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Microalgae as sources of green bioactives for health-enhancing food supplements and nutraceuticals: A review of literature

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ABSTRACT

The world population is ever increasing and so is the need to ensure food security. Food production needs to increase by about 70% within the next 40 years to cater for food consumption. Moreover, with increasing collective consciousness toward food supplementation for improving quality of health, the development of nutraceuticals has gained prominence in disease prevention, treatment, and overall health improvement. However, due to the constant controversial debate of food production for consumption against other uses, the search for better alternatives led to microalgae. Species such as *Spirulina*, *Chlorella*, *Scenedesmus*, and *Dunaliella*, among many others, are important sources of primary and secondary metabolites that play crucial roles in disease prevention and treatment. Understanding the significance of nutraceuticals and how microalgae can be used to produce those value-added molecules is necessary for any potential commercial exploitation. This review discusses the potential of microalgae to be exploited as promising sources of nutraceuticals. Here, essential biomolecules used as nutraceuticals are explored and their crucial roles in disease prevention, especially cancer, cardiovascular diseases, and strengthening the immune system. The composition of microalgae, which makes them suitable candidates to produce nutraceuticals, is discussed. Furthermore, the multifarious aspects of microalgae cultivation, in terms of cultivation systems and factors affecting biomass production and productivity regarding nutraceutical production, are reviewed. The multiple sustainable facets of microalgae culture, which can help in carbon sequestration, fast biomass production, and boosting health benefits, should interest stakeholders and potential commercial producers. Bioprocessing of microalgae for the extraction and purification of microalgae-based products is also reviewed, focusing on the key methods of pre-treatment, extraction, and purification of microalgal biomass.

Keywords: Nutraceuticals, Food supplements, Microalgae, Culture conditions, Secondary metabolites

NUTRACEUTICALS AS VEHICLES OF HEALTH IMPROVEMENT

Since the past few years, health consciousness among consumers has significantly increased due to more readily available knowledge and increasing cases of diseases, leading to positive changes in eating habits and overall lifestyle.[1] Moreover, the health industry's focus is being shifted more toward disease prevention than disease treatment,[2] hence, stimulating the search for alternative ways for disease prevention and treatment, and improvement of overall health. In light thereof, the terms "nutraceuticals" and "functional foods" have been developed, accentuating the famous quote from Hippocrates: "Let the food be thy medicine and medicine be the food."

An introduction to nutraceuticals

The concept of "nutraceutical" originated from a survey carried out in the United Kingdom, Germany, and France about the factors contributing to overall good health, and they concluded that consumers rated diet as the most important one. According to DeFelice,[3] nutraceutical is defined as "a food or part of a food that provides medical or health benefits, including the prevention and treatment of diseases." However, in some cases, nutraceuticals have been defined as formulated products ingested in doses or forms of pills and tablets,[4] or as food that provides the body with the recommended amount of essential biomolecules.[5] This definition may vary among authors, but the basis remains the same: A food (or part of) is considered as a nutraceutical when it provides additional health benefits other than its basic nutritional functions, such as disease risk reduction, treatment, and overall health improvement. On the other hand, food supplements can be defined as products containing one or more dietary ingredients required for optimal nutrition to supplement the diet by increasing the total daily intake of these specific dietary ingredients.[6] The difference between these two concepts is minimal since nutraceuticals are these dietary supplements utilized mainly for health purposes rather than only nutrition.

Even though nutraceuticals and pharmaceuticals appear similar, it is vital to understand their differences. The major difference is that pharmaceuticals are usually composed of high doses of a single compound, while nutraceuticals are generally composed of low amounts of a pool of compounds.[7] Unlike nutraceutical products that use food or specific food components to prevent or treat a specific disease, pharmaceutical products are chemically formulated drugs to achieve the same purpose.[8] Hence, nutraceuticals and pharmaceuticals aim to prevent or treat diseases, but only the latter have government sanctions and are more strictly regulated.[9] Nutraceuticals have gained significant attention as a potential alternative to pharmaceutical products due to the side effects associated with pharmaceutical drug products, the increasing cases of antimicrobial resistance, and the fact that nutraceuticals are comparatively more affordable.[10]

Composition of nutraceuticals

As stated in the previous section, not all the health benefits of nutraceuticals are backed by clinical trials. Nutraceuticals can be considered either potential or established ones,[11] whereby the former promises a specific health benefit and only becomes an established one after undergoing enough clinical trials to confirm the same. Nutraceuticals comprise of an overwhelmingly wide range of bioactive compounds derived from edible sources.[12] They can be classified into different main groups, as shown in [Table 1].[1,5,7,10,13-20] It should be noted that the list of nutraceuticals is continuously changing due to the ongoing research, interest of consumers, and effectiveness and safety usage of the nutraceutical itself.[6]

Table 1: Examples of bioactive compounds and their edible sources.

Bioactive compounds	Examples of bioactive compounds and their edible sources
Antioxidants	Curcumin – turmeric Catechin – green tea and berries Proanthocyanidins – grape seed Lycopene – tomatoes
Dietary fiber	Wheat bran, oats, and fruits
Fatty acids	Omega-3 and omega-6 PUFAs – salmon and algae
Peptides	Casein and lactoferricin – cow's milk
Probiotics	Bacterial strains: *Lactobacillus* spp. and *Bifidobacterium* spp.
Prebiotics	Inulin and fructooligosaccharides – onions, leaks, and asparagus
Minerals	Magnesium – almonds and banana Zinc – Shellfish and spinach
Vitamins	Vitamin A – Orange/yellow fruits and vegetables Vitamin C – citrus fruits

PUFAs: Polyunsaturated fatty acids

Antioxidants

Antioxidants inhibit oxidation and prevent damage caused to cells by free radicals. Flavonoids and carotenoids are the two primary antioxidant pigments. Flavonoids are among the most common polyphenols, further divided into flavones, flavanols, flavanones, and isoflavones [Figure 1].[16] Common examples of flavonoids include catechins and proanthocyanidins. Carotenoids are fat-soluble pigments copious in plants and algae, including lycopene, α-carotene, β-carotene, lutein, and zeaxanthin.[21] Both carotenoids and flavonoids are essential in lowering the risk of diseases due to their antioxidant and anti-inflammatory properties. For instance, lutein and zeaxanthin are the carotenoids present in the retina and prevent retinal degeneration by acting as photoprotectants.[22] Furthermore, some vitamins and minerals act as antioxidants, namely, retinol, ascorbic acid, tocopherols, magnesium, manganese, selenium, chromium, and zinc.[13,23,24]

Antioxidants are crucial for optimal cellular health and to prevent diseases associated with free radical damage, such as cancer, Alzheimer's disease, Parkinson's disease, and aging.[25-28] Among the various treatments against Alzheimer's disease, is a promising method where the aggregation of the 42-mer amyloid β protein (Aβ42)is prevented, which a desired condition. The latter was explored with the flavonoid kaempferol, which successfully suppressed the Aβ42 aggregation by preventing amyloid fibril elongation.[29] The potential of this compound was further evaluated on *Drosophila* flies expressing human

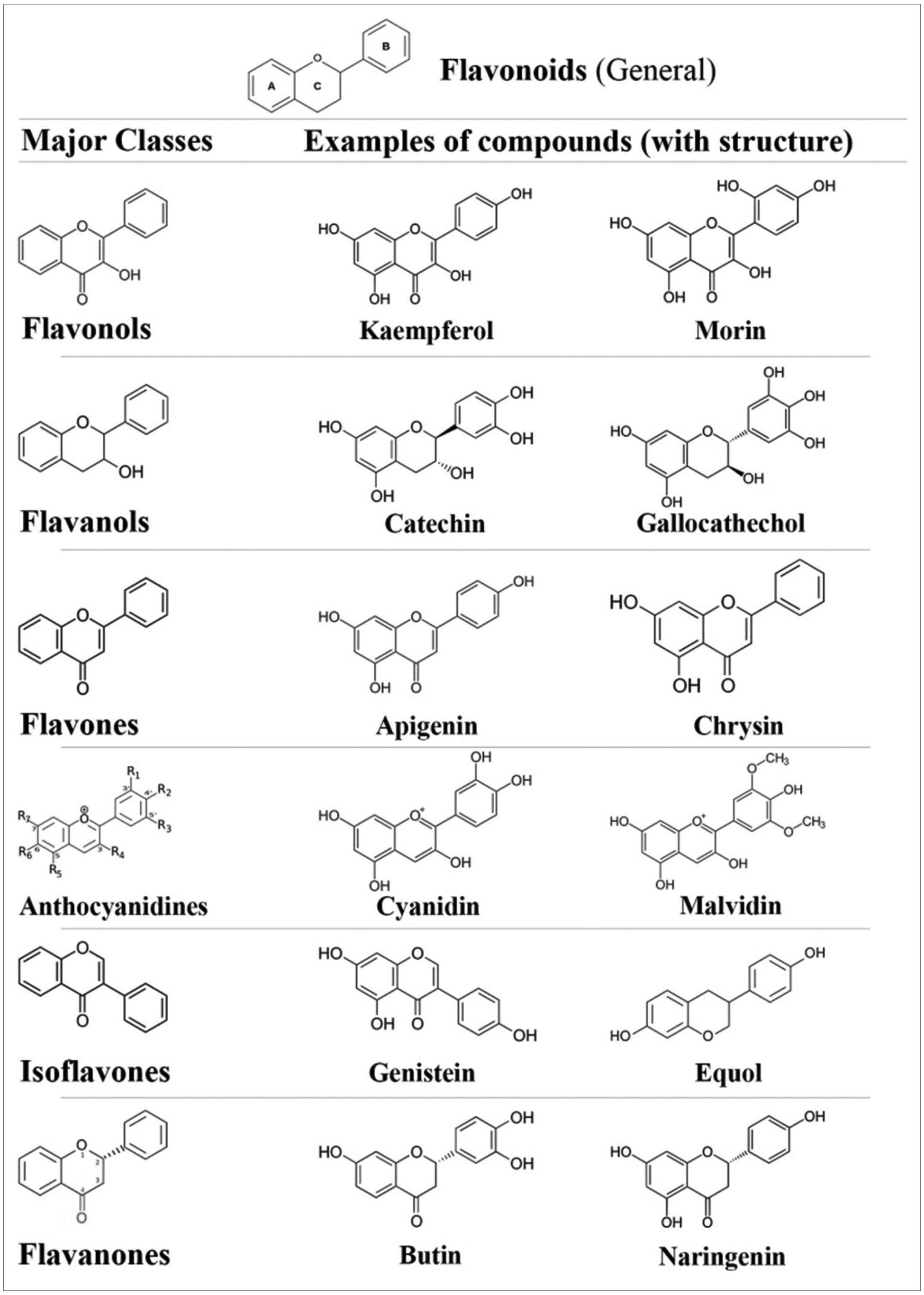

Figure 1: Major classes of flavonoids and some examples of their compounds (with structures).

amyloid beta-42 and resulted in a delay in memory loss, reduced oxidative stress, and acetylcholinesterase activity with 40 μM of kaempferol.[30]

Other medical conditions such as hyperlipidemia, a group of metabolic disorders, are denoted by hypercholesterol in the blood circulation, which may lead to cardiovascular disease (CVD). A naturally occurring anthocyanin called cyanidin-3-rutinoside has been recently identified as a lipid-lowering agent by inhibiting the formation of cholesterol micelles and inhibiting pancreatic cholesterol esterase by 5–18%.[31] A different metabolic disorder, Type 1 diabetes, is a prevalent health issue without a cure but can be controlled. The flavonoid naringin was identified as a beneficial compound considering its ability to mitigate complications of diabetic ketoacidosis by improving the fasting plasma insulin, hepatic glycogen content, and other related factors.[32]

Neurological diseases have been have been constantly and increasingly under study, sharing similar concerns regarding the lack of cure for metabolic disorders. A widespread disease in this category is status epilepticus (which can be described as a failure to terminate or initiate mechanisms leading to abnormally prolonged seizures).[33] A study that used astaxanthin on rats with status epilepticus indicated an alleviation of neuroinflammation in the brain and alleviated damage caused to their hippocampus.[33] Even neuropsychiatry medical conditions such as depression can be managed with lutein due to its antidepressant-like effect following corticosterone treatment in mice to induce depression.[34] Other conditions such as spinal cord injuries can also be managed (reduced oxidative damage, mitochondrial dysfunction, and cell apoptosis) with the use of different carotenoids such as lycopene due to its antioxidant capacity, as demonstrated in a study on rats administered with the compound (10 and 20 mg/kg).[35]

Carbohydrates and dietary fiber

Carbohydrates are essential primary metabolites that may occur as free sugars such as glucose, short-chain carbohydrates, or polysaccharides such as starch. They are the main source of energy to fuel the human body. Glucose acts as a short-term but immediate source of energy, while fiber, an indigestible carbohydrate, helps with digestion and maintains normal blood cholesterol levels. Important sources of carbohydrates are milk, sugarcane, tubers, rice, whole grains, fruits, and beans, among many others. Plants synthesize glucose during photosynthesis which is then converted to starch, cellulose, and hemicellulose.[36] Starch is the most commonly consumed carbohydrate by humans, while animals such as ruminants can also digest cellulose. Furthermore, carbohydrates are utilized in producing oral nutritional supplements as taste enhancers or inhibitors, energy sources in prolonged fasting, and color, flavor, and texture enhancers.[37] In aquaculture practices, carbohydrates in bread flour, cornflour, and cassava starch are used as energy sources and as binders in the feed production process.[38] Indian major carp is an extensively cultured fish in countries such as Bangladesh, and its feed formulation can contain up to 30% carbohydrates, as a cost lowering measure.[39]

Dietary fibers are non-digestible carbohydrates that the human body cannot break down. They exist as either insoluble fibers (cellulose and hemicellulose) or soluble fibers (pectin and gum) and are mostly present in fruits, vegetables, and whole grains.[5] Dietary fiber is crucial for maintaining a healthy digestive system. In a study conducted by Hou *et al.*,[40] dietary fibers help reduce inflammatory bowel diseases, namely, Crohn's disease and ulcerative colitis. Furthermore, soluble fibers have also been found to help regulate blood glucose and fat levels.[5]

Lipids and fatty acids

Fatty acids are the building blocks of lipids, a group of fats or fat-like substances characterized by their insolubility in water. Due to the diversity of lipids and their structural variations, a standard classification of lipids has been developed containing eight categories: Fatty acids, glycerolipids, glycerophospholipids, sphingolipids, saccharolipids, polyketides, sterol lipids, and phenol lipids.[41] This classification includes lipids from various sources such as animals, plants, bacteria, microalgae, and fungi. The intake of lipids can be achieved by consuming plants and plant-based products (walnut, fruits, vegetable oils, and grains), aquatic organisms (fish, crustaceans, and molluscs), or even animal-derived products such as milk, meat, and eggs.[42]

Fatty acids can be classified as either saturated fatty acids, monounsaturated fatty acids, or polyunsaturated fatty acids (PUFAs). The latter are widely used as nutraceuticals due to their positive impacts on overall health.[7] There are fatty acids synthesized in very low amounts by the body, called essential fatty acids, which must be supplied through the diet. Those are essentially the main n-3 PUFAs α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). These n-3 PUFAs have positive effects against cardiac disorders, Type 2 diabetes, and autoimmune diseases,[43-45] and they are also crucial for the growth and the healthy development and functioning of the brain and nervous system.[46] Fatty acids are highly involved in metabolic pathways, such as linoleic acid (LA) and ALA, which are the precursors to other PUFAs. LA is the most common and highly consumed PUFA in the diet and helps in growth and development. One of the primary uses of LA is its physiological ability to maintain the transdermal water barrier of the epidermis, and deficiency of this PUFA would lead to skin lesions.[47]

Moreover, n-6 PUFAs have also been found to be involved in health. For instance, arachidonic acid is vital in the healthy development of the central nervous system and retina of newborns and promotes healthy muscle growth in humans.[48] Gamma-linolenic acid, another n-6 fatty acid that can be obtained in human milk, plant seed oils, blackcurrant, and borage,[49] is successful in treating rheumatoid arthritis as it may increase the E-levels of prostaglandins, which, in turn, increase cAMP levels, thus suppressing tumor necrosis factor-α (TNF-α) synthesis.[50] Thus, the dietary intake of PUFAs, especially the n-3 and n-6 ones, is crucial for proper growth and optimal health.

Proteins and bioactive peptides

Proteins are macromolecules that consist of amino acid chains. Proteins and amino acids play dominant roles in the maintenance of body homeostasis. They are critical components for the muscles, enzymes involved in metabolic pathways, immune system (as immunoglobulins), and hormones that control various activities such as growth and many other functions.[51] Therefore, sufficient amounts of proteins in the diet are needed to meet the daily physical requirements.[52]

Bioactive peptides are organic compounds produced when proteins are fermented or digested by proteolytic enzymes.[5] Most bioactive peptides are derived from milk proteins, and they are believed to improve human health. For instance, lactoferrin possesses antimicrobial, anti-inflammatory, anticarcinogenic, antioxidant, and immunostimulatory activities.[53] Furthermore, some bioactive peptides have an opioid-like activity, and they are involved in different processes in the nervous system and the regulation of cytokines in immunomodulatory activity, decreasing blood pressure, and preventing hypertension.[54]

Probiotics and prebiotics

In general, the gut has an abundance of beneficial bacteria. However, intestinal dysbiosis may occur due to antibiotic therapies, poor diets, and stress.[55] Probiotics and prebiotics can help restore the gut microbiota healthy balance and, in turn, improve intestinal health. Probiotics are living microorganisms that benefit the gut microbiota when administered adequately. Probiotic organisms most commonly used include *Bifidobacterium*, *Lactobacillus*, and *Saccharomyces*.[24] It has been shown that probiotics can contribute to the prevention of diarrheal infections and reduce the length of the diarrheal spell in outpatient treatments.[56] On the other hand, prebiotics is non-digestible compounds on which intestinal microbes feed themselves for growth while beneficially impacting the host.[57] The most common prebiotics include oligosaccharides present in fruits, vegetables, and whole grains. Hence, probiotics and prebiotics work together in symbiosis to maintain healthy gut health.

Vitamins and minerals

Vitamins and minerals are considered micronutrients, required in smaller quantities by the body compared to macronutrients. Both are required for the body's normal functioning, except that vitamins are organic substances while minerals are inorganic ones. According to the World Health Organization (WHO), it is estimated that more than 2 billion people worldwide suffer from micronutrient deficiency. The latter can greatly increase the risk of several diseases and health complications: Anemia, reduced cognitive functions, increased risk of infections, and perinatal complications.[58] These micronutrients are mainly obtained from external food sources since most of them cannot be synthesized by the body and play pivotal roles in maintaining health [Table 2] and preventing many diseases.[59]

Table 2: Role of vitamins and minerals in the body.

Micronutrients	Role in the body
Vitamins	
Vitamin A	- Needed for healthy vision, skin, and hair
Vitamin B	- Aids energy release from food (B1) - Required for building and maintenance of tissues (B2) - Helps in proper development of the nervous system (B6 and B12)
Vitamin C	- Needed for healthy and strong gums, teeth, and bones - Acts as an antioxidant
Vitamin D	- Required for strong bones and teeth
Vitamin E	- Protects cell membranes from damage - Helps in blood production
Vitamin K	- Involved in blood clotting
Folic acid	- Helps in building proteins and DNA - Required for bone growth
Minerals	
Calcium	- Required for healthy bones and teeth - Involved in blood clotting
Potassium and sodium	- Regulation of osmotic balance in cells - Required for healthy nerve function
Magnesium	- Involved in protein synthesis and regulation of blood glucose - Required for healthy nervous and muscular systems
Iron	- Formation of red blood cells - Involved in oxygen transportation
Zinc	- Required for transportation of carbon dioxide - Involved in formation of enzymes

Nutraceuticals and health

As shown in Section 1.2, most nutraceuticals possess several therapeutic benefits.[6] Either being potential or established, nutraceuticals are famous for their potential roles in the treatment and prevention of CVDs, diabetes, hypertension, and cancer, as well as in anti-aging processes.

Strengthening the immune system with nutraceuticals

Viral diseases are presently one of the leading causes of death worldwide, and their ability to spread is escalating, which could result in pandemics, especially in the case of COVID-19. A healthy diet consisting of nutraceuticals can significantly boost the immune system, optimize cell functions, and fight against several viral infections.[20] Nutraceuticals that have been shown to have antiviral properties include resveratrol, quercetin, curcumin, and epigallocatechin gallate.[14,15,60,61]

In a research study, these nutraceuticals had demonstrated antiviral activities against coronaviruses by inhibiting the viral replication process through regulation of the protease, Mpro of the virus.[62] Moreover, Vitamins A, D, B, C, and E and minerals such as calcium, magnesium, and zinc have significantly strengthened and supported the immune system, allowing the body to combat viral invasions.[20,62-66]

Nutraceuticals against CVDs

CVDs are disorders related to the heart and blood vessels, including coronary heart diseases, cerebrovascular diseases, peripheral arterial diseases, rheumatic heart diseases, congenital heart diseases, and deep vein thrombosis.[67] According to the latest study published by the WHO, an estimated number of 17.9 million people died from CVDs worldwide in 2019. This alarming finding raises concerns and calls authorities to find effective ways in preventing and treating this disease. There is growing evidence showing that some nutraceuticals can lower the risks associated with CVDs. For instance, regular consumption of n-3PUFAs, especially LAs, EPA, and DHA, has been proven to lower the risks of CVDs and diabetes and can also enhance brain development.[7,68]

A study conducted in Japan with 18,645 patients demonstrated that EPA, supplied as 1.8 g in the diet, reduced coronary diseases by 18%.[69] Results were similarly reported in another study, where patients with percutaneous coronary intervention were given early EPA treatment, resulting in a significantly lower death rate at 0.8% than 4.2% in the control group.[70] Another study reported that patients who consumed *Annurca* apple polyphenol extract increased fecal cholesterol excretion by 35%.[71] Therefore, diets rich in nutraceuticals can potentially be used to prevent and treat CVDs.

Nutraceuticals for cancer prevention

Cancer is the second leading cause of mortality worldwide, and around 35% of these deaths are associated with diet.[1] Nutraceuticals in the categories of antioxidants, bioactive peptides, minerals, and vitamins have demonstrated the ability to prevent certain cancers. For instance, the carotenoids lycopene and β-carotene have antioxidant activity and can prevent cancer by decreasing oxidative stress and damage to DNA.[23,72] Moreover, it has been found that bioactive peptides can reduce the risk of developing certain tumors and enhance the effectiveness of anticarcinogenic therapies.[73] For example, the bioactive peptide lactoferrin possesses antitumor activities without damaging healthy body cells.[74,75] Therefore, a healthy lifestyle and a diet rich in these nutraceuticals mentioned above, along with selenium, Vitamin C, and Vitamin E, can help prevent cancer.[6] However, it should be noted that these studies have demonstrated that nutraceuticals mainly participate in preventing cancer rather than treating the disease, and hence, more research is still needed in this area.

MICROALGAE AS FOOD SUPPLEMENTS AND SOURCES OF NUTRACEUTICALS

Most nutraceuticals are produced from food sources such as fruits, nuts, and certain vegetables. One major drawback of using such food commodities to produce nutraceuticals is that it decreases their availability on the market as fresh food for consumption. Following the Sustainable Development Goals of the WHO, where there is a need to ensure food security and end hunger, food commodities should not be promoted for alternative uses. This tendency would trigger the need to bring more land into agricultural activities and thus lead to deforestation and biodiversity loss. A similar trend was encountered in biofuels production from plant biomass.[76]

Microalgae have been promoted as a biofuel alternative. However, a potential utilization of microalgae in the production of nutraceuticals has emerged. Microalgae are good sources of proteins, carbohydrates, essential fatty acids and amino acids, antioxidants, and some essential vitamins and minerals.[77-80] Due to their high nutritional values, microalgae have the potential to be incorporated into diets as nutrient sources and can also act as nutraceuticals to prevent and treat diseases and maintain optimal health. The following sections will further discuss these primary and secondary metabolites of microalgae.

DERIVING NUTRACEUTICALS FROM MICROALGAE

Microalgae as a source of essential metabolites

The screening of microalgae isolates has gained much attention over the past 15–20 years with the severe threat of global warming and greenhouse gas emissions. Given the depletion of fossil fuels and the need for sustainable renewable biofuels, microalgae are considered third-generation biofuels over plant biomass.[81] Many research studies have been conducted to obtain the species or strains that would produce most carbohydrates for bioethanol and lipids for biodiesel.[82] This situation has led to the publication of numerous research works in this field with information on the biochemical composition of biomass at the macro- and micro-nutrient levels. However, the data available from these works may explore their usage in other fields such as nutraceuticals and food industries.

Furthermore, concerning the biochemical contents, microalgae also look promising for their use as food additives/supplements for human consumption and feed additive for agricultural sectors such as aquaculture practices. With a fast-growing world population (currently of 7.9 billion people), global agricultural productivity has to increase by 70% to meet demands for food supply in the next three decades.[83,84] Food crops, being the base source of macro- and micro-nutrients, are partly diverted to other sectors such as biofuels production,[85] which can decrease the availability of marketable fresh food commodities. Natural disasters are a massive setback for agriculture, influencing food availability and nourishment.[86]

Microalgae may marginally improve food production in agriculture and aquaculture and as a direct source of nutrients. Microalgae are the primary producers in the aquatic food webs that produce carbohydrates, proteins, and lipids. The diversity of microalgae in the marine and freshwater environment is estimated to be more than 100,000 species, with more than 25,000 species already isolated and identified [Figure 2].[87] These photosynthetic organisms are essential components across food chains as they pass on macro- and micro-nutrients to the next level and so on. Carbohydrates such as glucose provide the energy, proteins help with hormones, muscle, and tissue development, while fats act as an energy source but are also involved in the brain and central nervous system development. Furthermore, the metabolic pathways differ considerably from species to species, allowing the synthesis of specific compounds such as fatty acids, carotenoids, and polysaccharides.[80]

Since many microalgae species may possess the ability to produce high-value components, it is more profitable to culture the most productive species or those displaying the most favorable composition. However, this depends on the desired end product and the ease of culture, harvesting, biomass processing, and other downstream processes. Research on microalgae for such end products has been ongoing for many decades to initiate economic growth in the pharmaceutical, aqua-cultural, biofuel, and food production sectors.[88,89] Therefore, before reaching the industrial utilization of microalgae, evaluating their biochemical composition is necessary as they vary from species to species.

Microalgae *Graesiella* spp. WBG-1 was successfully evaluated as a source of biofuel at the pilot scale.[90] Clinical studies have shown that *Spirulina* consumption ingested as 6 g/day improved oxygen uptake in high-intensity exercise apart from being a source of protein.[91] Regarding feed use, *Arthrospira platensis* (*Spirulina*) was an excellent alternative to improve fish immunity in aquaculture practices.[92]

The world's largest *Spirulina* producer is Hainan Simai Enterprising, located in China, and has an annual algal powder production of 200 tons. Worldwide, 20 countries (primarily China, Israel, Japan, Mexico, Taiwan, and Thailand) produce *Spirulina*-based products, such as tablets and powder. Microalgae are also researched for the bioremediation of wastewaters and biofuel production, for instance, *Chlorella* spp.[93]

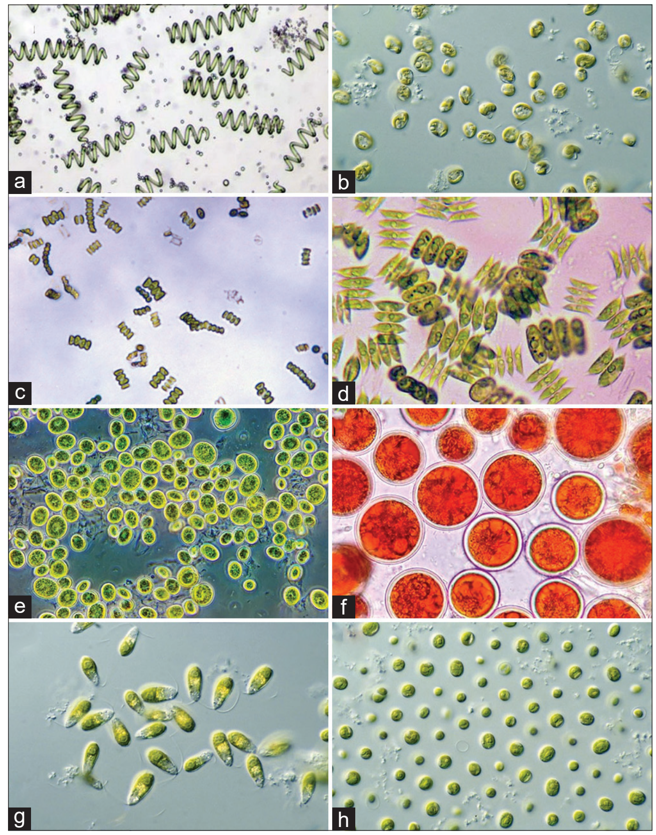

Figure 2: Microalgae of interest – (a) *Spirulina* spp., (b) *Pavlova* spp., (c) *Desmodesmus* spp., (d) *Scenedesmus* spp., (e) *Chlorella vulgaris*, (f) *Haematococcus pluvialis*, (g) *Dunaliella* spp., and (h) *Nannochloropsis* spp. *The images reproduced in this figure are assigned to the public domain through a Creative Commons Zero (CC0) license or similar release – Wikimedia Commons.*

Table 3: Biochemical composition of some microalgae species (values correspond to research parameters applied).

Microalgae species	Macronutrients (% dry weight)		
	Proteins	**Carbohydrates**	**Lipids**
Arthrospira platensis	62.9	15.6	8.1
Arthrospira platensis	76.7	6.5	2.5
Chlamydomonas reinhardtii	48.0	17.0	21.0
Chlorella vulgaris	31–36	8.8–20.8	24–33
Chlorogloeopsis fritschii	50.0	44.0	7.0
Dunaliella tertiolecta	8.3–31.3	46.5–50.6	18.0–23.5
Lyngbya limnetica	3.1	18.4	14.5
Phormidium autumnale	50.2	22.4	15.6
Porphyridium cruentum	28.0–39.0	40.0–57.0	9.0–14.0
Scenedesmus dimorphus	8.0–18.0	21.0–52.0	16.0–40.0
Scenedesmus obliquus	34.9	23.8	14.3

Understanding the biochemical composition of microalgae (from species to species) [Table 3] is important for commercial applications, especially in finding the most appropriate strain for a specific task.[94-105]

Proteins

Importance of proteins and microalgae

Microalgae diversity facilitates multiple combinations of primary and secondary metabolites.

Among these combinations, are proteins and amino acids, which play dominant roles in maintaining body homeostasis. Traditional protein sources include meat, eggs, milk, and soybean. From investigations, protein levels from microalgae are comparable to levels from traditional sources.[79] Furthermore, since proteins are composed of amino acids, the composition comparison between some microalgae species and some basic conventional food items share similar patterns.[95] Acquiring protein from microalgae is deemed more beneficial than traditional high-protein crops in productivity and nutritional value. Microalgae can produce a protein yield of 4–15 tons/Ha/year compared to soybean (0.6–1.2 tons/Ha/year) and wheat (1.1 tons/Ha/year).[106] Since microalgae are being prospected for biofuel production, the residual biomass would contain the proteins useful for feed formulations, following lipids and carbohydrate extraction.[88,107]

Commercially important microalgae for protein

Despite the production advantage, microalgae culture on a large scale as sources of food/feed is limited to a few countries due to the costs involved. However, research has identified suitable microalgae candidates that are suitable sources of proteins and amino acids and are currently being cultured at the industrial level. One popular microalgae is *Spirulina platensis*, a non-toxic cyanobacterium, often regarded as a complete food containing all essential amino acids and can reach up to 62% of protein levels.[108,109] More recent studies on *S. platensis* produced a protein concentrate that contained 75.97% proteins on a dry weight basis which could help in food/feed formulations.[110]

Chlorella spp. is also a very good source of proteins that can reach a content of 51–58% as dry weight. The amino acid composition of this *Chlorophyta* microalgae makes it an appealing source of proteins as it contains nearly all of the essential ones.[95] *Scenedesmus* spp. microalgae, typically investigated for biofuels, tend to yield protein contents between 30 and 55%, and the variations are due to type of strain, culture medium, among other factors.[111] Another commercially produced microalgae is *Dunaliella* spp., yielding up to 57% protein content.[95] However, this microalgae tends to be overlooked as a source of protein due to its importance in β-carotene production.[112]

High-value protein metabolites

Nonetheless, the microalgae proteins are released in various forms following biomass processing, such as glycoproteins and phycobiliproteins.[113] Early research on *Chlorella vulgaris* led to the discovery of a water-soluble glycoprotein with antitumor activity.[114,115] An investigation on *C. vulgaris* glycoproteins indicated a rare occurrence of modified oligomannosidic glycans that have not been found in either plant or vertebrate N-glycans, prompting the need for further investigations as these microalgae are sold as a source of protein.[116]

Other glycoproteins such as lectins are involved in various biological processes, including cell-cell communication, antiviral activities, and antibacterial activities, among many others.[117] In the phycobiliproteins group, phycocyanin and phycoerythrin are the most common compounds produced by microalgae. Phycocyanin is mainly derived from *S. platensis* and is a natural dye for food products and cosmetics. Phycoerythrin is used as a fluorescent agent for diagnostic purposes and is mainly derived from red algae such as *Porphyridium cruentum*.[118]

Microalgae-derived carbohydrates and valuable species

Polysaccharides derived from microalgae such as *P. cruentum* are essential for various applications such as viscosifiers, flocculants, and lubricants for the industrial sector and as antiviral agents in medical applications.[118] The microalgae species that offer the most useful carbohydrate content would be *Dunaliella salina*, *Dunaliella tertiolecta,* and *P. cruentum,* among many others that are commercially cultured [Table 3]. However, recent development in microalgae research has shown that other species possess even higher levels than those listed above.

An investigation of the filamentous microalgae *Tribonema* spp. demonstrated a sugar concentration of 18.8 g/L, equivalent to 81.48% extraction efficiency, following acid hydrolysis of the biomass.[119] Different microalgae from the *Rhodophyta* phylum, particularly *Rhodosorus* spp. SCSIO-45730, were investigated for its rich carbohydrate content and were found to produce total carbohydrates and β-glucans up to 242.6 ± 2.3 mg/L/day and 108.1 ± 4.0 mg/L/day, respectively.[120] Investigating the carbohydrate and starch production capacities of *Chlorella* spp. AE10 recovered productivities of 0.311 g/L/day and 0.421 g/L/day for starch and total carbohydrate, respectively.[121]

The most of the recent research on microalgae for carbohydrates is related chiefly to third-generation biofuels production in an attempt to produce bioethanol.[122] However, obtaining carbohydrates from microalgae biomass rely on releasing the sugars or starch from the cells. Although they are microscopic photosynthetic plants, microalgae do not possess lignified cell walls.[123,124] This aspect of microalgae makes it easier to extract intracellular contents such as carbohydrates or starch. Various pre-treatment methods exist, such as chemical hydrolysis, sonication, and microwaving.

Lipids from microalgae

PUFAs have multiple sources, most notably derived from marine and aquaculture, including fish, fish oil, fishmeal, crustaceans, and other products.[125] Based on the third level of dietary need, research is ongoing in finding microalgae that could replace fish oil, especially in terms of EPA and DHA.[126] These are produced from microalgae such as *Pavlova* spp., *Nannochloropsis* spp., *Crypthecodinium* spp., *Spirulina*, and *Porphyridium* spp.[80,118] Microalgae as a candidate for lipids production were also investigated, where EPA was produced at the peak of 13.1%, ARA at 30.5%, LA at 27.6%, and linolenic acid at 0.4% (under various conditions) with microalgae *P. cruentum*.[127]

While much research is driven for biodiesel production from microalgae lipids, the information also exists on PUFAs, which are undesirable for biofuels production due to oxidative stability and cold flow challenges.[128] Marine microalgae *Skeletonema costatum* were evaluated as a biodiesel feedstock, and the fatty acids profiling revealed the presence of ARA and EPA.[129] *Tetraselmis* spp. was also investigated for the same purpose and did show some content of PUFAs such as LA, linolenic acid, and EPA at peak values of 3.8%, 11.3%, and 4.9%, respectively.[130] These PUFAs, considered as essential fatty acids, contribute to the knowledge of lipids from microalgae.

The concentrations of the various biochemical compounds within the microalgae, such as PUFAs, highly depend on the environment.[127] Environmental changes in light, temperature, and nutrients affect microalgal growth and the accumulation of carbohydrates, proteins, and lipids.[131] Microalgal growth rate depends on pH and temperature for optimal lipid production, hypothesizing that various microalgae strains could yield higher PUFA content under specific conditions.[132]

Microalgae as a source of value-added secondary metabolites

Apart from the primary metabolites, microalgae are producers of biomolecules such as astaxanthin, lutein, beta-carotene, and chlorophyll-a.[133] Among the bioactive compounds, some tend to have antimicrobial properties. An increase in antimicrobial resistance undermines the efficacy of current therapies.[134,135] With only a small percentage of researched microalgae community, there is room for further discoveries of novel antimicrobial molecules. Microalgae such as *Scenedesmus obliquus*, *Chlamydomonas reinhardtii,* and *C. vulgaris,* among many others, are known to exhibit antimicrobial activities against Gram-positive and Gram-negative bacteria.[136]

Other high-value compounds are carotenoids such as astaxanthin and β-carotene. *Scenedesmus* spp. was found to be a potential source of these compounds when grown in wastewaters leading to increases of 2.8- and 5-fold against a control, respectively.[137] However, the richest source of microalgal astaxanthin is *Haematococcus pluvialis,* with yields reaching 1.5–3% of the dry weight.[138] This biomolecule provides perfect protection for the membrane phospholipids. In addition, it offers health benefits such as anticancer, immune modulating functions, and prevention of neurodegenerative disorders.[139]

Similarly, β-carotene, another carotenoid, is a valuable food colorant with antioxidant and cancer-preventive properties.[80] The major producer of this biomolecule is *D. salina,* which can accumulate up to 14% of this component as dry weight (up to 25 mg/L) under outdoor conditions.[140,141] *D. salina* production is currently at 1200 tons per annum in Australia, Israel, the USA, and Japan for the production of β-carotene.[142] Apart from those listed above, microalgae are also good producers of Vitamins B, C, and E, which are important to decrease fatigue, prenatal health problems, and antidiabetic compounds.[80] Commercially available *Chlorella* products have been shown to possess considerable amounts of Vitamin B2, C, niacin, and carotenoids, among many other constituents.[143]

The human body can only synthesize two vitamins (D and B3) out of the 13 essential vitamins. Microalgae have been found to contain all 11 essential vitamins and minerals required by the body. The cyanobacteria *Arthrospira*, also commonly known as *Spirulina*, was not labeled as a superfood coincidentally. Its micronutrient-rich profile can be seen in [Figure 3], with high B vitamins, iron, manganese, and sodium concentrations. Moreover, it has been found that *Dunaliella* spp. contains Vitamins A, B, C, and E and minerals such as Mg, Fe, K, Ca, Na, Zn, Cu, and Mn.[144,145] According to Fabregas and Herrero (1990), the levels of Vitamins A, B, and E were higher than conventional foods such as orange, wheat, corn, rye, and soya.[144] At a closer look, microalgae culture can simultaneously lead to various business opportunities as they contain value-added products from proteins to PUFAs.

CURRENT STATUS OF MICROALGAE CULTURE

Conventional culture conditions

Biomass production and biochemical composition of microalgae rely strongly on prevailing environmental conditions. It is known that variations in abiotic factors such as light, temperature, and nutrients can influence microalgae

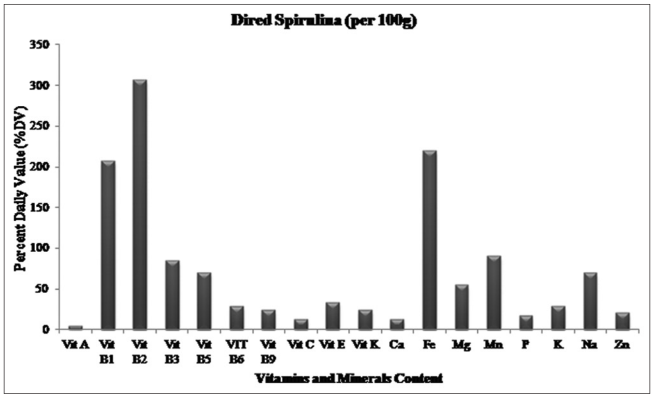

Figure 3: The micronutrient contents of dried *Spirulina* (*Arthrospira*). Adapted from.[146]

growth and the accumulation of carbohydrates, lipids, and proteins. The optimal parameters for microalgae growth are temperatures between 16 and 27 °C, adequate illumination, pH between 7 and 9, salinity between 20 and 24 gL^{-1}, and agitation.[76]

Mode of nutrition

Due to the diversity of microalgae species and the need to acquire commercially valuable ones, research has been able to help identify their mode of nutrition. These have been identified as follows: Photoautotrophy, heterotrophy, and mixotrophy. The mode of nutrition is an important aspect of microalgae culture to consider because it dictates the whole process of the culture from the choice of the microalgae, type of culture vessel, culture conditions (light, temperature, agitation, and among others), source of nutrients, and ultimately, the overall production costs. Therefore, understanding these modes of nutrition is crucial from an economic point of view to judge how much investment is necessary.

Photoautotrophy-based culture is considered the most utilized method and is extensively used in open ponds and photobioreactor (PBRs) systems.[147] It uses light as an energy source, CO_2 from the surface air, and aerators in artificial ponds.[148] The cells grow and multiply through photosynthesis, where solar energy is stored as adenosine 5'-triphosphate and nicotinamide adenine dinucleotide phosphate hydrogen. The Calvin cycle processes these to generate glucose as an energy source. The extensive use of this culture method is judged most economical since it utilizes sunlight as an energy source.

However, despite being abundantly available, sunlight is also considered a limiting factor as photosynthesis will only occur in the presence of light. To counter this issue, using artificial lightning shows potential, but it may prove very costly to implement and run. In addition, recent investigations on this method resulted in creating droplet-based PBRs that allow monitoring the algae growth under various concentrations of CO_2 and light intensities. This results in the rapid evaluation of the photoautotrophic growth of microalgae species and finding the most appropriate conditions.[149]

The second mode of nutrition is through heterotrophy, where an organic compound (consisting of carbon) is present in the media. It is then absorbed by the microalgae and subsequently metabolized. Through this method, biomass production does not require light, and the yield is much higher. Glucose is generally considered a primary carbon source, but other alternatives such as peptone and acetate have also been used.[150] The main disadvantages of this type of culture are the costs required to supply the carbon source and potential contamination with bacteria/fungi, resulting in waste of resources.

The growth of diatom *Cyclotella cryptica* was investigated under heterotrophic conditions while using glucose as the sole carbon source. The resulting biomass produced cellular carbohydrate, protein, and lipid contents of 360 mg/g, 260 mg/g, and 165 mg/g, respectively, showing promising levels of carbohydrates.[132] Another study with *Chlorella* spp. HS2 showed a 3-fold increase (from 5.58 g/L to 18.13 g/L) in biomass production when the media were supplemented with glucose at 72 g/L.[151] Such findings showcase the potential of this method of culture. However, recent studies have shown wastewater as a cost-effective organic source to culture microalgae.[152] A variation of heterotrophy is a photoheterotrophic culture where the condition is that light is needed to activate photosystem I while using sugar as the exclusive carbon source, but this leads to an expensive production.

Mixotrophic systems are designed to allow the occurrence of photosynthesis in the presence of different carbon sources, such as organic compounds and CO_2.[150] This method employs both methods described above in two stages. The preliminary stage is performed under heterotrophy in the presence of high organic carbon content. The second stage would then be performed by photoautotrophy when the supplied organic carbon source is no longer available.[153] However, mixotrophy does not always follow this pattern. When cultured under such conditions, *C. vulgaris* recorded yields of 140% and 170% of biomass and lipid productions, respectively, compared to autotrophic growth.[154] The authors pointed out that culture under such conditions was not a simple combination of heterotrophy and autotrophy. One of these metabolic pathways (either heterotrophy or autotrophy) was dominant, leaving the other one active at a much lower rate.

Each of these culture conditions has its advantages and disadvantages regarding the light and carbon sources, biomass yield, and costs. However, depending on the culture's goal, whether for feed production, biofuel production, value-added secondary metabolite production, or other applications, the culture parameters will depend on the microalgae strain/species, level of investments, and the desired end product.

Light

Light energy is necessary for fundamental biological processes such as synthesizing the cell's protoplasm. Microalgae thus require light for optimal growth and productivity. Light intensity and photoperiod are both important lighting parameters in microalgae culture. Light directly relates to the photosynthesis rate.[155] It has been established that microalgal growth increases with light intensity to a saturation point, beyond which a significant decline in growth is noted. This decline is attributed to photodamage and photoinhibition by intense and prolonged light.[156]

Besides biomass production, light also affects the biochemical composition of microalgae. A study investigating the effect of light intensity on pigment accumulation in *Phaeodactylum tricornutum* has revealed that low light intensity favored the light-harvesting pigment, fucoxanthin.[157] In fact, at low light intensities, microalgae respond by enhancing light-harvesting pigments such as phycobilins, primary carotenoids, and chlorophylls, probably to maximize the light utilization by the microalgae. This has been evidenced in a study where the accumulation of beta-carotene and lutein at low light intensity (140 $\mu molm^{-2}s^{-1}$) was demonstrated.[158] The same study showed an increase in lipid production under low light intensity.

Besides light intensity, photoperiod also impacts biomass productivity and biochemical composition. Continuous illumination, light flashing, and alternate light and dark cycles are used during microalgae cultivation.[159] *Rhodomonas* spp. showed enhanced production of EPA and DHA under 16:8 h (light: dark) light regime compared to continuous lighting and achieved greater biomass.[160] Decreasing the light duration from 16 h to 8 h promoted the production of phycoerythrin, phycocyanin, and allophycocyanin while increasing the light period enhanced the accumulation of carbohydrates and carotenoids in *Nostoc calcicola*.[161] Prolonged lighting is not always beneficial for biomass and metabolites production as excess light is dissipated as heat and causes damage to cells.[162] Excessive light unnecessarily contributes to the production cost, an important aspect when considering the profitability of the process.

Light source is equally important. Recent investigations on cost-effective light sources for the profitable production of microalgae included the use of red and blue light-emitting diodes (LEDs). A study investigated the effect of various combinations of red and blue LEDs in the culture of *Chlorella* spp., and the researchers recorded the highest biomass when light intensity was 500 lux.[163] They also recorded a significant increase from 30% to 60% (w/w) in lipid content when the light source was altered from white to red/blue LEDs. Another study with *C. vulgaris* using blue LEDs indicated a lipid content of 34.06% as the highest lipid content instead of cool white and red LEDs.[164] Such experiments signify the importance of using wavelength-based light sources as an economical step in microalgae culture.

Nitrogen and phosphorus

Nitrogen and phosphorus are critical macronutrients required for microalgae growth and play an essential role in the biosynthesis of important biomolecules. An inadequate supply of these nutrients can be detrimental to the cells' morphology, physiology, and biochemical composition. Microalgae can assimilate nitrogen in the form of nitrates, nitrites, ammonium, and urea. In general, microalgae's preferred form of nitrogen is ammonium as its assimilation is more energetically favorable, although some microalgae such as *Dunaliella* spp. and *Botryococcus* spp. prefer nitrates.[165] However, it has been established that microalgae do not tolerate high concentrations of ammonium (above 25 μM).[166] For this reason, nitrates and urea are most commonly used in culture media.

Although nitrogen deficiency limits the growth of microalgae, it is known to shift the system toward the accumulation of carbohydrates and lipids. Researchers have reported that nitrogen depletion considerably decreased the growth of *Isochrysis galbana* and protein content, significantly increasing carbohydrate content and saturated fatty acids.[167] Similarly, *Rhodomonas* spp. showed reduced cell growth but enhanced PUFAs accumulation under nitrogen starvation.[168] Nitrogen deficiency shifts the metabolism to the lipid metabolic pathway, leading to the accumulation of TAGs as energy sources. In terms of pigment production, chlorophyll content decreased while carotenoid content increased significantly in *Pycocystis salinarum* under nitrogen limitation.[169] Other researchers revealed similar findings where a decrease in phycobilins in *Rhodomonas* spp. and an increase in carotenoid content in *D. salina* were noted.[168,170] The impact of nitrogen concentration on key light-harvesting pigments also relates to the rate of photosynthesis, which is adversely affected under nitrogen limitations. Undeniably, nitrogen plays a critical role in biomass production and productivity of microalgae. Depending on the target compound, the nutrient levels should be adjusted accordingly.

Temperature

The temperature of the system strongly influences the growth and accumulation of biomolecules. Temperature affects crucial enzyme-mediated metabolic reactions.[171] Depending on the strain, microalgae have an optimum temperature ranging from 15 to 50°C, beyond which growth declines due to denaturation of enzymes and instability of pigments.[172]

Biochemical pathways for biosynthesis and accumulation of unsaturated and saturated fatty acids and lipids are susceptible to thermal variations.[173] Microalgae respond by increasing total fatty acids and omega-3-fatty acid content at low-to-moderate temperatures (10–20°C).[174] A study investigating the effect of temperature on eight microalgae strains demonstrated that EPA and DHA are produced at greater amounts at 14°C than 26°C.[175] PUFA content increased under low temperature (18°C) in *Nannochloropsis oceanica*.[176] In another study, a 40.7–52.9% increase in PUFAs was produced by *Heterochlorella luteoviridis,* when the temperature was increased from 22 to 27°C.[177] Enhanced lipid synthesis, especially of PUFAs, is an adaptive mechanism of microalgae at low temperatures aimed at maintaining the fluidity, functionality, and flexibility of the cell membrane.[178]

CO_2 supply

Carbon dioxide is a key requirement for photosynthesis in microalgae. In PBRs, the CO_2 concentration supplied through spargers can be easily controlled, while in open raceway ponds, microalgae can utilize atmospheric CO_2. The tolerance to CO_2 levels is species specific.[179] CO_2 concentration significantly affects microalgae growth as it influences the pH of the medium and the availability of bicarbonates.[180]

A study investigating the effect of CO_2 on polyculture of microalgae reported that growth of the cells increased with increasing CO_2, but beyond 400 mgL^{-1}, the growth rate reduced to half.[181] Similar observations were made by researchers who reported that the growth rates of *Desmodesmus* spp. and *Scenedesmus* spp. increased until a saturation point of 60 μM and 30 μM, respectively.[182] CO_2 influences enzymes involved in carbon metabolisms such as Rubisco and carbonic anhydrase.[183] Hence, increasing the concentration of CO_2 results in enhanced rates of photosynthesis, which has a positive effect on the growth of the cells. Consequently, elevated concentration may act as a stress factor and impede the growth rate. Increased dissolved CO_2 levels in the media decrease the system's pH, making it unfavorable for microalgae growth. It should be noted that optimal pH for microalgae lies between 7 and 9, above or below which inhibition of key enzymes involved in carbon fixation may occur.[184]

In terms of biochemical composition, CO_2 affects lipid, protein, and pigment contents. A different study suggested that high CO_2 favors the accumulation of chlorophylls, carotenoids, and lipids. Interestingly, one strain under study showed the accumulation of beneficial omega-6 fatty acid, LA.[180] In *Scenedesmus bajacalifornicus,* the maximum protein content was produced at 15% CO_2 and decreased beyond this CO_2 level.[185] The same study showed a significant increase in lipid and pigment content at 25% CO_2. Similar results were obtained in a different study.[181] In this study, a maximum protein content (30.41%) was obtained at 400 mgL^{-1} of CO_2, which decreased to 8.92% when CO_2 concentration was increased to 600 mgL^{-1}. Lipid content increased from 26.39% to 56% when CO_2 concentration increased from 200 mgL^{-1} to 800 mgL^{-1}. Increased levels of lipids at increased CO_2 were associated with stress-induced lipid synthesis due to a possible disruption in the system's pH.[186]

Mass production culture set-ups

The cultivation system of microalgae culture influences both biomass productivity and the production of essential algal products. Two cultivation systems can achieve large-scale production of microalgae; open raceway ponds and closed bioreactors, each having advantages and limitations. The choice of culture type depends not only on the microalgae strain but also on the purpose of the cultivation.

Open systems

Open cultivation systems include natural and artificial ponds such as the open raceway and circular ponds.[187] Open raceway ponds, also known as high-rate algal ponds (HRAPs), consist of a looped and closed channel driven by a paddle and baffles which guide the continuous liquid flow and promote agitation of the medium, preventing sedimentation.[188] These ponds are shallow (15–30 cm deep) to maximize light penetration and prevent self-shading of the microalgae.[189] Atmospheric CO_2 is the source of aeration, although aerators may be added as an additional CO_2 source in some cases.[190,191] Open ponds are ideal for pilot and commercial scale productions because of their low operation and maintenance cost and easy set-up.[192]

The set-up of raceway ponds does not require arable land and this is an important aspect as it does not compromise on land aimed at agricultural production. In fact, this culture system is widely utilized for the industrial production of microalgae cells for producing pigments, single-cell proteins, and beta-carotenes and accounts for 90% of microalgae biomass production worldwide.[193,194] HRAPs have been reportedly established in the USA, India, Australia, Israel, and China to produce nutraceuticals commercially.[195] An Australian firm achieved about 13 ta^{-1} productions of beta-carotene from microalgae grown in open unmixed ponds.[196]

However, the open ponds are limited by evaporation losses, the variation in weather conditions in terms of temperature and duration of sunlight, and contamination by predators and other heterotrophic microorganisms that compete with microalgae for nutrients and produce toxins that may contaminate the final product.[197] Furthermore, atmospheric CO_2 (0.04–0.06%) is suboptimal for microalgae growth, which impedes biomass production and productivity.[198]

The circular pond consists of a circular tank (30–70 cm deep) with a central rotating agitator to mix and prevent sedimentation.[199] This type of open system has the same demerits as the raceway pond.

Closed PBRs

Closed PBRs have been designed to counter the shortcomings of open ponds and represent an attractive option due to high biomass production and low contamination. Tubular PBR, flat panel PBR, and vertical column PBR are commonly used in nutraceutical production. The design, merits, and demerits of each PBR are described in [Table 4].[193,199,201] The commercial application of PBRs is manifold. For instance, Israel and Germany have employed the tubular PBR to cultivate *Haematococcus* spp. and *Chlorella* spp.[200] The flat panel PBR is used in the Czech Republic to produce astaxanthin.[199]

Overall, these PBRs offer better control and optimize culture parameters such as light intensity, temperature, agitation, low-energy consumption, and high photosynthetic efficiency.[189] For this reason, PBRs demonstrate significantly higher biomass production. The productivity potential of *Nannochloropsis* spp. in flat panel PBR was found to range between 3800 and 13,000 tkm$^{-2\ year-1}$, which was higher when compared to the open algal pond.[202] *Chlorella salina* could achieve 9.73 mgL$^{-1\ day}$-1 of lutein when cultured in an 8 L lab-scale air-lift PBR at optimized conditions.[203]

Another essential aspect of PBRs is a lower likelihood of contamination, favoring microalgae axenic cultures.[204] Prevention of contamination is critical in producing high-end nutraceuticals and biopharmaceuticals. The compactness of the design of PBRs is another advantage, as it does not take up as much space as open raceway ponds. Overheating, bio-fouling, and cleaning difficulties are some of the limitations of PBRs.[205] Another demerit of PBRs is difficulty in up-scaling due to the high costs associated with superior material and support systems.[206]

Biomass harvesting

Harvesting the cells involves the separation of the biomass from the growth medium and represents a critical step in downstream processing. Microalgae pose many challenges in harvesting as these processes are often restricted by the small size of the cells, low biomass concentrations, and negatively charged surface.[207] This makes the overall process energetically expensive, contributing considerably to the cost. Various harvesting techniques are currently used for biomass harvesting, each having merits and limitations.

Sedimentation relies on gravity to separate the biomass, with larger cells sedimenting faster than smaller ones.[208] A study on an inclined gravity settler showed that *Scenedesmus dimorphus* could be concentrated, leading to a biomass recovery of 80% and that the separation efficiency was independent of the biomass concentration.[209] However, the biomass recovery was only 55% with *C. vulgaris* leading the authors to hypothesize that this result was due to the morphological differences between the two microalgae. Other microalgae such as *Nannochloropsis* spp., *Cylindrotheca fusiformis*, *Tetraselmis suecica*, and *Ankistrodesmus falcatus* were harvested through sedimentation for 24 h, and the biomass recovery varied significantly between the isolates.[210] Although sedimentation is adopted for its low cost and easy scale-up, it can prolong harvesting time. It is also inappropriate for lipid-rich microalgae as they tend to float due to their low density.[211]

Table 4: The basic design, advantages, and drawbacks of the different types of PBRs.

PBR	Basic design	Advantages	Drawbacks
Tubular	Composed of long transparent tubes that can be placed vertically, horizontally, or diagonally	Tubes can be adjusted to maximize exposure to light and hence maximize photosynthetic efficiency, Employs spargers to produce air bubbles for agitation and aeration, thereby minimizing damage caused to cells	High costs associated with construction materials
Flat panel	Composed of rectangular compartments constructed with transparent material	Design allows for maximum light penetration and low oxygen accumulation, hence, supporting high photosynthetic efficiencies, Easy scale-up	Offers poor temperature control, Production of gas zones
Vertical column	Characterized by transparent vertical tubing	Simple design allowing for simple operating procedures and low-energy requirements, High mass transfer, Easy sterilization	Does not optimize sunlight penetration, High construction cost

Filtration is a highly efficient method for cell harvesting with 70–90% cell recovery.[212] Being chemical-free limits contamination of the final product, which is relevant to nutraceutical production.[213] However, high capital and operational costs restrict the use of this technique for harvesting. Furthermore, it is unsuitable for small and filamentous cells as they can lead to membrane fouling. To counter this limitation, ultra-filtration and counter-current filtration can limit fouling. Vibrating sieves are commonly used for filamentous microalgae like *S. platensis*.[214]

Centrifugation is a simple and efficient method for microalgae harvesting. This technique makes use of centrifugal force to cause sedimentation of the cells. Different types of centrifuges, such as disk centrifuge or multi-chamber centrifuge, are used and can operate both in batch and continuous modes by being integrated with the bioreactor.[213] High recovery of the cells of greater than 90% can be achieved by centrifugation.[194] The high-energy requirement for centrifugation increases the operational cost. Cell disruption due to gravitational and shear forces may cause the loss of valuable products in the medium, representing a disadvantage in producing high-end products.[215] A study has reported that increasing centrifugal force above 5000 g causes cell disruption and an overall 40% loss in glycerol content in *D. salina*.[216]

Flotation relies on transferring the cells to the medium's surface, from which they can be skimmed. Due to the presence of gas vesicles, some microalgae such as *Spirulina* and *Microcystis* naturally float.[217] Otherwise, techniques such as dispersed air flotation or dissolved air flotation help in producing air bubbles that attach to the cells and cause them to lift to the surface of the liquid.[207] It is generally preferred over sedimentation as it is rapid.[211] Ozone flotation is also used, although it is not recommended for harvesting cells aimed at protein and carbohydrate production. In fact, ozone disrupts the cells and releases proteins and carbohydrates, denaturation of which causes foaming. The latter acts as a surfactant and facilitates floating.[218] Nevertheless, this technique has been involved in enhanced lipid recovery.[208]

Flocculation involves using flocculants that neutralize the negative charge of the cell's surface and promotes agglomeration of the cells, facilitating harvesting. Flocculation is often used in conjugation with other techniques such as sedimentation to facilitate harvesting.[219] Inorganic multivalent cations such as aluminum sulfate, ferric sulfate, and ferric chloride are widely used as flocculating agents. However, these chemical flocculants are influenced by the pH of the system and have been associated with negative health effects such as cancer and Alzheimer's disease.[215] Considering this, their use in cell recovery for nutraceuticals is not recommended due to possible contamination. Furthermore, using these chemical flocculants prevents the reuse of the media.[220] Alternatively, natural flocculants such as chitosan have been explored. A study showed that mushroom chitosan could be an efficient flocculating agent with more than 95% efficiency for *Nannochloropsis* spp.[221] Other alternatives include *Moringa oleifera* seed extract, which has been proven to be an effective coagulating agent for removing *Chlorella*, *Microcystis*, *Oocystis,* and *Scenedesmus*.[222]

Combinations of harvesting techniques are often used to reduce the overall cost of the harvesting process. Cheaper techniques such as sedimentation are used as first-stage harvesting, followed by higher throughput techniques such as filtration and centrifugation to reduce energy input and cost. An example of such a process involves *Spirulina* harvested by simple filtration using a stainless steel mesh followed by vibrating sieving and vacuum belt.[223]

Despite the availability of various techniques for biomass harvesting, the choice will be very dependent on the investments made and the desired end product. However, once the biomass is available, it requires further treatment, but a specific pre-treatment phase is necessary just before that.

Pre-treatment of biomass

Pre-treatment often precedes the extraction of biomolecules to increase the recovery of the components. Pre-treatment can be categorized into mechanical, physical, and biological, as illustrated in [Figure 4].

Bead milling is extensively used for industrial cell disruption due to low cost, single-pass process, and ease of scale-up. A study has demonstrated that disruption increased the content of extractable lipids from *Parachlorella kessleri* and that bead milling yielded larger droplets that were more readily harvested.[224]

High-pressure homogenization (HPH) has become one of the main methods for cell disruption. This technique achieves high cell disruption efficiencies, even better than bead milling. A study has established that HPH is efficient and cost

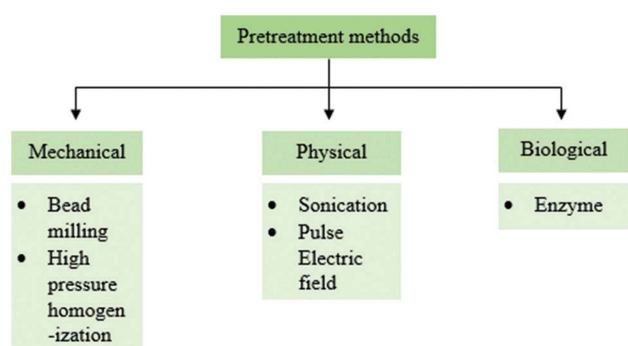

Figure 4: Pre-treatment methods for biomolecule recovery from microalgae.

effective in cell disruption, achieving 95% cell disruption and 50% protein recovery at even low pressures.[225] A limitation of HPH is related to the unspecific release of biomolecules which could complicate downstream processing.[226]

Sonication relies on the destructive effect of ultrasounds by acoustic cavitation resulting from the generation of high-pressure bubbles. Although it has been investigated for cell disruption, its utilization is limited as long sonication periods are required to achieve sufficient cell disruption for large amounts of cells. It is effective at high ultrasonic energy input, which can affect the functionality of some biomolecules.[227,228]

Pulsed electric field (PEF) is a gentler method of cell disruption. PEF has not been proven to be the most efficient method for protein recovery. A study has shown that on treatment with PEF, *C. vulgaris* retained 95% of the protein.[229] A different study has also made similar findings, claiming that a maximum of 13% protein has been recovered from *C. vulgaris* and *Neochloris oleoabundans* and could be considered an alternative to mechanical disruption.[230]

Enzymatic treatment is effective due to biological specificity and ease of scale-up.[231] Enzymes are used to disintegrate the complex components of the cell wall, thereby increasing the permeability of the cells. Some researchers had observed that carotenoid extraction was enhanced when *C. vulgaris* was pre-treated with a mixture of carbohydrate-active enzymes and sulfatases.[232] Autolysin treatment of *C. reinhardtii* was efficient in attaining 100% cell permeability and greatly improved protein and lipid extractability compared to ultrasound-assisted extraction.[233] The high cost of enzymes limits enzymatic treatment.[207]

Extraction of biomolecules

Organic solvent extraction is the most used and traditional method of biomolecule extraction. This method uses organic solvents such as methanol, chloroform, hexane, and acetone.[234] Having a high partition coefficient in organic solvents, pigments such as chlorophyll and carotenoids are frequently extracted by organic solvent extraction.[235] Using acetone as a solvent, some researchers have successfully extracted 3.9–4.3 mgL^{-1} of lutein from *H. pluvialis*.[236] However, the organic solvents used for extraction have been associated with adverse environmental effects. Solvent extraction might not be safe for extracting nutraceutical compounds due to the probability of contamination.[237]

Supercritical fluid extraction (SFE) has been explored to address the shortcomings of organic solvent extraction. SFE allows greater extraction selectivity, a shorter processing period, and avoids the presence of trace organic solvents in the final products.[238] Many compounds have been used for SFE, including ethanol, nitrous oxide, butane, and pentane. Nevertheless, CO_2 remains the most commonly used compound due to its cheap cost, non-toxicity, and inert nature.[239] Extraction of astaxanthin and lutein has been successfully achieved by supercritical CO_2 extraction in *H. pluvialis*.[236] Similarly, carotenoids have been commonly extracted by this method.[240] Ionic liquid solvents have also been explored as an alternative to organic solvents.[241]

Purification

After extraction, the desired products exist in a mixture of other cellular components and debris and extraction solvents and other metabolites. Therefore, the purification step aims to separate the required compounds to formulate the final product. The choice of the purification method strongly depends on the product's intended use. In the case of nutraceuticals, where high purity is usually desired, high-throughput techniques are used.

Precipitation and filtration are often used for the purification of proteins. This technique exploits the isoelectric point of proteins at which they show low solubility, causing them to precipitate.[228] About 80.6% protein recovery was achieved through isoelectric point precipitation in *A. platensis*.[242] However, this method results in high-protein recovery due to its specificity. Precipitation by calcium hydroxide or sulfuric acid, hydrochloric acid, and acetic acid has been previously reviewed for precipitation of chlorophyll from beta-carotene and astaxanthin extracts.[235] Membrane filtration, such as ultrafiltration and diafiltration, has also been employed for purification, especially protein. Other studies have explored filtration for the concentration of functional proteins from *T. suecica*.[243] Large volume input, high yield, and low cost are among the many benefits of filtration.[227] They are limited by the biofouling of the membranes and by the membrane's limited pore size, which prevent the accurate separation of desired proteins.[244]

Otherwise, for final stage purification, modern techniques such as expanded bed chromatography, high-performance liquid chromatography, ion-exchange chromatography, affinity chromatography, size exclusion chromatography, and dialysis have been widely used for further purification steps.[107] Given the high cost of these techniques, they are applied to purify more valuable products. Ion exchange relies on the affinity of the biomolecules for a charged resin.[231] This technique is not widely used to purify pigments such as carotenoids and chlorophylls as they are not charged molecules but are widely used for protein purification.[227,245] It has, nonetheless, been applied in separating protein-based phycoerythrin in *Gracilaria gracilis*.[246] Gel filtration chromatography has been applied to purify bioactive polysaccharides from *Neochloris oleoabundans*.[247]

CONCLUSION

Over the past few years, nutraceuticals have gained significant attention as alternatives to pharmaceuticals in preventing and treating diseases. The wide diversity of compounds showing promising and positive health improvement effects is constantly being reviewed and assessed. Nutraceuticals can be categorized as antioxidants, carbohydrates, and dietary fiber, fatty acids, proteins and peptides, probiotics and prebiotics, vitamins, and minerals. These nutraceuticals demonstrated significant improvement in overall health by strengthening the body's immune system, fighting against CVDs, and preventing various cancers. The constant controversial debate of food production for consumption versus other uses led to the search for better alternatives in microalgae. Stakeholders and pharmaceutical industries shifting toward sustainable productions are offered numerous advantages with microalgae culture. Biomass production can assist in tackling multiple problems related to climate change, rising CO_2 levels, and food security while promoting health benefits. With the dire need to increase food production by about 70% within the next 40 years, the concept of microalgae culture looks pretty appealing. They possess a variety of biomolecules, which have great potential as supplements, including astaxanthin, phycobiliproteins, beta-carotene, vitamins, and PUFAs, all having beneficial properties for disease prevention and treatment. These compounds can be incorporated into food products to enhance their nutritional and health benefits. *Spirulina* has been widely explored for its role in nutraceutical production, and its nutritional profile makes it a superfood. It is a rich source of vitamins, proteins, carbohydrates, and essential fatty acids such as EPA and DHA. *Chlorella* spp. are also excellent sources of vitamins, carotenes, and niacin. However, several other strains have been exploited for nutraceutical production, such as *Dunaliella* spp., *Haematococcus* spp., *Tetraselmis* spp., and *Scenedesmus* spp. Microalgae culture for nutraceutical production requires scrutinous examination before being launched. The whole process depends on the desired end product and its purity since such conditions will likely dictate the investments required and the final product's price. As elaborated previously, many culture vessels, such as the open pond and closed PBRs, can be adopted based on the targeted biomolecule. However, the downstream processing of the biomass still requires further investigation as this section in microalgae culture is considered the most expensive part. Nonetheless, microalgae as sources of nutraceuticals exist and can be further refined.

Declaration of patient consent

Patient's consent not required as there are no patients in this study.

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Conflicts of interest

There are no conflicts of interest.

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