Microalgal Food

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Food Ingredients and Nutraceuticals from Microalgae: Main Product Classes and Biotechnological Production Microalgal products are an emerging class of food, feed, and nutraceuticals. They include dewatered or dried biomass, isolated pigments, and extracted fat. The oil, protein, and antioxidant-rich microalgal biomass is used as a feed and food supplement formulated as pastes, powders, tablets, capsules, or flakes designed for daily use. Pigments such as astaxanthin (red), lutein (yellow), chlorophyll (green), or phycocyanin (bright blue) are natural food dyes used as isolated pigments or pigment-rich biomass. Algal fat extracted from certain marine microalgae represents a vegetarian source of n-3-fatty acids (eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), γ-linolenic acid (GLA)). Gaining an overview of the production of microalgal products is a time-consuming task.

Keywords: food ingredient; microalgal pigments; n-3-fatty acids; microalgae cultivation; growth conditions; cultivation systems

1. Introduction

Microalgae are microscopic photosynthetic organisms that are found in marine, as well as in freshwater, environments. These unicellular organisms have a size ranging from a few to several hundred micrometers, depending on class and species. Most microalgae have photosynthetic mechanisms similar to land-based plants but generally more efficient in the photoautotrophic conversion of solar energy into biomass. This is since the cellular structure of microalgae is less sophisticated, and the normal environment is aqueous with easy access to CO2 and further nutrients [1].

Microalgae are a resource for food and feed with high potential. Currently, the most cultured species are Arthrospira platensis and Arthrospira maxima, Chlorella vulgaris, Dunaliella salina, and Haematococcus pluvialis, which can be grown photoautotrophically with sunlight as an energy source. The carbon source is CO2, which can be supplied by gassing or supplementing the water with soluble carbonates. Heterotrophic cultivation on organic carbon sources such as sugars is used for the production of n-3 fatty acids with marine organisms (Crypthecodinium cohnii, Schizochytrium sp. TC 002, and Ulkenia sp. strain TC 010) in fermenters excluding sunlight ^[2]. For feed for fish aquaculture, the main cultivated species are: Chlorella vulgaris, Isochrysis galbana, Pavlova sp., Phaeodactylum tricornutum, Chaetoceros sp., Nannochloropsis oceanica, Skeletonema sp., Thalassiosira sp., Haematococcus pluvialis, and Tetraselmis suecica ^{[3][4][5]}. However, the technology to produce microalgae is still immature. Research and development have been done in recent years and continue on cultivation systems. A leap in the development of microalgae technology is required; on a practical level, the scale of production needs to increase with a concomitant decrease in the cost of production ^[1]. Microalgae are cultivated in a wide range of different cultivation systems that can be placed outdoors or indoors. Cultivation systems range from open shallow raceway ponds to closed photobioreactors. The systems mostly used on a large scale and on a commercial basis are open systems ^[6].

2. Microalgae Production

2.1. Microalgae Cultivation

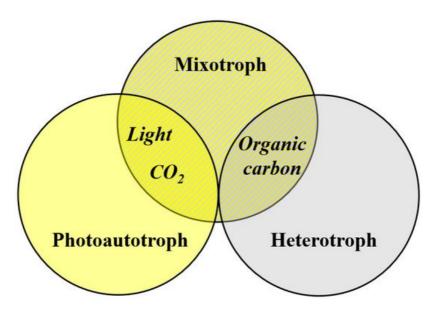
2.1.1. Illumination

Low cell densities and moderate growth rates are the major obstacles towards a broad commercial use of microalgal products. The most important cultivation parameters are light and nutrient supply. Decisive factors of light supply are light intensity, spectral range, and photoperiod.

2.1.2. Diverse Growth Conditions

Microalgae show diverse metabolic pathways. Other than the dominant photoautotrophy, secondary heterotrophy and mixotrophy is encountered (**Figure 1**). All microalgae are photoautotrophs, with autotrophic cultivation being the main

method for biomass production $^{[Z]}$. Photoautotrophs use light as an energy source to fix carbon via photosynthesis. Large-scale, photoautotrophic cultivation is mostly realized in open systems. Strains that tolerate/require high salinity or alkaline pH are used so that cultures are protected from contaminants. Prominent examples are Arthrospira platensis and Dunaliella salina $^{[S]}$. Generally, lower biomass concentrations are obtained with microalgae cultivated photoautotrophically compared to mixotrophic and heterotrophic cultivation strategies $^{[S]}$. Microalgae capable of mixotrophic growth can metabolize CO2 and light simultaneously with organic carbon sources but can also switch to sole phototrophic or sole heterotrophic growth. These microalgae show higher growth rates under mixotrophic conditions as compared to sole heterotrophic or photoautotrophic conditions $^{[10]}$. A prominent example of mixotrophic cultivation is astaxanthin production by H. pluvialis (e.g., $^{[11]}$). Generally, mixotrophic growth requires less light compared to photoautotrophic growth. Heterotrophs can grow on organic carbon in the absence of light (no illumination required). Again, growth rates under heterotrophic conditions are higher than those under photoautotrophic conditions. However, other microorganisms compete with heterotrophic microalgae for the carbon source, and sterile conditions are required to avoid culture contamination and product loss $^{[S][S]}$.

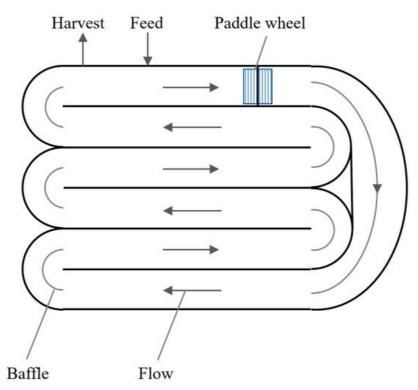


 $\textbf{Figure 1.} \ \, \textbf{Energy and carbon sources of microalgae}.$

2.2. Cultivation Systems

2.2.1. Open Ponds

Classical algae cultivation uses open ponds in natural habitats or shallow artificial ponds often mixed by a rotating arm or by paddle wheels (**Figure 2**).



2.2.2. Closed Photobioreactors

PBRs facilitate increased biomass and product concentrations. PBRs protect the algal culture against (microbial) contaminations, provide nutrients, illumination, pH and temperature control, and outgassing of the produced oxygen. Mixing and illumination are again the most important parameters. Optimal cultivation conditions provide gentle mixing so that cells, carbon dioxide, and other essential nutrients are evenly circulated. Cell movement facilitates constant illumination and prevents cell growth on vessel walls (see also **Figure 3**). The simplest PBRs consist of transparent plastic bags or plastic columns operated as bubble columns and airlift reactors (also referred to as vertical tubular reactors) (**Figure 3**).



Figure 3. Culture of Arthrospira platensis in a bubble column type bioreactor. Slight foam formation and cell growth on the vessel walls were experienced (photo: M. Murkovic).

2.3. Contaminations

A major bottleneck in the industrial production of products from algae is that algae cultures are prone to contaminations. To avoid contamination, closed systems can be used; however, in open systems (large scale), contamination is unavoidable. These are either organisms that graze upon and feed on the algae or undesired algae strains that take over the cultivation media. Contaminations can lead to culture crashes and product loss, and also the formation of toxins [12][13]. Therefore, effective strategies to avoid, repress, monitor, and control contaminants without interfering with the growth of the target microalgae are required. Grazers and contaminants experienced (especially in open-pond cultivations) are mainly insect larvae, protozoans (ciliates, amoeba, dinoflagellates), viruses, fungi, bacteria, and other algae strains [14].

2.4. Downstream Processing

Similar to the majority of bioprocesses, downstream processing constitutes a considerable cost factor. Specifically, the cost for the harvest of microalgae was calculated as 20–30% of the total production cost of cheap bulk products such as biodiesel [15].

In the majority of applications, subsequent energy-intensive dewatering and biomass drying steps are required. Discussion of further downstream processing is highly strain and product-specific. Extraction of oils and dyes requires cell rupture, centrifugation, and/or solvent extraction $^{[16]}$. The conditions of cultivation, harvesting, and subsequent processing clearly influence the nutritional composition of microalgae. For example, protein loss by the drying of Arthrospira platensis was previously reported. With freeze-drying, 5% of protein was lost, whereas spray, convective, and infrared drying led to protein losses of 10 up to 25% $^{[1Z]}$.

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3. Rentability of Microalgae Production

One of the major concerns relates to the rentability of microalgal products. Key challenges are the high production costs of products made up of commercial-scale cultivation, harvesting (dewatering), and finally, product quality assurance. The value of microalgal products spans from exceptionally high market values (e.g., EPA 4600 USD/kg) to products in the middle price segment (e.g., biomass for food and feed 10 to 50 USD/kg) down to fishmeal replacement (fishmeal price is approximately 2 USD/kg) [18]. Several algal-based foods have been launched in the last years: Microalgae biomass is used in the form of powders, tablets, and capsules. Especially in Western countries, the number of snacks and drinks containing microalgae doubled in the past few years. Most new products launched were bakery, meals, and chocolate confectionery [19]. Improvements in capital and operational costs shall enable economic production of low-value food and feed products going down to fishmeal replacement in the future economy [18][20].

4. Nutritional Composition of Microalgae

The composition of the microalgae is similar to other bacteria. Usually, they contain high amounts of protein, with all essential amino acids present. Especially the microalgal species Arthrospira platensis, Chlorella vulgaris, Dunaliella salina, Haematococcus pluvialis, and Scenedesmus obliquus show a very high content of protein with values higher than soybean, corn, and wheat (**Table 2**). Polyunsaturated fatty acids are occurring in significant amounts, including linoleic acid, γ-linolenic acid (GLA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (**Table 3**).

Table 2. Macronutrient composition of commercially important microalgae (extracted from [1][21][22][23].

| Species | Protein Carbohydrate (wt%) (wt%) | | Lipid (wt%) | |
|--------------------------|----------------------------------|-------|----------------|--|
| Nannochloropsis sp. | 29–32 | 9–36 | 15–18 | |
| Nannochloropsis oceanica | 29 | 32–39 | 19–24 | |
| Botryococcus braunii | 70 | - | - | |
| Arthrospira platensis | 53–70 | 12-24 | 6–20 | |
| Chlorella vulgaris | 49–55 | 7–42 | 3–36 | |
| Haematococcus pluvialis | 48 | 27 | 15 | |
| Isochrysis galbana | 27 | 17 | 17 | |
| Dunaliella salina | 57 | 32 | 6 | |
| Scenedesmus obliquus | 50-56 | 10–17 | 12–14 | |
| Porphyridium cruentum | 28–39 | 40–57 | 9–14 | |

Table 3. Polyunsaturated fatty acids in microalgae (extracted from [21][23]; given in % of total fatty acids).

| Species | LA | ALA | GLA | STA | AA | EPA | DPA | DHA |
|--------------------------|---------|------|-------|--------|---------|--------|--------|-----|
| Chlorella vulgaris | 3–10 | 2-21 | 15–24 | Traces | Traces | 3 | 3 | 21 |
| Arthrospira platensis | 10-21 | 1 | 1–25 | 1 | 0.3-0.4 | 2–3 | - | 3 |
| Isochrysis galbana | 1 | 0.5 | 0.5 | 1 | 1 | 2 | Traces | 19 |
| Scenedesmus obliquus | 1–2 | 4–22 | 0.1–4 | 1–3 | 0.1-0.2 | - | - | - |
| Haematococcus pluvialis | 4 | 15 | 0.1 | - | - | - | - | - |
| Porphyridium cruentum | 0.4–25 | - | - | - | 1–35 | 1–27 | - | 6.1 |
| Nannochloropsis oceanica | 0.6-0.8 | - | - | - | 1.4-1.9 | 8.4–11 | - | - |

LA: linoleic acid, ALA: α-linolenic acid, GLA: γ-linolenic acid, STA: 18:4n-3 stearidonic acid, AA: 20:4n-6 arachidonic acid, EPA: 20:5n-3 eicosapentaenoic acid, DPA: 22:5n-3 docosapentaenoic acid, DHA: 22:6n-3 docosapentaenoic acid.

5. Food Ingredients and Nutraceuticals from Microalgae

5.1. Microalgae Used as a Food Ingredient

When using microalgae as a food ingredient, some limitations are obvious. This is primarily because of the intensive green color that is added to the recipes and a fishy aroma $^{[24]}$. As the flavor is one of the biggest disadvantages of algae-containing food, a lot of work was invested in masking this flavor by using intensive spices $^{[25]}$ or by using the aroma of the microalgae to bring the typical aroma of seafood out. A commercial product $^{[26][27]}$ was developed that shows microalgae can be used as an ingredient in foods with a typical seafood aroma. Many products from algae are commercialized directly as biomass. These are sold as nutritional supplements since the microalgae, mostly Arthrospira platensis, contain high amounts of protein and n-3 fatty acids. Other products derived from Haematococcus pluvialis are used for supplementing the carotenoids astaxanthin and lutein. Since microalgae are also known for having high concentrations of n-3 fatty acids (mainly Chlorella vulgaris and Arthrospira platensis containing γ -linolenic acid, eicosapentaenoic acid, docosapentaenoic acid, docosahexaenoic acid, Table 2), extracts are developed that might be used to upgrade foods with these fatty acids.

5.2. Isolated Products from Microalgae for Enrichment of Foods

5.2.1. n-3 Fatty Acids

Some of the cyanobacteria are known for their high content of n-3 fatty acids (**Table 3**). Since the alimentary supply of n-3 fatty acids is generally limited due to limited availability of fish oils, a series of efforts are made to increase the content of these fatty acids in foods. From a nutritional point of view, the uptake of n-3 fatty acids from microalgal oil is comparable to fish oil with the possible advantage of a better supply with carotenoids $^{[28]}$. In addition to adding the n-3 fatty acids directly to the food, it is possible to enrich animal products by supplying the unsaturated fatty acids with the feed. This was shown, for example, by Lemahieu and a co-worker $^{[29]}$, who could increase the DHA content of eggs when feeding Isochrysis galbana to the chicken. Polyunsaturated fatty acids (PUFAs), particularly the n-3 fatty acids having five (EPA) or six (DHA double bonds, are prone to oxidation due to the lability of the hydrogens linked to the methylene groups between the double bonds. These PUFAs require stabilization against oxidation. Shen and co-workers could show that a combination of octyl gallate with tea polyphenol palmitate showed the most effective protection $^{[30]}$.

5.2.2. Carotene and Other Carotenoids

Microalgae are known for producing high amounts of carotenoids. Dunaliella salina can produce β -carotene up to 13% of dry weight. Astaxanthin can be found in Haematococcus pluvialis, which produces up to 7% of the dry weight. Canthaxanthin was found in Coalstrella striolata containing close to 5% of dry weight. Lutein, zeaxanthin, fucoxanthin, echinenone, and violaxanthin could also be identified in different species $\frac{[31]}{2}$.

5.3. Microalgae as Food Supplements

As mentioned earlier, the nutritional composition of microalgae is of high quality with respect to amino acids, fatty acids, and minor components such as vitamins and pigments. With these high concentrations of nutrients, microalgae are predestined for the manufacturing of food supplements. The main microalgae that are marketed as dried powder, capsules, or pressed pills are Chlorella vulgaris and Arthrospira platensis. A series of positive health effects of microalgae are described ranging from an influence on the cholesterol level and associated coronary heart diseases, antiviral, antimicrobial, and antifungal activity. The presence of sterols (e.g., clionasterol) implies all the potential positive health effects that are related to an intake of these compounds [32].

5.4. Toxicological Relevance of Microalgal Products

Some of the cyanobacteria species are known for their potential of producing potent toxins. When these microalgae contaminate the cultures for food production, the toxins pose a severe health risk. Some examples are, microcystin and related structures are produced by Microcystis aeruginosa, saxitoxin by Aphanizomenon flos-aquae, and anatoxins by Anabaena flos-aquae. The chemical structures comprise cyclic peptides (microcystin), alkaloids (anatoxin, saxitoxin), and lipopolysaccharides affecting the liver, nerve axons, and synapse, as well as the gastrointestinal tract [33]. The group of Dietrich published an overview on the presence of algal toxins in commercially available food supplements. In their study, they only found microcystins, which were present in products of Aphanizomenon flos-aquae. Extracts from all the tested samples showed cytotoxic effects, which leads to the conclusion that additional components should be present that have the potential to induce adverse effects in consumers [34].

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