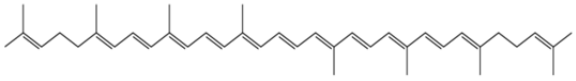
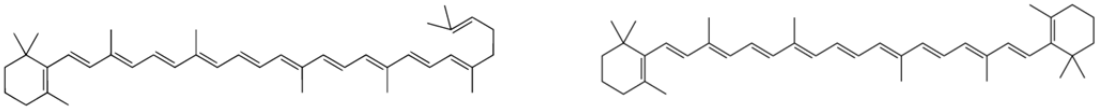


Figure 1. Lycopene.

At the ends of the hydrocarbon chain, acyclic or cyclic groups can be formed: *γ*-carotene, *β*-carotene, etc., Figure 2.



a) b)

Figure 2. a) *γ*-carotene; b) *β*-carotene.

At the double bonds between the polyene carbon atoms, in the chain, the configuration is trans (*E*) and must be indicated in the name, e. g.: (*all-E)-lycopene*. Based on the chemical structure, carotenoids are divided into two major classes:

1) **carotenes** – are the carotenoids composed only from atoms of carbon and hydrogen: *α*-carotene, *β*-carotene, *γ*-carotene and lycopene;
2) **xanthophylls –** oxygenated carotenoids: lutein, contains hydroxyl groups (alcoholic); canthaxanthin, with carbonyl groups (ketone); astaxanthin contains both functional groups (alcoholic and ketone); fucoxanthin with esteric groups, Figure 3.

Apart from these two major classes, carotenoids with less than 40 carbon atoms in the molecule are also known: apocarotenoids, which are formed by oxidative cleavage, as would be the abscisic acid (plant hormone), retinol (vitamin A_1), retinoic acid, etc. [5]. *C50* Carotenoids, with more than 50 carbon atoms in the molecule, have a complex structure, often being glycosylated. For example, soil bacteria (*Corynebacterium glutamicum*) are unique in the production of the *C50* decaprenoxanthin carotenoid and its glycosylated forms [36, 37], Figure 3.

Carotenoids are also classified into **provitamins A**, which can be converted in the body into retinol, such as *α*-carotene, *β*-carotene, *γ*-carotene and *β*-cryptoxanthin and **non-provitamins *A***, such as lutein, zeaxanthin and lycopene [36].

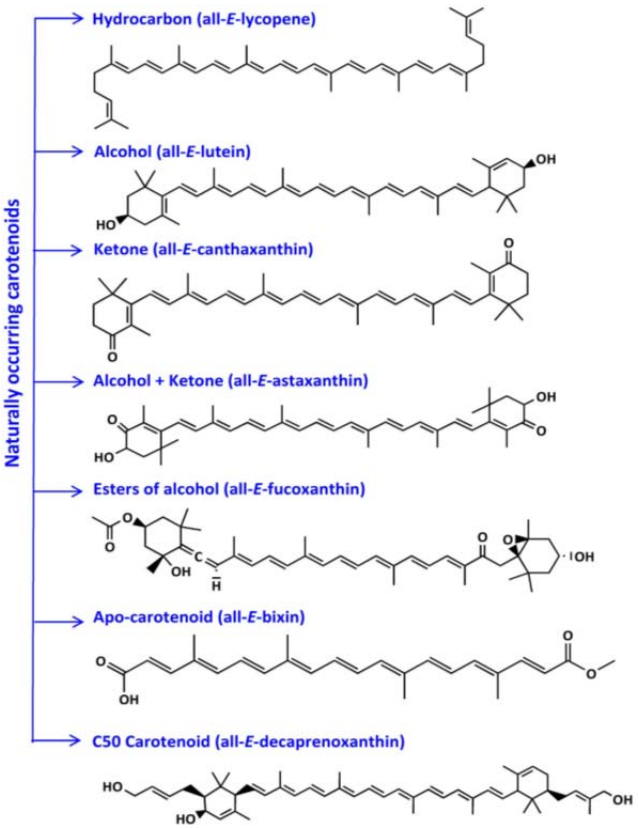


Figure 3. Carotenoids widespread in nature [36].

The biological activity of the carotenoids

Carotenoids are lipophilic pigments, soluble in lipids and non-polar organic solvents, with a high antioxidant activity, playing a key role in plant photosynthesis. The structure of carbon chains with conjugated double bonds determines the biological role of the carotenoids, which consists in sending the energy from the absorbed light straight to the chlorophyll molecules or in transporting the energy from one chlorophyll molecule to another, moreover, carotenoids protect plant cells from the effects of excess light exposure by scavenging reactive oxygen species (*ROS*), such as singlet oxygen molecules and free radicals in the process of photosynthesis [37, 39, 40].

Carotenoids from plants are important biologically active phytonutrients for the animal organisms, having multiple effects, including provitamin *A* activity. In the human body, *β*-carotene is converted into two molecules of vitamin A, while α-, γ-carotene and β-cryptoxanthin in one molecule of vitamin *A* [40, 41]. In the human body, the carotenoids supplied from food have pronounced antioxidant activity and reduce the oxidative stress by capturing free radicals [42]. It is well known that *ROS*, formed under stress conditions, damage tissues, causing so inflammation, attack the neutrophils and form superoxide radicals that lead to lipid peroxidation and cell membrane lysis [43 - 45]. Carotenoids inhibit free radicals by electron transfer (which occurs due to the conjugated double bonds), by assignment of hydrogen atoms to free radicals or by binding the free radicals. Scientific research has proved that carotenoids can interact directly with some types of free radicals, eliminate the *ROS* from the environment, at the same time preventing their further formation [43, 46, 47].

The therapeutic value of carotenoid has been elucidated [47], and it includes the prevention and treatment of the chronic inflammatory diseases [43, 47], cancer, cardiovascular [48], renal, pulmonary, liver, intestinal diseases, the treatment of metabolic disorders, autoimmune diseases, HIV, sepsis, multiple sclerosis, atherosclerosis, etc. [49 - 55]. It was established that in the treatment of cancer, cardiovascular diseases and eye disorders, the administration of *β*-carotene, lycopene, lutein and zeaxanthin is extremely important [56]. However, it has also been established that administering synthetic *β*-carotene to smokers stimulates the lung cancer [57].

Carotenoids contained in the eye retina – lutein and zeaxanthin, prevent retinal damage and protect it against light and ultraviolet radiation [55, 56]. The same photoprotective effect slows down the aging process of the body and skin and prevents photodermatitis and skin cancer [58]. Due to the antioxidant, photoprotective properties, carotenoids are widely used in the cosmetic industry [1, 4, 5].

Carotenoids from microalgae

Characterized by diversity, some structures not being found in the traditional sources, Tables 4, 5. Microalgae synthesize both carotenoids and xanthophylls found in terrestrial sources (*β*-carotene, lutein, zeaxanthin, antheraxanthin) as well as microalgae specific pigments (e.g., astaxanthin, fucoxanthin, diatoxanthin, diadinoxanthin) [4, 11].

Microalgae carotenoids can be classified into two categories. Primary carotenoids, which are the components of the photosynthetic apparatus, essential for survival and secondary carotenoids, which are produced under specific environmental conditions (high light intensity, *UV* radiation, nutrient variation, salinity, etc.), for cell protection. Lycopene, *α*-carotene and *β*-carotene are primary carotenoids. Secondary carotenoids are synthesized from primary carotenoids under specific conditions of cultivation or under the action of abiotic factors. When triggering the photoprotective mechanism, called the xanthophyll cycle, lutein, zeaxanthin, astaxanthin, canthaxanthin, antheraxanthin, violaxanthin, neoxanthin, fucoxanthin, diadinoxanthin, diatoxanthin, etc. are formed [59].

In comparison with traditional sources, microalgae have the ability to synthesize carotenoids with a varied structure in much higher concentrations Table 4. In the *Phormidium autumnale* microalgae were identified 24 types of carotenoids, of which *β*-carotene, lutein and zeaxanthin with major content, reaching a concentration of 225.44 $\mu g.g^{-1}$, 117.56 $\mu g.g^{-1}$ and 88.46 $\mu g.g^{-1}$ of biomass, respectively [60]. A single species of microalgae is able of producing several active components; for example, *Chlorella Sorokiniana* produces carotenoids with the mass of 0.59% from the dry matter, and *α*-tocopherol, *β*-carotene and lutein content of 112, 600 and 4300 $\mu g.g^{-1}$ of dry matter, respectively [61].

The market demand for carotenoids obtained from natural sources is constantly increasing, especially for *β*-carotene, lutein, astaxanthin, zeaxanthin and fucoxanthin [3,9]. Currently, the largest microalgae producing companies are widely cultivating the *Spirulina, Chlorella, Dunaliella, Haematococcus, Aphanizomenon flos-aquae* species and the red microalgae of the *Porphyridium* genus [11] both for their high nutritional value, as well as for the increased content of pigments and other biologically active substances.

The commercial source of lutein is the petals of Marigold flowers (the genus *Calendula*), although the research has shown that microalgae have 3 - 6 times more lutein per unit mass [62]. The green species of *Chlorella* produce lutein, violaxanthin and

zeaxanthin [63]; the green algae *Dunaliella salina* produce a large quantity of carotenoids that can be converted to pro-vitamin *A* in the human body [27 - 29]. Usually, most microalgae synthesize *β*-carotene and other types of carotenoids, some are major components in the biomass extracts [12], Table 4.

Table 4

Carotenoids produced by microalgae

Microalgae Species	Major Carotenoid	Other Carotenoids	Concentration % (TC/DW)
Chlorella sp.	Lutein	Astaxanthin	0.23
Chlorella ellipsodea	Violaxanthin	Antheraxanthin, zeaxanthin	-
Chlorella Sorokiniana	*β*-carotene	Lutein	0.59
Chlorella pyrenoidosa	Lutein	Violaxanthin, Loroxanthin, *β*-carotene, *α*-carotene	0.2-0.4
Chlorella vulgaris	Canthaxanthin, lutein	Astaxanthin, violaxanthin	-
Chlorella zofingiensis	*β*-carotene	Canthaxanthin, astaxanthin	3.7
Chlorococcum humicola	Violaxanthin	Astaxanthin, lutein, zeaxanthin, *β*-carotene, *α*-carotene	-
Coelastrella striolata var. Multistriata	Canthaxanthin	Astaxanthin, *β*-carotene	4.75
Dunaliella salina	*β*-carotene	Zeaxanthin, lutein, *α*-carotene	10-13
Haematococcus pluvialis	Astaxanthin	*β*-carotene, lutein, canthaxanthin, neoxanthin, violaxanthin, zeaxanthin, echinenone	6.0
Isochrysis aff. galbana	Fucoxanthin		1.8
Odontella aurita	Fucoxanthin	Diadinoxanthin, *β*-carotene	2.2
Phaeodactylum tricornutum	Fucoxanthin	Diadinoxanthin, zeaxanthin, neoxanthin, violaxanthin, *β*-carotene	1.65
Porphyridium cruentum	Zeaxanthin	*β*-carotene	Zeax. 97.4 from TC
Scenedesmus sp.	Lutein	Astaxanthin	0.18
Spirulina maxima	*β*-carotene	Astaxanthin, lutein, *β*-cryptoxanthin, zeaxanthin, echinenone, oscillaxanthin, myxoxanthophyll	<0.8
Spirulina pacifica	*β*-carotene	Cryptoxanthin, zeaxanthin	-

Note: TC—Total carotenoids; DW—Dry weight of microalgae

β-carotene

It is the orange-red colour pigment, soluble in non-polar solvent (carbon disulfide, benzene, hexane, chloroform). Currently it is obtained from *Dunaliella salina* and *D. bardawil*

microalgae, where it is contained in a larger quantity (3-5% of the dry mass), compared to other sources [65 - 67]. Recent studies show that the *β*-carotene quantity produced by microalgae can be increased by modifying the cultivation conditions – high salinity, high light intensity, *UV* radiation, extreme temperatures and varying the nutrient content of the environment [28, 66, 67]. Researchers have determined that with modifying the cultivation conditions, *Dunaliella salina* can accumulate up to 10 - 14% of *β*-carotene in dry mass [27]. Both *cis-* and *trans-* forms of *β*-carotene, with a high bioavailability and bio efficiency [68] as well as oxygenated carotenoids (xanthophylls) [28] are contained in the *Dunaliella* species. *Spirulina* species accumulate 0.8–1.0% of *β*-carotene dry mass and *Rhodophyta* accumulate *α* and *β*-carotene and their hydroxylated derivatives [65].

The health benefits of the *β*-carotene derived from microalgae have been proved [41]. The natural form of *β*-carotene obtained from microalgae is easily assimilated by the body, has higher bio efficiency and has no adverse effects as compared to a synthetic form [69, 70]. Some researchers claim that the natural sources contain only one or two types of carotenoids in low concentrations and that they do not meet all the requirements [12]. Nevertheless, microalgae contain different carotenoids in high concentrations with a broad applicability. The purified *β*-carotene extract of *D. salina,* for example, is accompanied by other carotenoids with pronounced beneficial effects, in particular: lutein, neoxanthin, zeaxanthin, violaxanthin, cryptoxanthin and *α*-carotene Table 4. Research has shown the adverse effects of synthetically obtained *β*-carotene, which administered in high pharmacologic doses (30mg) for a long period of time, increase the probability of lung cancer in smokers. The reason might be *β*-carotene's tendency to form apocarotenal by oxidative cleavage, suspected of causing cancer [71].

Research has shown that the administration of *β*-carotene from *Dunaliella* microalgae inhibits the oxidation of low-density lipoprotein (*LDL*) and influences plasma triglycerides, cholesterol and high-density lipoprotein (*HDL*) levels [16, 17, 72], prevents atherosclerosis, protects the cells from oxidative stress [73], boosts the immunity [74], prevents cancer, macular degeneration, asthma and other degenerative diseases [6, 19, 75]. *β*-carotene and other carotenoids, such as phytoene and phtytofluene, from *Dunaliella*, provide health benefits through antioxidant effects, photoprotection against the UV radiation, prevention of premature aging and other disorders [27, 29]. It was proved that the oral intake of *β*-carotene from *Dunaliella spp*. can prevent UV-induced erythema in humans [76].

The fields of application of carotenoids derived from microalgae are diverse, *β*-carotene being one of the most important carotenoids, which besides the pronounced antioxidant properties, when in the human body, converts into two molecules of vitamin *A* [41]. It is included in multi-vitamin complexes and other supplements. Oily, purified extracts of carotenoids from microalgae are sold in containers or in capsules [64]. Due to its antioxidant and photoprotective properties, along the suppressing of the aging effect the UV radiation has on the skin, microalgae derived *β*-carotene is largely used in the cosmetic industry, in creams, skin care lotions, hair care and as a natural dye [77]. Dry microalgae biomass and *β*-carotene enriched extracts are used to feed animals, cattle, poultry, fish, shellfish, etc. Microalgae is behind the color of the aquatic organisms (salmon, shellfish) and the egg yolk [5, 8, 12].

Nowadays, the commercialized *β*-carotene, used in pharmaceutical and food industries is chemically synthesized. *β*-carotene is the most common colorant and natural

food additive, with E number *E160a*, used to increase the appeal of foods, beverages, dairy, bakery, confectionery, spices, etc. [3, 9, 16].

Astaxanthin

Keto-carotenoid, a red-orange pigment, lipid-soluble, with a major content in the freshwater microalgae *Haematococcus pluvialis*, with a 4-6% of its dry matter [78,79]. This keto-carotenoid has a higher degree of stability. Astaxanthin is largely contained in the thick-walled *Haematococcus* alanospore cells and to make it bioavailable, the cell wall has to be destroyed [35, 36].

Cultivating *H. pluvialis* species outdoors may lead to water contamination with other microorganisms; therefore, researchers prefer to use photobioreactors [17]. *Chlorella zofingiensis* microalgae, under stress conditions (light, radiation, nitrogen limitation) synthesize astaxanthin, but to a lesser extent than *Haematococcus* [80].

Astaxanthin, promoted as a multi-benefit dietary supplement, has a 100-fold higher antioxidant activity compared to α-tocopherol, regarding the protective effects against lipid peroxidation [77, 78, 81] and a 10-fold higher compared to β-carotene as a scavenger of various reactive species, being considered the super vitamin *E* [82]. Astaxanthin is not converted to vitamin *A* in the human body so it is completely nontoxic if given orally. Besides, when antioxidant activity of various microalgae extracts was tested in Human Umbilical Vein Endothelial Cells cells, the antioxidative cell protection was almost 90 times higher with the natural astaxanthin containing esters than with the synthetic xanthophyll [83]. Astaxanthin is also an antiglycant and is able to protect proteins from glycation [84].

Research has shown that astaxanthin reduces the carcinogenic effect of aflatoxins [85], protects cells from radiation [75], prevents atherosclerosis and cardiovascular diseases [78, 81, 86]. A study carried out on humans in an age group of 25–60 years showed that 12 weeks of astaxanthin administration significantly decreased serum triglyceride levels, while significantly increasing *HDL*-cholesterol levels. However, *LDL*-cholesterol levels remained unchanged [87]. Several studies have shown the chemo preventive effect of astaxanthin and its role in fighting chronic inflammation, metabolic disorders and eye diseases [88]. Some researchers have reported that *Helicobacter pylori*-infected mice fed with astaxanthin extracted from the microalga *H. pluvialis*, showed reduced levels of gastric inflammation [89]. It has been proved that astaxanthin has an immunomodulatory effect, suppresses the development of carcinogenic cells, boosts the production of immunoglobulin and antibodies in the body [74, 82]. Several studies have reported that astaxanthin has significant anti-cancer effects on certain cancer types such as prostatic hyperplasia and prostatic cancers. Astaxanthin mainly inhibits the enzyme 5-α-reductase, which is involved in abnormal prostate growth [78]. An investigation on the differential effects of algal extracts (containing 14% astaxanthin) and synthetic astaxanthin on cancer cells in culture showed that treatment with both, algal extracts and synthetic astaxanthin, can protect cells against UVA-induced *DNA* damage [90]. In another study, the occurrence of colon cancer induced by azoxymethane in *F344* rats was significantly lower in rats fed with 500 ppm astaxanthin or canthaxanthin for 34 weeks [91]. It was also reported that both topical and oral use of astaxanthin can suppress skin hyper-pigmentation, inhibit synthesis of melanin, and improve the condition of all skin layers [92].

Astaxanthin can be used in diabetes treatment, research reports the anti-hyperglycemic effects of astaxanthin [93, 94]. Other researchers suggest that astaxanthin

may be applied in the prevention of neuronal disorders associated with age-related macular degeneration, in the prevention of diseases such as Alzheimer, Parkinson and other disorders of the nervous system [88]. Furthermore, the oxidative protection of the brain and the neuroprotective effect of astaxanthin may also be due to the fact that this carotenoid can cross the blood-brain barrier as it has been observed in rats' brain tissue and in other experimental animals [95].

Nowadays, astaxanthin is used in various fields: in aquaculture as a pigmentation source, as well as in nutraceuticals, food and animal feed industries. In the fish industry, astaxanthin is used as food for salmon, shellfish and is considered an essential vitamin for the development of the fish brood. Microalgae extracts enriched with astaxanthin are a natural source of pigments, which increase the immunity of the aquatic organisms and improve their color [96]. Using astaxanthin enriched extracts in poultry feed leads to skin and egg yolk pigmentation.

Currently, astaxanthin for aquatic organisms and animal feed is synthetically produced The European Commission considers it food dye and it is given the E number *E161j*. The distinction between natural and synthetic astaxanthin is a matter of stereoisomerism. *H. pluvialis* produces only the (*3S, 3´S*) stereoisomer, while in a synthetic form, (*3R, 3´R*) stereoisomers are also present. Natural compounds differ from the synthetic ones in bioavailability and bio efficiency [97].

Astaxanthin is a nutraceutical with therapeutic effect. Astaxanthin represents 75% from the total extracts of carotenoids from *Haematococcus pluvialis,* sold as capsules [64]. Astaxanthin extracts are used as a natural dye in the cosmetic industry and ingredients for moisturizers, skin and hair lotions and serums due to their photoprotective properties and for slowing down the aging processes [78].

Canthaxanthin

It is the reddish-orange pigment, a lipid-soluble keto-carotenoid (also called Lucantin red). Large amounts of canthaxanthin are produced by *Coelastrella striolata* and *Chlorella zofingiensis* microalgae under salt stress and nitrogen-deprivation conditions [98, 99]. It is also contained in *Dunaliella salina* [100], *Chlorella vulgaris* and *Scenedesmus komareckii* [101] microalgae. Small quantities of canthaxanthin are also produced by *Haematococcus pluvialis* and *Botryococcus braunii* [102].

Research has shown that canthaxanthin has antioxidant, anti-immflamatory and neuroprotective properties [103]. It can be found in the egg yolk and it has the role of protecting the embryo from oxidative stress [104]. It was also reported the anti-cancer activity of cathaxanthin, this inhibiting significantly the growth of melanoma and fibrosarcoma tumor cells at a concentration of 100 mM [105]. Treatment with canthaxanthin also induced apoptosis in human colon adenocarcinoma and melanoma cells lines in a dose- and time-dependent manner [106]. The effects of canthaxanthin on chemically induced mammary carcinogenesis in mice showed that dietary intake of canthaxanthin for three weeks prior to the induction of cancer with dimethylbenzanthracene could reduce the occurrence of cancer by 65% [107].

Canthaxanthin is associated with E number *E161g* and is approved for use as a food coloring agent in different countries, is primarily obtained through synthetic methods; it is stable at *pH* 2-8 and as the majority of carotenoids, it is light sensitive and oxidizes in the presence of oxygen. It is used as a feed for poultry, to render a golden color to the birds'

skin and egg yolk as well as feed for some salmon and fish species. It is also used as food dye in bakery, confectionery, beverages and meat products [3, 9].

Ingestion of canthaxanthin stimulates tanning, leaving the skin with a golden shade. If consumed in large quantities, it crystallizes in the retina and causes canthaxanthin retinopathy, the process is reversible, however [108].

Cathaxanthin was not obtained from microalgae for practical application, due to its reduced content, it is found in the form of a mixture with other carotenoids – purified carotenoid extract.

Lutein

The lipid-soluble pigment, it appears yellow at low concentrations and orange-red at high concentrations, is one of the most important carotenoids for the human being. Lutein is isomeric with zeaxanthin, differing only in the placement of one double bond Table 5. A greater quantity of lutein is synthesized under conditions of temperature variation in the *Scenedesmus almeriensis* and *Muriellopsis* species of microalgae [109, 110]. An accumulation of 0.55% of lutein in the dry biomass of *Scenedesmus almeriensis* when varying the light intensity, temperature and nutrient concentration, has been identified in some research [110]. The production rate of lutein in *Chlamydomonas zofingiensis, Chlorella protothecoides* and *D. salina* varies with the environment's *pH* [1, 5, 63].

Being one of them most important carotenoids found, lutein is essential to the macula lutea (or yellow spot) in the retina and lens of the eye, several reports indicate that dietary supplementation with lutein alone or lutein together with other nutrients can improve visual function in patients suffering from atrophic age-related macular degeneration [111].

Research has shown the therapeutical effect of lutein in treating cancer, cardiovascular diseases, in preventing the retinal degeneration, cataract and in protecting the eye against light and ultraviolet radiation [1, 5, 6].

As a food additive, lutein has the E number *E161b*, is used in the pharmaceutical industry and in producing vision supplements; as a dye in the cosmetic industry and an ingredient in moisturizers and tanning lotions. In the food industry, lutein is used both as an additive and a colorant. It is also used as a supplement in poultry feed for egg yolk coloring; also used in the fish industry and in animal feed [3, 5, 6]. Currently, lutein is obtained from the flower petals of Marigold (the genus *Calendula*), although microalgae contain 3-6 times more lutein per unit of mass [62].

Zeaxanthin

An isomer of lutein has the same colour, similar properties and manifests health benefits. It is the photoprotective pigment in plants and it is accumulated in the mutated *D. salina* microalgae [28]. *Spirulina* microalgae also accumulate zeaxanthin, but it is rapidly converted into astaxanthin [11]. In *Porphyridium cruentum,* zeaxanthin accounts for 97.4% of the total of carotenoids [12]; the unicellular *Nannochloropsis* microalgae produce fatty acids and a significant quantity of zeaxanthin, astaxanthin and canthaxanthin [112].

Zeaxanthin exhibits a pronounced antioxidant activity, and similar to lutein, protects the retina, the eye from light and UV radiation and improves the vision. In vivo studies have shown that administration of lutein-zeaxanthin for 8 weeks increases the amount of macular pigment in humans [113]. Zeaxanthin reduces the oxidative stress in the body, prevents cardiovascular diseases, cancer and has an anti-inflammatory effect [114]. Lutein,

in association with zeaxanthin (considering a daily intake of 6 mg in humans), protects tissues from free radicals and can prevent atherosclerosis, cataract, diabetic retinopathy and age-related retinal degeneration [114].

As a food additive, zeaxanthin has the E number *E161h*. It is used in the pharmaceutical industry, as a food additive, dye (yellow-red pigment). The use of zeaxanthin producing microalgae in the food of shellfish, salmon and poultry increases the quality and color of the product [5].

Fucoxanthin

It is the pigment found in diatomic microalgae and chloroplasts of brown algae. It gives the microalgae a brown or olive-green color. It was isolated for the first time from *Fucus, Dictyota* and *Laminaria* marine algae [115]. It accumulates in the *Phaeodactylum tricornutum* microalgae species [116]. Due to its unique structure Table 5, it exhibits pronounced antioxidant, anti-inflammatory, antidiabetic, antiphotoaging and neuroprotective properties [115, 116]. Research has shown that fucoxanthin reduces the body weight and lipid levels in blood [117]. The study reports that fucoxanthin significantly decreases the serum glucose and plasma insulin levels in diabetic/obese mice and reduces hyperglycemia [118]. Moreover, it was also proved to have an anticancer effect in different types of cancer, including colon cancer and leukemia in animals [119]. As a result of the study of the effect of fucoxanthin (at 5 and 10mM concentration) on the viability of 6 types of cancerous cells, it was proved that incubation for 72 hours reduces the viability of 5 lines of cancerous cells [120]. The anti-cancer effect of fucoxanthin was much stronger compared to that of lycopene, at the same concentration. Also, after incubating the prostate cancer cell lines with fucoxanthin at a concentration of 20mM for 48 hours, the percent of apoptotic cells exceeded 30% [121]. A study to compare the effects of carotenoids such as β-carotene and astaxanthin, with those fucoxanthin on human colon cancer cells has showed that the xanthophyll carotenoid, fucoxanthin, has higher anti-cancer activity than the other carotenoids. Taken together, the study clearly showed that the fucoxanthin metabolites (halocynthiaxanthin and fucoxanthinol) have greater anti-cancer activities than fucoxanthin [119].

Another study showed that administering fucoxanthin supplements can prevent osteoporosis and rheumatoid arthritis [123].

The biological activity, the health effects and the bioavailability of fucoxanthin have only begun to be investigated. The *P. tricornutum* carotenoid extract is the main supplement with a major content of fucoxanthin, sold at the moment.

Diadinoxanthin, **diatoxanthin, violaxanthin, neoxanthin, loroxanthin, etc**

The listed xanthophylls, although not sufficiently studied, are of great interest to researchers. The content of xanthophylls in microalgae varies and is highly dependent on the cultivation conditions. As it was mentioned before, they are synthesized for protecting the cells from the oxidative stress and the influence of abiotic factors. Usually, microalgae accumulate several types of carotenoids, some of which are major components in biomass extracts, Table 4.

The study of the biological activity, elucidation of health benefits and practical implementation of the above mentioned xanthophylls are the main objective of research conducted in various fields.

Chlorophylls

Chlorophylls *a* and *b* are liposoluble, green pigments, present in almost all of the terrestrial and aquatic photosynthetic organisms. Chlorophyll *c* has a greenish-blue color and is more often found in seaweed while chlorophyll *d* is found in red algae and cyanobacteria. Microalgae are a rich source of chlorophyll, which is 0.5-1.5% from dry matter [124]. Several types of chlorophyll can be found in microalgae, for example *Phordium autumnale* contains both chlorophyll *a* (2.7 $\mu g.g^{-1}$) and *b* (0.7 $\mu g.g^{-1}$) [125].

Chlorophyll and its derivates manifest beneficial health effects due to their antioxidant, anticarcinogenic, antigenotoxic and antimutagenic properties. Several studies have shown that chlorophyll is a detoxifying agent, stimulates bile secretion, improves the metabolism of proteins, carbohydrates and proteins [5, 9].

The potential of microalgae as a source of chlorophyll for food dye with a nutriacetic effect has not been fully studied, however, the semi-synthetic derivative of chlorophyll, chlorophyllin (additive *E141*), which contains copper instead of ions of magnesium (characteristic for the natural form) is used and can have adverse health effects for the human body.

Phycobiliproteins

Are hydrosoluble proteins in *Spirulina*, *Porphyridium, Rhodella, Galdieria* microalgae, in cryptophytes and glaucophyte [125 - 128]. It is a family of fluorescent proteins, highly soluble in water. From the point of view of the chemical structure, they are made of chromophores - bylines (linear tetrapyrrole prosthetic groups) covalently linked via thioether bonds to an apoprotein Table *5*, capable of absorbing wavelengths between 470 and 660 nm. They have the mission to capture the light rays and pass them on to the chlorophyll during the photosynthesis process. Four groups of phycobiliproteins are known: phycoerythrin, phycoerythrocyanin, phycocyanin and allophycocyanin. The red phycobiliprotein - phycoerythrin and the blue phycobiliprotein- phycocyanin can serve as natural colorants in food, cosmetics and pharmaceuticals [127]. Currently, phycobiliproteins are used in health sectors as antioxidant, anticancer, antiviral, anti-inflammatory, anti-allergic, and neuro-protective material [5, 126, 127].

Phycoerythrin

The red *Porphyridium* microalgae are the main sources of the pinkish-red pigment – phycoerythrin [128]. These microalgae, grown in a usual aquatic environment, after three days accumulate 200mg of dye per liter of culture, with a concentration of phycoerythrin in the dye of 15%. Under optimal cultivation conditions, the phycoerythrin concentration may reach 30%. The color is stable at 60^0C for 30 minutes and has a long shelf life at *pH* 6-7. It is very stable as an ingredient in dry food preparations, stored in low humidity conditions [127]. Phycoerythrin, besides the red color, has a yellow fluorescence.

This dye is not currently used as it has not been sufficiently tested, but it is known that rats fed with *Porphyridium* microalgae biomass, had a normal development, without any adverse effects.

Phycocyanin

It is one of the most accessible phycobiliprotein that has attracted most attention for its use in animal feed, foods, and health. Phycocyanin cannot be made synthetically but is synthesized in *Spirulina platensis* [126], *Porphyridium aerugineum* and in the majority of

cyanobacteria. *P. aerugineum* microalgae, for example, after 4 days of growth, accumulate 100mg of dye per liter of culture with a 60% content of phycocyanin. *P. aerugineum* microalgae extract has a blue color and a red fluorescence [127, 128], which, with the change of the environment's *pH*, doesn't change and it is light-resistant but sensitive to the heat.

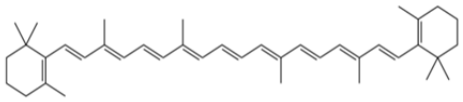
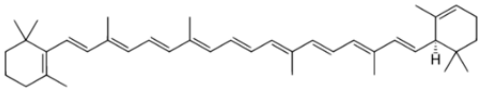
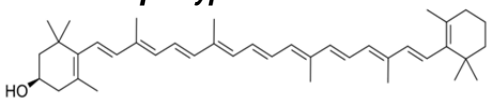
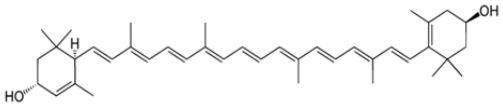
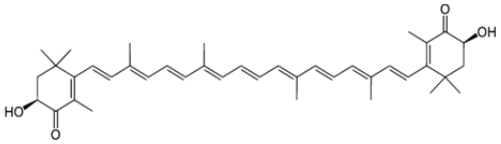
The phycocyanobilin groups are nutraceuticals which provide antioxidant and radical scavenging activity. Potential health effects related to phycocyanin include anti-inflammatory effects, anti-platelet aggregation, anti-cancerogenic effects, prevention of cholesterol-induced atherosclerosis, kainic acid-induced neural damage, kidney stone formation, thioacetamide-induced hepatic encephalopathy [126, 127].

Nowadays, phycocyanin is obtained from *S. platensis* microalgae and is applied as food dye in the United States of America to confer color to sweets, desserts, beverages, fermented dairy products, etc. [64].

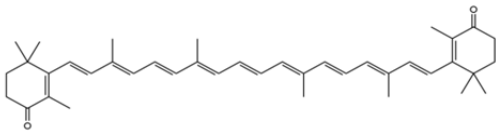
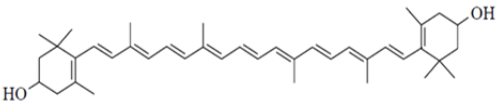
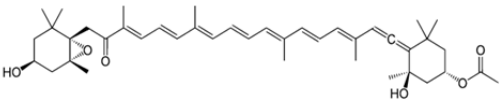
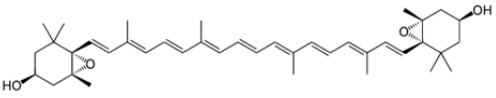
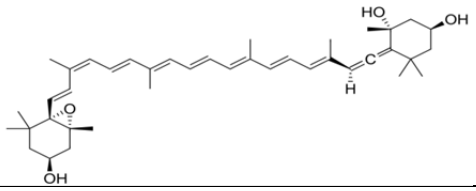
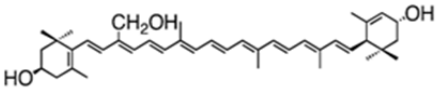
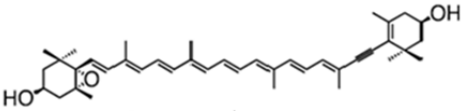
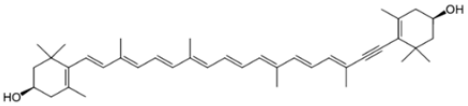
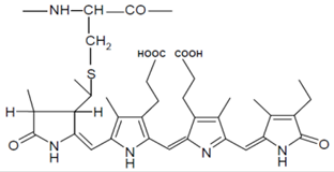
Within the European Union, the phycocyanin enriched *Spirulina platensis* extract is allowed to be used as a food dye.

Table 5

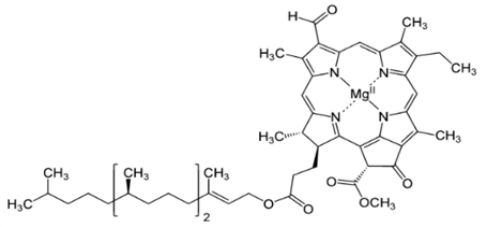
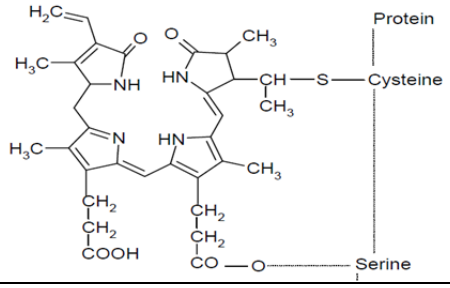
Pigments from microalgae

Carotenoids	Microalgae strains	References
β-carotene 	*Dunaliella sp.: D. salina, D. bardawil, D. tertiolecta, Haematococcus pluvialis, Scenedesmus almeriensis, Spirulina sp., Chlorella sp, Chlorococcum sp., Chlamydocapsa sp., Tetraselmis sp.*	[11-13,18, 65-67]
α-carotene 	*Chlorella sorokiniana, Chlorococcum humicola*	[1,5,9,11,12]
β-cryptoxanthin 	*Spirulina pacifica*	[1,5,9,13,16]
Lutein 	*Botryococcus braunii, Chlorococcum sp., Chlamydocapsa sp., Chlorella sp.: C. acidophila, C. fusa, C. protothecoides, C. pyrenoidosa, C. sorokiniana*	[1,17,18,63, 109,110]
Astaxanthin 	*Haematococcus sp., Chlorella sp., Coelastrella striolata, Monoraphidium sp., Chlorococcum sp., Chlamydocapsa sp., Neospongiococcum sp.*	[12,78-80]

Continuation Table 5

Cantaxanthin 	*Chlorella spp., Coelastrella striolata, Chlorococcum sp., Chlamydocapsa sp., Scenedesmus komareckii, Haematococcus lacustris, Neospongiococcum sp.*	[2,3,98-102]
Zeaxanthin 	*Scenedesmus almeriensis, Nannochloropsis oculata, Chlorella ellipsoidea, Chlorella nivalis, Dunaliella salina, Spirulina pacifica.*	[11,13,16,28, 65,112]
Fucoxanthin 	*Phaeodactylum tricornutum, Chaetoceros gracilis sp., Odontella aurita, Cylindrotheca closterium, Nitzschia sp, Ochromonas sp., Sarcinochrysis marina, Isochrysis sp., Corallina officinalis, C. elongata*	[12,17,18, 114,116]
Violaxanthin 	*Chlorella ellipsodea, Phaeophyceae, Chlorococcum humicola, Dunaliella salina. Oedogonium intermedium*	[5,9,12-18]
Neoxanthin 	*Dunaliella salina, Oedogonium intermedium Chlamydomonadales (Chlorophyta)*	[11,13,65-67]
Loroxanthin 	*Oedogonium intermedium Euglenia sanguinea*	[131,132]
Diadinoxanthin 	*Euglenia sanguinea, Phaeodactylum tricornutum*	[132,133]
Diatoxanthin 	*Euglenia sanguinea, Phaeodactylum tricornutum*	[132,133]
Phycobiliprotein-Phycocyanin 	*Spirulina sp. Arthrospira platensis, Rhodella, Galdieria, cryptophyta and glaucophyta*	[5,63,126-128]

Continuation Table 5

Chlorophylls 	*Formed by photosynthesis in most microalgae*	[1,9,124,125]
Phycobiliprotein -Phycoerythrin 	*Porphyridium sp., Agardhiella subulata, Polysiphonia morrowii*	[126-128]

Taking into consideration the beneficial health effects of the natural phycobiliproteins pigments, or of the phycoerythrin or phycocyanin enriched microalgae extracts, studying the biological activity, elaborating methods of obtaining, solving the technological requirements and implementing these dyes in real life are the major objectives for researchers from various fields, among which, the food industry as well.

Conclusions

Microalgae are a promising source of bioactive ingredients for producing functional food, increasingly used for their content of proteins, essential amino acids, unsaturated fatty acids, pigments, vitamins, polysaccharides, minerals, etc. In the last decades, microalgae biomass and phytonutrients enriched extracts are used not only as pills, capsules or powders, but also used for enriching pasta, bakery, meat and confectionery products, sweets, beverages, dairy, protein drinks and baby food [26].

Microalgae are also an excellent source of food for fish and aquatic organisms, animals, cattle, swine, poultry, etc. Pigments accumulated in microalgae have beneficial health effects and they could replace the chemically synthesized food dyes.

The synthetic sources of food dyes are substances of petrochemical origin, organic acids and inorganic substances. Today, the majority of the food dyes are synthetically produced; their impact on the human health is often a negative one, with various side-effects [134]. The carotenoids obtained from microalgae are easily digested by the body, have a high bioavailability and no side-effects if compared to their synthetic alternatives [69 - 71, 97]. Taking all the things above into consideration, elaborating methods of obtaining pigments and other bioactive substances from nontraditional sources, such as microalgae, and improving the existing ones, obtaining and establishing the chemical structure of the new compounds, studying their biological activity, solving the technological requirements regarding the optimization and application of phytonutrients in food production, are objectives drawing more and more attention.

Moreover, microalgae as renewable sources of biologically active substances have not been fully explored, less than 40 strains being exploited today, thus the potential of thousands of species remaining undiscovered[11, 13, 17].

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