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Exploiting the beneficial properties of microalgae for food and feed use

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1. SUMMARY

By 2050, 9.8 billion people are projected to live on Earth, which means that we need to double our current food production to keep pace with such a large population increase. In addition, rising greenhouse gas emissions and the associated climate change are placing a significant strain on the planet's ability to sustain itself. However, in order to increase the quantity of proteins of plant origin, it is necessary to increase crop production areas, harvesting frequencies and the quantity of crops produced. Unfortunately, the optimization of these factors is already very close to the available maximum in the current situation. The developed cultivation systems and maximum utilization of the soil power leads to very serious environmental problems, soil destruction, loss of biodiversity and serious environmental pollution through the transport of the produced plant raw materials.

This poses a serious challenge to food security and further increases the risk of hunger. There is therefore a need for agricultural practices that can lead to the cultivation of food and feed crops that have better sustainability indicators and are more resilient to climate change, which can be used to safely produce health-promoting feeds, as well as novel and value-added foods. Within this group, a particular problem is presented by the protein supply of the population, as currently about one billion people do not have adequate protein intake. However, conventional protein sources are not sufficient to meet growing protein needs.

As mentioned above, food and feed proteins are based on plant proteins. In recent years, a prominent role has been played by the research into alternative proteins and the mapping of their positive and negative properties. Among alternative proteins, special attention has been paid to various yeasts, fungi, bacteria, algae, single cell proteins (SCPs) and insects. In this paper, we focus on the presentation of algae, particularly microalgae, which are of paramount importance not only because of their significant protein content and favorable amino acid composition, but also because they are also sources of many valuable molecules, such as polyunsaturated fatty acids, pigments, antioxidants, drugs and other biologically active compounds. It is important to learn about microalgae biomass in order to be able to develop innovative health food products.

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2. Introduction

By 2050, the population of Earth will grow to nearly 10 billion, an increase of about 25% compared to today's population. In addition, the significant depletion of our Earth's water resources also makes it necessary to restructure our diet, as the amount of water needed to produce 1 kg of food is 13,000 liters for cattle, 5,520 liters for chicken, while only 50 liters for peas or lentils. All this means that we can expect a significant increase in the price of foods of animal origin, which will mean that we will have to reduce the proportion of them in our diet significantly.

The different plant protein sources make a positive contribution to the protection of the environment and the fight against climate change due to their more efficient use of water. On the other hand, legumes require 30 to 70% less synthetic fertilizers due to their nitrogen-binding properties, they increase soil power and have a positive effect on soil biology. It is also a known fact that due to nutrient transformation losses, the production of 1 kg of animal protein requires at least 6 to 16 times more cultivable area. In addition, the carbon dioxide footprint of the production of foods, especially of those based on beef, is about 10 times more than that of plant-based foods.

The structure of food consumption in Europe is characterized by a 59% proportion of the daily protein intake coming from protein sources of animal origin (meat, fish, milk), with proteins of plant origin representing only 41% of the total. More than 50% of the latter is wheat protein. As a result, a few cereals (wheat, corn, rice) could become staple foods, leading to geographical homogeneity of foods, dietary monotony and nutritional imbalances, increasing the risk of micronutrient deficiencies, overweightness and chronic obesity, as well as NCDs (Non Communicable Diseases), including cardiovascular diseases, stroke, cancer and diabetes.

Based on all this, it is becoming increasingly important to identify and investigate alternative plant and other protein sources, in addition to the protein sources mentioned above, which can contribute to meeting the protein needs of an increasing human population, as well as to addressing the unbalanced nutritional status of them.

An important group of protein crops are legumes (e.g., dried beans, runner beans, chickpeas, horse beans, lentils, grass peas, black-eyed peas, dried peas, autumn and spring peas) with a high protein content of 20 to 40% on average. Dry leguminous seeds are rich in protein and lysine, but low in sulfur-containing amino acids. At the same time, field crops with good nutritional values but low protein content (e.g., sunflower, canola, corn, sorghum, rice, wheat) are low in lysine and rich in sulfur-containing amino acids. Taking into account the positive nutritional values of the two plant groups, products containing complete plant protein can be developed by their combined use.

Table 1. Protein content of various crops [1]

Protein plant	Protein content of the seed
Wheat	8-15%
Rice	7-9%
Corn	9-12%
Barley	8-15%
Sorghum	9-17%
Soy	35-40%
Pea	20-30%
Chickpea	20-25%
Canola	17-26%
Lupine	35-40%

The protein content of various crops (**Table 1**) may show significant variability not only between species but also within a given species. In addition, protein content can also be altered by environmental factors and the food processing technology.

Other alternative protein sources include single cell proteins (SCPs) produced by fermentation technologies, seaweeds living in saltwater, duckweed species living in freshwater and various insect species. Protein content values of the different sources can vary widely depending on the species, the cultivation technology and the nutrient supply (**Table 2**).

Table 2. Protein content of various alternative protein sources [1]

Protein sources		Protein content
SCP	Microalgae	40-60%
	Yeasts	45-65%
	Filamentous fungi	35-50%
	Bacteria	40-60%
Seaweed		5-47%
Duckweed		20-35%
Insects		20-76%

3. Characterization and occurrence of microalgae

Algae, also called seaweeds, are eukaryotes capable of photosynthesis. Algae represent one of the oldest life forms on Earth, having existed on our planet for about 3 billion years. They produce one-third of Earth's living matter and about 50% of its organic carbon [1]. These plants have survived all geological epochs and climate changes. Algae still account for 90% of Earth's oxygen production. These organisms have allowed life to form on Earth, and they use the power of sunlight to produce organic food from inorganic materials through photosynthesis. In many respects, algae are the most diverse living things in the world. They have the simplest structure and are closely related to bacteria. The most complex ones, *Charophyceae* species, resemble kelp to the point of confusion. The smallest algae are picoalgae with a size of 0.5 µm, while the largest are 50 to 100 m long *Macrocystis* species (*Phaeophyceae*). They occur under the most extreme conditions in fresh and salt water, hot springs, on snow and ice surfaces, in the soil and in the upper layer of some rocks [2]. Algae are mostly eukaryotes, typically classified as „lower” plants that have no true stems, roots and leaves, and are generally capable of photosynthesis. Algae are widely classified into *Rhodophyta* (red algae), *Phaeophyta* (brown algae) and *Chlorophyta* (green algae) and are classified as macroalgae and microalgae by size. Macroalgae (seaweeds) are multicellular, large size algae that are visible to the naked eye, while microalgae are microscopic single-celled organisms and may be prokaryotes, similar to cyanobacteria (*Chloroxybacterium*), or eukaryotes, similar to green algae (*Chlorophyta*).

Microalgae, as excellent sources of various organic carbon compounds, can be used in the manufacture of health supplements, drugs and cosmetics [2]. they can also be used in wastewater treatment, atmospheric CO₂ reduction and the production of biofuels. A wide range of bio-products can be extracted from microalgae, such as polysaccharides, lipids, pigments, proteins, vitamins, bioactive compounds and antioxidants [3]. In addition to all this, they are playing an increasingly important role in the feed and food industries (**Figure 1**).

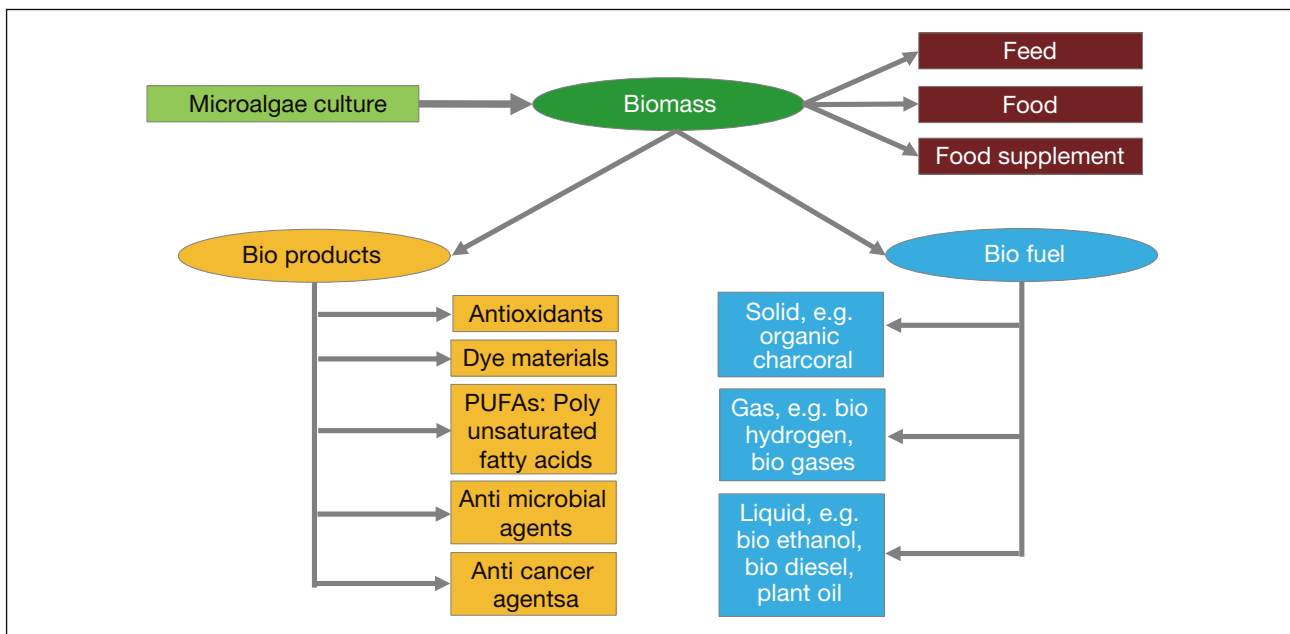


Figure 1. Applications of microalgae [3]

4. General composition of microalgae

As with all other higher plants, the chemical composition of algae varies depending on the type of cultivation, such as environmental parameters, temperature, illumination, pH and mineral content of the medium, CO₂ supply and mixing speed: 9-77% protein, 6-54% carbohydrate, 4-74% lipid (**Table 3**)

Table 3. Comparison of the protein, carbohydrate and fat content of some food ingredients and microalgae

Food ingredient	Protein (%)	Carbohydrate (%)	Fat (%)
Baker's yeast	39	38	1
Meat	43	1	34
Egg	47	4	41
Milk	26	38	28
Rice	8	77	2
Soy	37	30	20
Microalgae			
<i>Anabaena cylindrical</i>	43-56	25-30	4-7
<i>Chaetoceros Calcitrans</i>	36	27	15
<i>Chlamydomonas reinhardtii</i>	48	17	21
<i>Chlorella pyrenoidosa</i>	57	26	2
<i>Chlorella vulgaris</i>	51-58	12-17	14-22
<i>Diacronema vlkianum</i>	57	32	6
<i>Dunaliella salina</i>	39-61	14-18	14-20
<i>Euglena gracilis</i>	10	40	41
<i>Haematococcus pluvialis</i>	48	27	15
<i>Isochrysis galbana</i>	50-56	10-17	12-14
<i>Porphyridium cruentum</i>	8-18	21-52	16-40
<i>Scenedesmus obliquus</i>	6-20	33-64	11-21
<i>Scenedesmus dimorphus</i>	60-71	13-16	6-7
<i>Spirulina maxima</i>	46-63	8-14	4-9
<i>Spirulina platensis</i>	52	15	3

4.1. Protein and amino acid content of microalgae

Based on research results it can be stated that algae are a source of protein with an amino acid composition similar to that of plant proteins. Examination of the net protein utilization, i.e., of the proper amino acid composition, digestibility and biological value, also led to a similar result.

Several microalgae species produce large amounts of various essential amino acids and proteins that can be used in foods and feeds, one of the main reasons they occupy a prominent place among alternative proteins. Certain species of microalgae can produce as much protein as other rich protein sources, e.g., eggs, meat and milk [6].

In addition, the amino acid pattern of almost all algal species is very similar to the protein pattern of many foods. Of the amino acids, they are only low in cysteine and lysine. Since cells are able to synthesize almost all amino acids, they can be used to provide the essential amino acid needs of both humans and animals [7]. The composition of amino acids synthesized by microalgae, especially the amount and composition of free amino acids, varies greatly, depending on the species, growth conditions and the growth phase [8].

In addition, microalgae proteins are easily digestible and have a relatively high nutritional value. Microalgae produce 2.5 to 7.5 tons/ha/year protein [9], for example, the green microalgae *Chlorella* is a rich source of different types of marketed proteins. Another protein-rich microalga is *Arthrospira*. Proteins from microalgae lower cholesterol levels by activating cholecystokinin. They also have other important enzymatic effect [10]. For example, the microalga called *Lyngbya majuscula* produces microcholine-A, a protein with immunosuppressive effects [11]. The microalga *Nostoc* produces a protein called cyanovirin, which is known to have an antiviral activity against both HIV and the influenza virus [12]. At the same time, *Anabaena* and *Porphyridium* species produce the enzyme SOD (superoxide dismutase), which protects against oxidative

damage, while *Isochrysis galbana* produces the enzyme carbonic anhydrase, which plays a key role in the conversion of CO₂ to carbonic acid and bicarbonate. *Microcystis aeruginosa* produces a number of amino acids, including proline, serine, glycine and valine.

4.2. Fatty acids

Polyunsaturated fatty acids play an important role in tissue protection and have a beneficial effect on health. Omega-3 and omega-6 fatty acids are especially important for humans, but the human body is unable to produce these fatty acids. Therefore, intake from an external source, such as various foods, is essential. Docosahexaenoic acid (DHA), linoleic acid, eicosapentaenoic acid (EPA), arachidonic acid and gamma-linolenic acid have been shown to lower cholesterol levels, delay aging, protect membrane integrity and prevent cardiovascular diseases [13,14]. Several species of microalgae capable of synthesizing these valuable fatty acids have been studied. These studies have shown that *Pavlova lutheri* produces large amounts of polyunsaturated fatty acids [15], *Arthrospira platensis* mainly produces and accumulates stigmaterol, sitosterol and γ -linolenic acid [16], while *Porphyridium* produces arachidonic acid, *Nannochloropsis*, *Phaeodactylum*, *Nitzschia*, *Isochrysis* and *Diacronema* species produce eicosapentaenoic acid and *Cryptocodinium* and *Schizochytrium* microalgae produce docosahexaenoic acid in significant amounts [17, 18, 19].

The polyunsaturated EPA and DHA are also pharmaceutically very important omega-3 fatty acids. They play a key role in the treatment of inflammatory diseases, heart problems, arthritis, asthma and headaches, among other things [20, 21, 22].

4.3. Polysaccharides

Polysaccharides are widely used in the food industry, primarily as gelling and thickening agents. Many polysaccharides used in the food industry, such as agar, alginates and carrageenans are extracted from macroalgae, e.g., *Laminaria*, *Gracilaria* and *Macrocystis* species [8]. One of the most promising microalgal species, the unicellular red alga *Porphyridium cruentum* produces a galactan exopolysaccharide, that can replace carrageenan in many cases. *Chlamydomonas mexicana* also produces significant amounts of polysaccharides, and it is used in the United States as a soil improver. Sulfated algal polysaccharides also have pharmacological properties and they play a prominent role in stimulating the human immune system [23].

4.4. Photosynthetic pigments

It can be said in general that each species of algae has a specific pigment combination that creates its characteristic colour. In addition to chlorophylls, the primary photosynthetic pigments, supplementary or secondary pigments, such as phycobilins and a number of carotenoids are also produced by microalgae. These natural pigments have the ability to improve the efficiency of light energy utilization and provide protection for algae from the harmful effects of solar radiation. They are used preferentially when added to foods and feeds as natural antioxidants and colourants [24].

4.4.1. Carotenoids

Carotenoids are naturally occurring pigments that play a role in the formation of the colour of fruits, vegetables and other plants [25]. They are typically isoprenoid polyene pigments derived from lycopene, ranging in colour from yellow to red, and are produced by *de novo* photosynthetic organisms and some other microorganisms [8]. Carotenoids ingested with foods or feeds are either accumulated or metabolized by the body. Carotenoids can be found in the meat of various animals, eggs, fish skin (trout, salmon), crustaceans (shrimp, lobster, Antarctic krill, crab) and subcutaneous fat, skin, egg yolk, liver and the feather of birds (e.g., poultry) [26].

In algae, carotenoids primarily play the role of sunscreen and light collector, i.e., they protect the photosynthetic apparatus from light damage [24]. They also play a role in phototropism and phototaxis. Some microalgae undergo carotenogenesis in response to various environmental effects (e.g., light, temperature, salts, nutrients). During this, algae stop their growth dramatically alter their carotenoid metabolism, resulting in the accumulation of secondary carotenoids [27].

There are more than 600 carotenoids in nature, about 50 of which show provitamin A activity. These include α -carotene, β -carotene and β -cryptoxanthin [28]. A β -Carotene protects membrane lipids from peroxidation, thus preventing and reducing the development of many serious and fatal diseases, such as cancer, cardiovascular disease, Parkinson's disease and atherosclerosis [29, 30, 31].

Relatively few carotenoids are used in the food and feed industries: β -carotene and astaxanthin, lutein, zeaxanthin, lycopene, etc. Among microalgae, the main carotenoid-producing species are *Dunaliella salina* and *Haematococcus pluvialis* which produce significant amounts of β -carotene and astaxanthin, respectively. The microalga *Dunaliella salina* produces β -carotene in an amount that accounts for 10 to 14% of its dry matter content [32].

β -Carotene serves as an essential nutrient, mainly as a food colouring, and is increasingly used in various dietary supplements due to its health-protective effects, but is also used preferentially by the cosmetics industry [33].

In the food industry, β -carotene is also used regularly in various soft drinks, cheeses, butter or margarines due to its beneficial physiological effect, as it possesses a provitamin activity [34].

Astaxanthin has a number of beneficial properties, including improving eye health, muscle strength and endurance, protecting the skin, reducing premature aging, inflammation and damage caused by UVA radiation. It also plays an important role in animal feeding, as it promotes growth and reproduction, improves vision, has an immunostimulatory effect and also aids in post-injury regeneration [35, 36].

Numerous studies have shown that daily intake of astaxanthin protects cells and tissues from oxidative effects and that its effect on free radicals is significantly, about 500 times more intense than that of vitamin E. The microalga *Haematococcus pluvialis* produces 4-5% astaxanthin on the basis of dry biomass [37], so its dried biomass is marketed as a rich source of astaxanthin and is sold on the market at a price of about 2,500 US \$/kg.

4.4.2. Chlorophyll

Each alga contains one or more chlorophylls. Their primary photosynthetic pigment is chlorophyll-a, and it is also the only chlorophyll in Cyanobacteria (blue-green algae) and red algae (Rhodophyta). Like all higher plants, Chlorophytes (true green seaweeds) and Euglenophytes (flagellate seaweeds) also contain chlorophyll-b; chlorophyll-c, -d and -e are found in many other marine algae. The quantity of chlorophylls is usually up to 0.5-1.5% of the dry matter content [38].

In addition to being used as a food and pharmaceutical colourant, chlorophyll derivatives also have a health-protective effect. They are also traditionally used because of their wound healing and anti-inflammatory properties [39]. Epidemiological studies conducted in the Netherlands (Cohort Study) have shown a significant correlation between chlorophyll consumption and a reduction in the risk of colon cancer [40].

4.4.3. Phycobilins

In addition to chlorophyll and carotenoid lipophilic pigments, Cyanobacteria (blue-green algae), Rhodophytes (red algae) and Cryptophyta algae also contain so-called phycobilins, which are coloured, fluorescent pigments. Like chlorophylls, they bind to proteins (phycobiliproteins) which, contrary to chlorophyll-protein complexes located in the membranes, are water-soluble proteins and are important components of the photochemical system. Significant amounts of phycobilins, mainly blue phycocyanin and red phycoerythrin are found in *Spirulina* algae and *Porphyridium*, respectively.

The use of phycobilins is quite widespread. In addition to being widely used in clinical immunofluorescence studies to detect fluorescently labeled antibodies as a fluorescent marker [38], phycocyanin is currently used in Japan and China as a natural colourant in foods, such as chewing gums, candies, dairy products, jellies, ice creams and soft drinks. It is also widely used in the cosmetics industry, for example, in lipsticks, eyeliners and eyeshadows [41].

According to a study, phycocyanin is one of the most versatile blue colourant, providing a bright blue colour to various jellies and coated soft candies [42], while a number of pharmacological properties are also attributed to phycocyanin, including antioxidant, anti-inflammatory, neuroprotective and hepatoprotective effects [43, 44, 45].

4.5. Tocopherols and sterols

Tocopherols are widespread in nature, occurring in both lower and higher plants as parts of the photosynthetic system.

Research in this area has revealed that *Euglena* has the highest content of tocopherols among the various microalgae species [46].

The sterols produced by plants are called phytosterols. Microalgae can make a major contribution to the production of phytosterols, they can be considered as efficient and promising sources for their large-scale production. Some microalgae contain high levels of sterols. Microalgae sterols have health-protective,

cholesterol lowering and anti-inflammatory properties, and are effective in the treatment of certain neurological diseases, such as Parkinson's disease [47, 48], and are increasingly used in the food industry as dietary supplements and food ingredients [49, 50]. Some microalgae, such as species belonging to the genera *Pavlova* and *Thalassiosira*, are rich in sterols [51, 52].

4.6. Vitamins, minerals

Microalgae biomass is a valuable source of almost all essential vitamins, as it contains vitamins B₁, B₂, B₃, B₅, B₆, B₁₂, C, E and H, among other things, and its mineral content (e.g., Na, K, Ca, Mg, Fe, Zn and trace elements) is also significant [53]. The vitamin B₁₂ and iron content of some microalgae, such as *Spirulina* species, is particularly high, therefore, they are often used in foods and dietary supplements made for vegetarians.

The vitamin content of algae depends on the genotype, as well as the stage of the growth cycle, the nutrition of the algae and the light intensity. Thus, their vitamin content can be increased by selecting the right species, choosing the right culture conditions and/or by genetically modifying them. However, the vitamin content of the cells can be significantly reduced by using inappropriate environmental conditions, harvesting and biomass drying methods [54].

4.7. Antioxidants

Microalgae are photoautotrophic organisms, that is, organisms that depend on light as the energy source to produce organic molecules from inorganic molecules. This process is known as photosynthesis, and the food chain is usually based on these organisms. During their development, these organisms have developed an effective defense system against various abiotic effects affecting them, such as high levels of free radicals and reactive oxygen species [23]. Due to the high antioxidant content of certain algae species (e.g., *Isochrysis galbana*, *Chlorella vulgaris*, *Nannochloropsis oculata*, *Tetraselmis tetraethele*, *Chaetoceros calcitrans*), their use has been increasing in some cosmetics (e.g., sunscreens) and in functional foods.

The research of Natrah et al. [55] has shown that the methanolic extract of some fresh/untreated microalgae exhibits an antioxidant activity that is higher than that of α -tocopherol, but lower than that of the synthetic antioxidant BHT (butylhydroxytoluene). However, the latter and BHA (butylhydroxyanisole) being synthetic antioxidants, their safe use is questionable, as their use in high doses may be carcinogenic and tumorigenic [56, 57].

4.8. Other biologically active components

Microalgae are undoubtedly a large repository of versatile compounds with significant biological activity, as well as unique and interesting structure and function [58].

In recent decades, marine microorganisms, especially Cyanobacteria, have become the focus of medical research aimed at developing new drugs and antibiotics. Data published up to 1996 revealed about 208 Cyanobacterial compounds exhibiting biological activity. By 2001, the number rose to 424. The compounds identified include various lipoproteins (40%), alkaloids, amides, etc. [59], many of which have cytotoxic, antitumor, antimicrobial (antibacterial, antifungal), antiviral (e.g., anti-HIV) activities, as well as biomodulatory, for example, immunosuppressive and anti-inflammatory effects [59, 60].

Numerous studies have shown that microalgae may also contain compounds that are effective in treating cancer and tumors by inhibiting angiogenesis. Angiogenesis is a physiological process during which new blood vessels emerge from existing blood vessels. Although angiogenesis is a normal process, pathological conditions can develop under certain conditions, such as cancer, atherosclerosis, arthritis, diabetic retinopathy and ischemic stroke. Pathological angiogenesis promotes the development and growth of tumors [61, 62]. Fucoxanthin and fucoxanthinol, found in many species of microalgae, have been shown to inhibit the process of angiogenesis in the aortic ring of rats by reducing the formation and growth of microvessels [63]. Fucoxanthin has also been shown to protect DNA from photooxidation [64]. Microalgae, especially blue-green algae, are currently considered to be potential sources of active ingredients that can be used in the treatment of cancer, as several studies have demonstrated their anti-cancer effects [65].

5. Some major microalgal species

Although many indigenous microalgal populations have been used for various purposes for centuries, their large-scale cultivation has only begun in the last few decades [66]. Of the assumed roughly 30,000 species of microalgae, only a few thousands are kept in stock collections [67, 68], of which a few hundred are considered more important due to their chemical composition, and very few are grown in industrial quantities [69].

The biotechnologically most relevant microalgae include green algae (Chlorophyta), such as *Chlorella vulgaris*, *Haematococcus pluvialis*, *Dunaliella salina* and *Spirulina maxima*, which belongs to the phylum of Cyanobacteria. Their cultivation, marketing and use are very significant, mainly as dietary supplements and animal feed additives.

5.1. *Spirulina species*

Spirulina (Arthrospira) algae are a tiny, filamentous, freshwater, spiral-shaped, blue-green algae species that is abundant in the alkaline lakes of Mexico and Africa and has been consumed by the local population since ancient times [59]. Its characteristic feature is that its cell membrane is very weak, and this makes it easy to utilize. It is also one of its important physiological features that it can easily become a colloidal solution when exposed to moisture and is very easy to digest. *Spirulina* is widely grown all over the world (3,000 tons/year) and is used as a food and feed supplement due to its high protein content (60 to 70%, including 18 amino acids, 8 of which are essential) and its excellent nutritional value. For example, its γ -linoleic acid content is remarkably high [70, 71]. Its digestibility and absorption are superior to both animal and plant proteins. It contains the vitamins important to the body (C, B₁, B₂, B₅, B₆, B₉, B₁₂, A, E), trace elements, of which iron, iodine, calcium, sodium, potassium, copper, magnesium, manganese, zinc, phosphorus, selenium, chromium and vanadium are the most significant. It is a particularly good source of iodine and potassium. It stimulates the immune system greatly due to its high content of β -carotene, chlorophyll and γ -linolenic acid. Its polyunsaturated fatty acid content is significantly higher than that of marine fish. *Spirulina* contains high levels of GLA (gamma-linolenic acid), which is found in greater amounts only in breast milk. *Spirulina* has a number of health-protecting effects: it lowers high blood fat levels, cholesterol levels, high blood pressure, elevated blood sugar levels, is suitable for treating kidney failure, and promotes the growth of probiotics, such as *Lactobacilli*, in the gut [19]. It is the main source of natural phycocyanin, used as a natural blue colourant in foods and cosmetic products, and also as a biochemical tracer in immunoassays [70, 71, 72].

5.2. *Chlorella vulgaris*

This species of algae is one of the oldest, simplest plants on Earth. Its nearly 4% chlorophyll content, strong cell wall and high pigment and cellulose content make the detoxifying effect of *Chlorella* unique. It binds and removes heavy metals from the body, cleanses the intestinal flora. By improving liver function, it helps to remove other contaminants, in addition to heavy metals, and to detoxify the body.

Its physiological effects are similar to those of *Spirulina*: it is high in protein, contains all the essential amino acids, and it is a storehouse of various vitamins, trace elements and minerals. *Chlorella vulgaris* has been used in the Far East since ancient times in alternative medicine, as well as in the preparation of various traditional foods. It is widely cultivated and used, primarily in animal feeding, aquaculture and as a dietary supplement, in many countries, including China, Japan, Europe and the United States. The health-protecting effects of *Chlorella* are manifested, for example, in the rapid healing of stomach ulcers and other wounds, and it is useful in the treatment of constipation, anemia, hypertension, diabetes, infant malnutrition and neurosis. The prophylactic role of the glycolipids found in *Chlorella* against the development of atherosclerosis and hypocholesterolemia has been demonstrated by research [58]. However, one of the most important substances in *Chlorella* is β -1,3-glucan, which is an active immunostimulant, it binds free radicals and reduces the amount of blood fats [19].

The γ -linolenic acid (GLA) content of *Spirulina* and *Chlorella* is very high. The role of GLA in the functioning of the body is extremely diverse. On the one hand, it is important for the proper functioning of the immune system, and on the other hand, it has an anti-inflammatory effect, lowers blood pressure and improves blood circulation. It prevents platelets from sticking together, thus reducing the risk of formation of blood clots. It has a positive effect on cholesterol levels, thus reducing the risk of atherosclerosis. It improves nervous system function and eliminates excess fluid from the body.

5.3. *Haematococcus pluvialis*

This freshwater microalgae, with a size of barely 0.1 mm, attracted the interest of researchers early on. *Haematococcus pluvialis* is the plant with the highest astaxanthin content (1.5-3.0% dry weight) based on previous research. This carotenoid pigment has a very strong radical scavenging effect that exceeds the antioxidant properties of β -carotene, vitamin C and vitamin E. The astaxanthin production of the algae is a natural reaction to environmental stress. Thanks to the protective functions of astaxanthin, in a state of deep sleep, these algae can survive without food and water for more than 40 years, so they can easily survive the heat of summer or the icy cold of winter. They will only wake up again and regain their original green, active state when living conditions are right again. As a result, algae defied the harshest environmental conditions even at the early stages of Earth's history. The ability of certain algae species to survive both droughts and ice ages is due to their astaxanthin shield. Astaxanthin is a bioactive antioxidant that has been shown to be

effective against Alzheimer's disease and Parkinson's disease, as well as macular degeneration in both animal and human experiments. In some cosmetics, the astaxanthin used can slow down the aging process of the skin. In addition, the immune-boosting and anti-inflammatory effects of astaxanthin have been reported, as well as its beneficial effects on the development of cardiovascular diseases and atherosclerosis.

Haematococcus pluvialis is currently a natural source of this pigment, its commercial utilization is outstanding, especially in aquaculture (salmon and trout farming) [73]. There is another natural source of astaxanthin, however, the yeast *Xanthophyllomyces dendrorhous* requires large amounts of expensive nutrients for proper pigmentation [36].

5.4. *Dunaliella salina*

Dunaliella salina is a halotolerant microalgae, its natural habitats being salt lakes. It is able to accumulate large amounts of β -carotene, which is why this species of algae is sought after mainly as a food colourant. Research has shown that the *Dunaliella salina* community in Pink Lake, Victoria, Australia, can produce up to 14% carotenoids [74] and some *Dunaliella* algae can contain up to 10% in cultivated cultures.

Higher β -carotene content can be achieved with adequate nutrient supply under high salt and light conditions [75, 76]. Similarly to *Haematococcus* algae, *Dunaliella* contains significant amounts of astaxanthin. However, *Haematococcus* is a freshwater algae that is difficult to grow in outdoor cultures because it is easily infected, requiring a closed system, and the extraction of astaxanthin is more complicated than in the case of *Dunaliella*, as *Haematococcus* has a thick cell wall that has to be disrupted by physical methods.

6. Use for animal feed

Today, many species of microalgae (e.g., *Chlorella*, *Tetraselmis*, *Spirulina*, *Nannochloropsis*, *Nitzschia*, *Navicula*, *Chaetoceros*, *Scenedesmus*, *Haematococcus*, *Cryptocodinium*) are used to feed farm animals, pets and fish.

Even a small amount of microalgae biomass has an immunostimulatory effect, which results in growth stimulation, disease resistance, has antiviral and antibacterial effects, improves absorption and the colonization stimulation of probiotic cultures such as Lactobacilli, and thus results in an increase in reproductive performance and weight [77]. By providing feeds that contain algae, the appearance of the animals improves visibly, which is manifested in a healthy skin and a shiny coat, both in the case of farm animals (poultry, cows, breeding bulls) and in the case of pets (cats, dogs, rabbits, ornamental fish and birds) [78].

Feed is the main exogenous factor influencing animal health and accounts for a significant part of the major cost of animal husbandry, and so it is very important to identify high quality, chemical and toxic substance free alternative protein sources that can replace or complement traditional protein sources [26]. The results of a large number of nutritional and toxicological evaluations have demonstrated the suitability of algal biomass as a valuable feed supplement [38]. Currently, about 30% of the global algae production is sold for feed purposes [53].

Becker et al [53] performed feeding experiments on broiler chickens, in which conventional proteins were replaced with species of different microalgae, namely *Chlorella*, *Euglena*, *Oocystis*, *Scenedesmus*, *Spirulina*, usually at a rate of 10%. In laying hens, no differences were found in egg production and egg quality (size, weight, shell thickness, egg solids, albumin index, etc.) and in feed conversion efficiency between birds fed with algae-containing feeds and the control birds.

However, *Haematococcus* microalgae can also be used as a natural colourant in the feeding of broiler chickens, making the skin of the birds yellower and the egg yolk more orange [79]. Studies were performed on chickens that were fed the biomass (5% or 10%) of red microalgae (*Porphyridium* species). Although no difference was found in the body weight and egg count of the chickens, the composition of the meat and eggs showed decreased cholesterol levels (by 10%) and healthier fatty acid composition and increased linoleic acid and arachidonic acid levels (by 29% and 24%, respectively). In addition, the colour of the egg yolk was darker due to a carotenoid content that was 2.4 times higher than average [80]. At the same time, it was observed that chickens fed with algal biomass consumed 10% less food in the case of feeds containing either 5% or 10% algae, and had serum cholesterol levels significantly lower (by 11% and 28%, respectively) than that of the control group.

Microalgae biomass is a feed with excellent nutritional values and is eminently suitable for breeding pigs. They can be used to replace traditional proteins such as soy flour or fish meal, and their acceptance is not difficult for the animals [38].

It is hypothesized that algae may be an excellent food source for ruminants, as these animals are able to digest even the cell wall of unprocessed algae. However, only a limited number of experiments have been performed with these animal species, as these procedures are expensive and large amounts of algae are required to perform appropriate feeding experiments. However, some experiments have shown that sheep, lambs and cattle were unable to digest the carbohydrate fraction efficiently when fed certain algal species (e.g., *Chlorella*, *Scenedesmus obliquus* és *Scenedesmus quadricauda*) [81, 82]. Better digestibility was achieved when *Spirulina* accounted for 20% of the total sheep feed, and it was observed that in calves fed a diet containing *Scenedesmus* algae, there were only minimal differences when compared to animals fed the control feed [83].

Microalgae feeds are currently used mainly to supplement and replace zooplankton used for the breeding of fish, fish fry and other aquatic animals (crustaceans, etc.) [84, 85]. The species most often used in aquaculture are *Chlorella*, *Tetraselmis*, *Isochrysis*, *Pavlova*, *Phaeodactylum*, *Chaetoceros*, *Nannochloropsis*, *Skeletonema* and *Thalassiosira* [86, 87].

Microalgae contain nutrients that are essential for aquatic animals, and these determine the quality, growth, health and disease resistance of the farmed animals. Mixed microalgae cultures have been shown to be useful for animal growth to provide adequate protein composition, vitamin content and high levels of polyunsaturated fatty acids (mainly EPA, AA and DHA), which are vital for the survival and growth of many freshwater and marine animals in the early stages of life [88]. One of the beneficial effects of algae is attributed to the fact that they increase the food intake of marine fish offspring, which enhances their growth and survival, and also improves the quality of fish meat [89]. In addition, the presence of algae in the breeding tanks of European sea bass larvae has been shown to increase digestive enzyme secretion [90]. When many aquatic species, such as Salmonidae (salmon and trout), shrimp, lobster, marine vertebrates, goldfish and koi carp are kept under intensive conditions, carotenoid colourants are added to their feed to achieve their characteristic muscle colour. Carotenoids, such as astaxanthin and canthaxanthin, have beneficial effects on animal health, growth and reproduction, by promoting the development of larvae [33].

7. Food use

In the early 1950s, microalgae were used to replace certain foods and were often used as single cell proteins in the diets of malnourished children and adults. Today, in human nutrition, microalgae are marketed in the form of various dietary supplement pills, capsules and liquids [91].

Gross et al conducted research in which *Scenedesmus obliquus* algae was added to the normal diet of children (5 g/day) and adults (10 g/day) during a four-week test period. Blood panel, urine composition, serum protein and uric acid concentration and weight change were measured, but the parameters analyzed did not deviate from normal values, only a slight increase in body weight was observed.

The same authors subsequently performed a three-week study in both slightly (Group I) and severely (Group II) malnourished four-year-old children. Four-year-old children in Group I (10 g algae/day) showed a significant weight gain (27 g/day) compared to children in the control group who received a normal diet, and no adverse symptoms were experienced. Group II was fed a diet enriched with 0.87 g algae/kg body weight, replacing only 8% of the total protein with algae protein, and the daily weight gain was approximately seven times that of children in the control group, while all anthropogenic parameters were normal. The authors concluded that the significant improvement in health can be attributed not only to algae protein but also to other important health-protective and immune-enhancing components [92].

Large-scale production of microalgae suitable for human consumption has been growing worldwide. There are many forms of microalgae and other health-protective dietary supplements on the market, such as various pills, powders, capsules, lozenges and liquids [23, 93]. Microalgae are also used in the preparation of various foods, such as algae pastries, biscuits, breads, snacks, candies, yogurts and soft drinks, which also provide the health and immunomodulatory effects associated with microalgae biomass [94].

Despite some reluctance on the part of consumers over novel foods in recent decades, there is a growing consumer demand for natural, health-enhancing foods today. Thus, functional foods containing microalgae biomass are also becoming increasingly popular. These products are also proving to be very attractive and varied from a sensory point of view, and their consumption also brings health benefits, satisfying consumer needs in all respects [23].

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9. References

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