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REVIEW ARTICLE

Freshwater Microalgae as Promising Food Sources: Nutritional and Functional Properties

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Abstract:

A number of researchers have predicted that the current food crisis is predicted to worsen in 2050. The prediction of this crisis is aligned with climate change causing increases in some basic foodstuff prices. Therefore, everyone should prepare to consume alternative foods at an early stage. Alternative foods have been widely developed, one of which involves microalgae. However, the type of microalgae produced by some countries on a large scale consists of only oceanic/seawater microalgae. This will have an impact on and hinder development in countries that do not have these resources. Therefore, it is necessary to explore the use of microalgae derived from freshwater. Unfortunately, freshwater microalgae are still rarely investigated for use as alternative foods. However, there is considerable potential to utilize freshwater microalgae, and these algae are very abundant and diverse. In terms of nutritional properties, compared to oceanic / seawater microalgae, freshwater microalgae contain nearly the same protein and amino acids, lipids and fatty acids, carbohydrates, and vitamins. There are even more species whose composition is similar to those currently consumed foods, such as beef, chicken, beans, eggs, and corn. In addition to dietary properties, freshwater microalgae also have functional properties, due to the presence of pigments, sterols, fatty acids, and polyphenols. Given the potential of freshwater microalgae, these aquatic resources need to be developed for potential use as future food resources.

Keywords: Bioactive, Food, Freshwater, Functional properties, Microalgae, Nutrition, Underexplored.

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1. INTRODUCTION

Food security is a topic that continues to be a topic of interest in all countries and has been linked to several factors, such as growing populations and rising food prices [1]. According to the Global Network Against Food Crises, the severity of the food crisis worsened in 2020 due to conflict, the economic impact of coronavirus disease (COVID-19) and extreme weather. In 2021, the world will experience a food crisis [2]. The effects of climate change, such as increased temperature, decreased water availability, and increased carbon dioxide, have decreased crop productivity. These phenomena will worsen the condition of areas where people are already vulnerable to hunger and malnutrition [3]. Regions in which these effects are especially concerning include sub-Saharan Africa and South Asia. It is estimated that countries will experience an average crop decline of up to 8% by 2050. The

harvest is expected to change by -17% (wheat), -5% (corn), -15% (sorghum) and -10% (millet) in Africa and by -16% (corn) and -11% (sorghum) in South Asia [4]. The food crisis will become dangerous if every country does not provide other alternatives to meet food demands. Therefore, new potential food sources are needed to meet nutritional requirements.

Microalgae can be used as alternative foods due to their nutritional contents. In China, people have long used microalgae as food sources. Commonly used microalgae include *Arthrospira*, *Nostoc*, and *Aphanizomenon*. More than 2000 years ago, algae were already used as food [5]. It is also known that the Aztecs consumed *Spirulina* in the 14-16th centuries. The production of microalgae as food stocks began to be encouraged in the Second World War, during which time Japan, America, and Germany were facing a food crisis [6].

Some countries have begun expanding and producing large-scale microalgae as health supplements, pharmaceuticals, and biofuels. Biofuels from microalgae are environmentally friendly and nontoxic. Carbohydrate-rich microalgae such as

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Chlamydomonas reinhardtii and *Chlorella vulgaris* have been widely used for biofuel production [7]. Developing countries such as those in Africa with a coastline stretching for 1420 km have succeeded in increasing the production of seawater microalgae from 0.08 million tons to 0.14 million tons. In addition, microalgae such as *Spirulina* have been used as foods and supplements [8]. In addition to Africa, Europe already has countries where algae (67%) and microalgae (33%) are produced. *Spirulina* producers include 23 countries, with the most significant biomass produced in Norway, France, and Ireland [9]. However, many countries develop only seawater microalgae, so development is limited if these countries are not adjacent to seawater. Therefore, the freshwater microalga is an attractive solution to address this issue.

Microalgae are photosynthetic microorganisms that utilize carbon dioxide and sunlight to form biomass and produce approximately 50% of the oxygen in the atmosphere. There are four types of microalgae, namely, *Bacillariophyceae* (diatoms), *Chlorophyceae* (green algae), *Chrysophyceae* (golden algae), and *Cyanophyceae* (blue algae). Although many countries in the Tropics have a high diversity of microalgae, their potential is still underexplored. In many countries, microalgae have been used as biofuel production agents because microalgae can produce high levels of fatty acids and carbohydrates [10 - 14]. Through the esterification process, microalgal fatty acids can be converted to biodiesel.

Research and discussions related to microalgae are still dominated by their application in wastewater treatment, bioenergy, and the pharmaceutical industry [15 - 17]. However, microalgae have long been used as foods [18, 19]. This review

shows freshwater microalgae's nutritional quality and functional properties and their potential to be developed as food sources to address the global food crisis.

2. POTENTIAL AND DIVERSITY OF FRESHWATER MICROALGAE

The abundance of algal species is estimated to be nearly ten million. In terms of classification of algae, they can be broadly assigned to 11 main phyla: *Cyanophyta*, *Chlorophyta*, *Rhodophyta*, *Glaucophyta*, *Euglenophyta*, *Chlorarachniophyta*, *Charophyta*, *Cryptophyta*, *Haptophyta*, *Heterokontophyta*, and *Dinophyta* [20].

Several freshwater microalgae have been reported. However, many researchers are only interested in studying their physiology and how they respond to environmental change. As a result, many freshwater microalgal species are still unknown. The classes Zygnematophyceae, Euglenoidea, and Chlorodendrophycea are among the most widely found. Details of the species commonly explored by researchers are listed in Table 1.

According to Matos *et al.* [21], microalgae are sources of bioactive ingredients and compounds for the most promising new food products. They can increase the nutritional value of food due to their balanced chemical composition. The addition of microalgae to food products is an excellent way to supplement nutrients and biologically active compounds. *Chlorella* is the most explored genus in terms of the suitability of its species compared to other species as dietary supplements [22 - 24].

Table 1. Classification of commonly investigated freshwater microalgae.

No.	Class	Species				
1	<i>Chlorophyceae</i>	<i>Scenedesmus obliquus</i>				
		<i>Chlamydomonas reinhardtii</i>				
		<i>Haematococcus pluvialis</i>				
		<i>Monoraphidium</i> spp. <i>Ankistrodesmus falcatus</i> <i>Oscillatoria</i>				
2	<i>Cyanophyceae</i>	<i>Nostoc commune</i> var. <i>sphaeroides</i>				
		<i>Aphanizomenon flos-aquae</i> <i>Synechocystis</i> spp. <i>Anabaena cylindrica</i>				
		<i>Nannochloropsis limnetica</i>				
3	<i>Eustigmatophyceae</i>	<i>Nannochloropsis limnetica</i>				
4.	<i>Trebouxiophyceae</i>	<i>Chlorella pyrenoidosa</i> <i>Chlorella vulgaris</i> <i>Chlorella sorokiniana</i> <i>Micractinium conductrix</i> <i>Choricystis minor</i> <i>Botryococcus braunii</i>				
		5.	<i>Euglenoidea</i>	<i>Euglena glaciris</i>		
		6.	<i>Bacillariophyceae</i>	<i>Pinnularia</i> spp. <i>Navicula</i> spp. <i>Frastulia</i> spp. <i>Didymosphenia</i> spp.		
				7.	<i>Zygnematophyceae</i>	<i>Spirogyra</i> spp.
				8.	<i>Chlorodendrophyceae</i>	<i>Tetraselmis cordiformis</i>

3. NUTRITIONAL PROPERTIES

The use of microalgae as food sources has been known for a long time. Microalgae are considered potential new food sources due to their high nutrients content. The nutrient composition of microalgae varies, and even for the same species, the nutritional content of individual microalgae can differ significantly due to the growth conditions, such as the composition of media and temperature for growth. The main nutritional components are mainly proteins and lipids, as well as vitamins and minerals, all of which are known to have a positive impact on human health [25]. Important macronutrient components (proteins, carbohydrates, and fats) can be found in high proportions. In general, the macro nutritional components of various freshwater microalgae are summarized in Table 2.

The nutritional value of algal species for a particular organism depends on the size of the algal cells, their digestibility, their production of toxic compounds, and their biochemical composition. Although there are striking differences in the composition of classes and microalgal species, proteins are always the primary organic components, usually followed by lipids and carbohydrates, regarding the percentage of dry biomass weight. Protein, fat and carbohydrate levels range from 12-35%, 7.2-23%, and 4.6-23%, respectively [36].

3.1. Proteins and Amino Acids

Protein is an essential macronutrient in food. Microalgae are considered a viable source of protein. Some freshwater microalgal species contain proteins similar to those of traditional protein sources, such as beef, eggs, chicken, corn, and beans. Microalgae protein is high and has a high nutritional value [37]. However, the quality of proteins varies, generally depending on the availability of essential amino acids in the proteins. Familiar sources of essential amino acids are eggs, poultry meat, red meat, milk, soy, and fish. These sources have complete suites of amino acids. However, there are consumption constraints for vegetarians. Vegetarians generally consume plant proteins with lower nutritional value due to the lack of essential amino acids.

On the other hand, microalgae are excellent sources of essential amino acids. Approximately 70% of the mass weight of *Chlorella* spp. reportedly comprises protein [37]. According to WHO / FAO / UNU recommendations, microalgae such as *Chlorella* spp. have balanced contents of essential amino acids needed for human consumption [38]. Some freshwater microalgal species have higher protein contents than traditional food sources (Table 2). For example, *A. flos-aquae* species have 62% more protein than other microalgal species [32]. The freshwater microalga with the lowest protein content is *E. gracilis*—29% [27]. The protein content in *A. flos-aquae* is higher than the protein content in beef (43%) [39]. Although *E. gracilis* has the lowest protein content among the microalgal species, the protein content of *E. gracilis* (with an average of 26%) is higher than the protein content in nuts [40].

Differences in protein values among microalgae are caused by several factors, such as temperature, light intensity, and

nutrient composition in culture media [41, 42]. Temperature and light are considered the most crucial factors because microalgae are cultivated outdoors to obtain direct sunlight exposure, so there are also variations in daily and seasonal temperatures [43, 44]. Furthermore, microalgae grow in the temperature range of 15-35 °C [45]. Occasionally, the use of high temperatures induces protein degradation, disrupts enzyme regulation, and breaks down protein structure, resulting in decreased protein content [46].

As mentioned, temperature and light intensity influence the protein content of microalgae. For example, Baiee and Salman [47] showed that high light intensity increases the protein content in *C. vulgaris*. On the other hand, in *Chlorella* spp., the high intensity of light causes a decrease in protein content. According to He *et al.* [48] the need for light intensity on species varies. In *C. vulgaris*, fluorescent lamps are used during the day (40, 200 and 400 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) without a dark period, while in *Chlorella* spp., fluorescent lamps with different light intensities are used (125, 268, and 300 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), and the photoperiod has no effect.

The composition of nutrients in microalgae media also plays a vital role. Since nitrogen is a building block of proteins, nitrogen availability significantly influences protein content [38]. In addition, nitrogen concentration is related to the temperature of the treatment. Protein content increases under high nitrogen concentrations and low temperatures. The highest protein content (138 mg.g^{-1}) was found in cultures with a high nitrogen concentration (60 $\text{mg L}^{-1} \text{N-NO}_3$). The highest nitrogen concentration was 45% higher than the lowest nitrogen concentration (12 $\text{mg.L}^{-1} \text{N-NO}_3$) when both cultures were at 27 °C. At low nitrogen concentrations, the protein content increases with temperature. Regarding high nitrogen concentrations, the protein content decreases as the temperature increases [49].

Microalgae have been identified as potential sources of high-protein foods needed by malnourished people [50]. Microalgae consumption as dietary supplements usually occurs through pills, tablets, powders, or pastes. In recent years, microalga-derived proteins have been incorporated into biscuits, candy, bread, noodles, and beverages [40].

In general, the nutritional value of plant-based protein sources is lower than that of animal protein sources. One of the main factors determining these qualities is that not all proteins contain essential amino acids in adequate amounts (called complete proteins). Amino acids produced by seven freshwater microalgae species have different essential and nonessential amino acids (Table 3). The species with a high average essential amino acid content is *N. limnetica*; compared with other species, this species contains essential amino acids such as histidine, isoleucine, leucine, methionine, and phenylalanine with higher contents. For example, the leucine content was 9.16% higher than the leucine content in eggs (8.8%) and soybeans (7.7%). In addition, the value of phenylalanine was 5.10% greater than that of eggs (5.8%) and soybeans (5%). The highest lysine content occurs in *C. vulgaris*, 8.4%, and higher than eggs (5.3%) and soybeans (6.4%).

Table 2. Macronutrient properties of freshwater microalgae (% of dry matter).

Species	Proteins	Lipids	Carbohydrates	Media and Growth Condition	References
<i>C. vulgaris</i>	51-58	14-22	12-17.6	- Media: Bold's Basal Medium (BBM) - Temperature: 30 ± 1°C - Light intensity: cool white fluorescent lamps with an intensity of 50 μmol ⁻¹ m ² s ⁻¹ with 16:8 h photoperiod - Incubation time: 10 days	Wolker et al. [26]; Ramaraj et al. [27]
<i>C. pyrenoidosa</i>	44	6	27	- Media: BG-11 and EG - Temperature: 28 °C - Light intensity: light intensity of 34 μmol ⁻¹ m ² s ⁻¹ under continuous light illumination - Incubation time: 4 days	Yadavalli et al. [28]
<i>E. gracilis</i>	29	10	38	- Media: BG-11 and EG - Temperature: 28 °C - Light intensity: light intensity of 34 μmol ⁻¹ m ² s ⁻¹ under continuous light illumination - Incubation time: 4 days	Yadavalli et al. [28]
<i>H. pluvialis</i>	13.78–21.23	8.29–16.17	47.20–64.22	- Media: BG-11, modified BG-11, and medium of Šetlik - Temperature: 21,5 – 40,5°C - Light intensity: photon flux density of 2 x 132 μmol ⁻¹ m ² s ⁻¹ - Incubation time: 4 days	Gacheva et al. [29]
<i>Scenedesmus quadricauda</i>	34.69	0.63	15.99	- Media: BG-11 - Temperature: 21 ± 2°C - Light intensity: three cool white fluorescent lamps with a combined light intensity of 1.90 Klux on a 12:12 h light: dark cycle. - Incubation time: 22 days	Arguelles [30]
<i>S. obliquus</i>	30.8-37.7	22-42.6	20–42.6	- Media: Bold's Basal Medium (BBM) and Bristol's medium - Temperature: natural condition (outdoor; day: 34 ± 2,1°C; night: 27± 1,7°C), control condition (in a room 25 ± 1°C) - Light intensity: natural condition (under shade and based on natural sunlight), control condition (2000) - Incubation time: 24 hours	Khattoon et al. [31]
<i>Selenastrum bibrainum</i>	38.9–44.7	9.4–38.9	8–38.2	- Media: Bold's Basal Medium (BBM) and Bristol's medium - Temperature: natural condition (outdoor; day: 34 ± 2,1°C; night: 27± 1,7°C), control condition (in a room 25 ± 1°C) - Light intensity: natural condition (under shade and based on natural sunlight), control condition (2000) - Incubation time: 24 hours	Khattoon et al. [31]
<i>A. cylindrica</i>	43-56	4-7	25-30	- Media: BG-11 - Temperature: 25 ± 1°C - Light intensity: under illumination from cool white fluorescent tubes (92.5 μmol photons m ² s ⁻¹) with 14:10 hours of light-dark cycle - Incubation time: 5 days	Becker [32, 33]; Patel et al. [34]
<i>A. flos-aquae</i>	62	3	23	- Media: BG-11 - Temperature: no reported - Light intensity: no reported - Incubation time: no reported	Becker [32, 33]
<i>C. reinhardtii</i>	32.7–48	17.8–21	15.5–17	- Media: Modified Bold 6N (MB6N) - Temperature: 23°C - Light intensity: 50 μmol photons m ² s ⁻¹ (white fluorescent lamps) - Incubation time: 48-72 hours	Becker [32, 33]; Nakanishi [35]

N. limnetica also has a high content of the nonessential amino acids glutamine, glycine, proline, and cysteine. *N. limnetica* contains more glycine and proline than eggs and soybeans. *H. pluvialis* has a higher arginine content than eggs and soybeans do. Chronakis and Madsen [38] explained that edible algae generally present similar patterns of amino acids.

The algae protein has a high content of essential amino acids such as valine, leucine, lysine, and phenylalanine. According to Kay and Barton [54], microalgae generally have low sulfur concentrations, so the amino acid contents of cysteine, methionine, and lysine are low. The concentration of amino acids with methionine and lysine becomes low if the

microalgae are processed with heating or in the presence of reducing sugars (the Maillard reaction). Altogether, the average composition of essential amino acids in an algal protein is better than in vegetables.

3.2. Lipids and Fatty Acids

Lipids are indispensable components of cells and are precursors to many important molecules. Hundreds of microalgal strains capable of producing lipids have been reported [55]. Most of them are marine microalgae. Recently, Torres-Tiji *et al.* [25] reported several freshwater microalgae that accumulate high amounts of lipids, such as *Chlorella protothecoides*, which comprise up to 70% lipids (*dry biomass*). According to Ambrozova *et al.* [56], freshwater microalgae have a higher lipid content than seaweed. Allard and Templier [57] reported that various freshwater and marine microalgae have various lipid contents (1 to 26%). Traditional sources of lipids other than microalgae include fish and seafood. Fish contain a relatively high abundance of omega-3 fatty acids, as fish consume plankton and algae. Algae produce essential long-chain polyunsaturated fatty acids (PUFAs). Several freshwater microalgae along with their lipid content, are listed in Table 2.

Based on the information in Table 2, the microalgal species with the highest lipid content is *S. obliquus*, the content of which ranged from 22-42.6%. Moreover, the lowest lipid

content was *A. flos-aquae*, with a value of 3%. Differences in lipids are affected by several factors during the cultivation process, such as temperature, light intensity, incubation time, and nutrient composition in the growth medium [56, 58].

The effect of light intensity on the lipid content of microalgae varies. In some microalgae species, an increase in light intensity results in a higher lipid content. For example, *B. braunii* and *H. pluvialis* showed increased lipid levels in cultures of high intensity; *H. pluvialis* showed a double increase in lipids [58, 59]. Conversely, low-light intensity conditions led to a high lipid content in other species such as *Nannochloropsis* spp. and *A. falcatus* [60, 61].

The temperature of the culture also affects the lipid content of the microalgae. For example, increased culture temperature of *S. obliquus* can increase the lipid content [62]. However, in *C. vulgaris*, the lipid content increases when the temperature of the culture is reduced [63].

The length of microalgal cultures is also one of the factors that affect differences in lipid content. Mir *et al.* [64] showed that the lipid content of *Cyclotella* spp. with a 15-day culture time was the highest—33.06% (w/w). The availability of nutrients in the growth medium is essential for the growth and production of macronutrients. Nitrogen limitations increase lipid accumulation or storage [65]. Similarly, increasing CO₂ concentrations can increase the amount of lipids produced by microalgae [66].

Table 3. Amino acid values of microalgae.

Food Source (amino acid (g/100 g dry matter))	Eggs [40]	Soy [40]	<i>C. vulgaris</i> [40]	<i>C. pyrenoidosa</i> [51]	<i>Aphanizomenon</i> spp [33].	<i>H. pluvialis</i> [41]	<i>S. porticalis</i> [52]	<i>S. obliquus</i> [33]	<i>N. limnetica</i> [53]
ESSENTIAL AMINO ACIDS									
His	2.3	2.6	2	0.82	0.9	0.31	0.9	2.1	2.14
Ile	6.6	5.3	3.8	1.64	2.9	4.32	0.02	3.6	4.63
Leu	8.8	7.7	8.8	3.33	502	3.64	0.81	7.3	9.16
Lys	5.3	6.4	8.4	4.91	3.5	2.68	0.96	5.6	5.27
Met	3.2	1.3	2.2	1.14	0.7	0.65	0.66	1.5	1.69
Phe	5.8	5	5	1.16	2.5	1.4	0.94	4.8	5.1
Thr	5	4	4.8	1.75	3.3	5.47	0.66	5.1	3.72
Try	1.7	1.4	2.1	-	0.7	-	-	0.3	-
Val	7.2	5.3	5.5	3.21	3.2	2.45	0.02	6	5.85
NONESSENTIAL AMINO ACIDS									
Tyr	4.2	3.7	3.4	1.65	-	2.22	-	3.2	2.97
Ala	-	5	7.9	3.63	4.7	5.6	1.31	9	7.72
Arg	6.2	7.4	6.4	3.21	3.8	10.26	0.21	7.1	5.16
Asp	11	1.3	9	2.58	4.7	-	0.33	8.4	7.33
Glu	12.6	19	11.8	6.33	7.8	10.41	0.04	10.7	16.7
Gly	4.2	4.5	5.8	3.19	2.9	6.61	0.75	7.1	8.68
Pro	4.2	5.3	4.8	3.6	2.9	1.24	0.96	3.9	8.17
Ser	6.9	5.8	4.1	4.52	2.9	3.34	0.62	3.8	4.26
Cys	2.3	1.9	1.4	0.54	0.2	0.25	0.23	0.6	1.45

Table 4. Fatty acid content in several microalgae (mg/g).

Microalgal Species	FFA	SAFA	MUFA	PUFA	References
<i>C. vulgaris</i>	340	22.0	3.5	73.4	Li et al. [69]
<i>Chlorella kessleri</i>	137.38	46.5	16.0	37.38	Ambrozova et al. [56]
<i>N. limnetica</i>	129.7	48.3	36.2	15.4	Marrez et al. [53]
<i>S. obliquus</i>	100	25.5	16.0	43.3	Salama et al. [70]
<i>B. braunii</i>	120	15.9	13.6	70.5	Lee-Chang et al. [71]
<i>A. falcatus</i>	79.5-86.9	32.1-36.2	27.7-31.1	25.9-27.9	Jayanta et al. [72]
<i>Oscillatoria</i> sp.	83.6	34.74	60.63	4.30	Irmak and Arzu [73],
<i>H. pluvialis</i>	83.41-93.68	27.81-30.36	18.96-20.07	43.15-47.23	Damiani et al. [58]

The fatty acid groups of several freshwater microalgae are shown in Table 4. *N. limnetica* has the highest saturated fatty acid (SAFA) value (48.3%) [53]. The SAFA group consisted of myristic acid (C14:0) at 3.34%, palmitic acid (C16:0) at 30.6%, and stearic acid (C18:0) at 6.06%. SAFAs are saturated fatty acids that contribute to many health-related functions. For example, cholesterol contributes to increasing low-density lipoprotein (LDL) levels [67]. Furthermore, SAFAs such as butyrate, caprate, lauric, myristic, palmitic, and stearic acid are useful for mammals' growth, development, and survival [68] (Table 4).

The highest monounsaturated fatty acid (MUFA) content was found in *Oscillatoria* spp., with a value of 60.63% [71]. MUFAs have the function of improving plasma lipid profiles, including those of both LDL and HDL concentrations [74]. The highest polyunsaturated fatty acid (PUFA) content was 73.4%, recorded in *C. vulgaris* [69]; PUFA consisted of palmitoleic acid (C16:2) (2%), linoleic acid (C18:2) (3.8%), linolenic acid (C18:3) (3.8%), arachidonic acid (C20:4) (2.7%), eicosapentaenoic acid (C20:5) (35.1%) and docosatetraenoic acid (C22:4) (16.5%). PUFAs such as linolenic acid and linoleic acid are essential fatty acids beneficial for the body, one of which is preventing coronary heart disease [75].

Fatty acids are also major components of the phospholipid present in cell membranes [76]. Moreover, fatty acids are an energy storage material and act as signaling molecules that regulate cell growth, differentiation, and gene expression [77, 78].

3.3. Carbohydrates

Microalgae are good sources of carbohydrates. Carbohydrates in microalgae are found in the cytosol, but also in organelles [79]. Carbohydrates in microalgae have several functions, such as acting as backup energy storage and structural components in the cell wall. Furthermore, the carbohydrate content can reach as much as 50% (w/w) of microalgal dry weight under high photoconversion efficiency. Therefore, some microalgae species have a high carbohydrate content (Table 2). However, the algal metabolism and carbohydrate composition vary from species to species. Therefore, choosing an algal type with a high carbohydrate content for human consumption is necessary.

The highest carbohydrate content of freshwater microalgal species is found in *H. pluvialis*, with a range of 47.20-64.22% [29]. In contrast, the lowest carbohydrate content is found in *C.*

vulgaris, with a range of 12-17% [26]. Differences in carbohydrate content can be affected by nutrients and light intensity [80]. For example, at a low light intensity, some microalgae synthesize proteins utilizing stored carbohydrates and this condition causes an overall decrease in the carbohydrate content [31, 81].

Sugars composing microalgae biomass include xylose, arabinose, glucose, maltose, galactose, and mannose [82]. The biomass of carbohydrates depends on the type of microalgal species, the method of cultivation, and the environmental conditions. Therefore, microalgal species can be manipulated to produce increased amounts of carbohydrates. Furthermore, the carbohydrates in microalgae can potentially be used for food [83].

3.4. Vitamins

Vitamins are micronutrients with essential functions in metabolic processes, are needed in small amounts and must be supplied from food. Vitamins have multiple functions that help regulate metabolism; prevent chronic diseases, and maintain appetite, mental health, and immunity. In general, vitamins act as coenzymes or as carriers of electrons or protons that are active in the process of macronutrient breakdown [40].

Algae are vitamin-rich foods. Some microalgae have high amounts of vitamins, such as provitamin A, vitamin E, vitamin B1, and folic acid. *Dunaliella tertiolecta* can synthesize vitamin B12, B2, E, and provitamin A [84], whereas *Tetraselmis suecica* is an excellent source of vitamin B1, B3, B5, B6 and C. Similarly, *Chlorella* spp. is good source of vitamin B12. *C. pyrenoidosa* accumulates 415 µg of vitamins per 100 g dry weight [85].

E. gracilis is also a vitamin-rich microalga, producing provitamin A, β-carotene, vitamin A, and vitamin E. Vitamin production in microalgae can be increased by applying a two-step culture. First, increased biomass of *E. gracilis* is achieved through photoheterotrophic culture. However, an increase in vitamin content is achieved through culture under photoautotrophic conditions. These conditions have been shown to increase the content of β-carotene, vitamin E and vitamin C to 71.0 mg/L, 30.1 mg/L, and 86.5 mg/L, respectively. Furthermore, adequate light applied to this species can increase the content of vitamin E by up to 100% [86].

Microalgae can synthesize all the vitamins that plants can produce. The accumulation of vitamins in microalgae is more

significant than that in soybeans and cereals but can vary depending on species, season, algal growth stages, and environmental parameters. For example, *Porphyridium cruentum* can accumulate high levels of tocopherols; specifically, this microalga contains α - and γ -tocopherol contents equal to 55.2 and 51.3 $\mu\text{g/g}$ dry weight. Tocopherol (vitamin E) is a fat-soluble antioxidant considered an essential nutrient due to its ability to protect membrane lipids from oxidative stress. Chlorella is the richest source of vitamin B12 and can serve as an alternative source of vitamins for vegetarians [87].

4. METABOLITES

4.1. Pigments

Pigment production in microalgae is affected by many factors. The availability of nutrients, pH, temperature, and light has been known to affect pigment production. Light and temperature are the most important limiting factors for enzyme production [88]. Pigments are located in the thylakoid membrane. In eukaryotic microalgae, this membrane is found within the chloroplast. However, in cyanobacteria (prokaryotic microalgae), the membrane is located near and parallel to the cell surface [89]. Some groups of freshwater microalgae known to produce pigments are listed in Table 5.

Pigments are grouped into three classes: chlorophyll (chl), carotenoids, and phycobiliproteins [102]. Although the composition of pigments that function in photosynthesis differs among microalgal species, all photosynthetic organisms have chl a as part of their core photosynthetic reaction center. Chl b, c, or d are also accessory pigments. All chlorophyll molecules exhibit two light absorption bands at 450–475 and 630–675 nm [103].

β -Carotene is present in most microalgae as an accessory pigment and has been extensively explored. β -Carotene production can be affected by abiotic factors such as light, intensity, salinity, temperature, and nutrients. The intensity of light can lead to high production of secondary pigments, one of which is β -carotene [104]. Another way to increase the production of β -carotene is to control the availability of nutrients in culture media. Nitrate and sulfate deficiency can induce higher β -carotene production [32]. Compared with inorganic fertilizers, Guillard media were better at inducing

high amounts of total carotenoids in *Hyaloraphidium contortum* and *C. vulgaris* [105].

Astaxanthin can be produced by freshwater microalgae, green microalgae, and *H. pluvisialis*. *H. pluvisialis* has two stages in its life cycle [106]. Optimal conditions for the growth of *H. pluvisialis* include a temperature of 20 °C, a light intensity of 250 mol photon $\text{m}^2 \text{s}^{-1}$, a CO_2 concentration of $\sim 2.2 \text{ mg L}^{-1}$, a nitrate concentration of up to 10 mM, and a pH of approximately 7.5–8.0 [107–109]. Moreover, in the red phase, pigment accumulation can be triggered by temperature increases to those higher than 27 °C, a light intensity of 500 mol photon $\text{m}^2 \text{s}^{-1}$, and nitrate concentrations of $\sim 3 \text{ mM}$ [84, 106, 109, 110].

4.2. Sterols

Sterols play an essential role in the human body. Sterols are the main constituents of cell membranes. In addition, many sterols surrogates help improve cholesterol metabolism by competition, primarily lowering the content of low-density lipoproteins (LDL). This property is associated with a decrease in cardiovascular disease in humans. Furthermore, there may be a strong association between the so-called phytosterol consumption and an improved immune system [111].

Sterol composition in microalgae show large differences, mostly as a consequence of their phylogenetic variety. Additionally, the pool of sterols may be affected by the precise growth condition. Major sterols present in Microalgae are either cholesterol or β -sitosterol [110], but minor amounts of other sterols may be also present [112]. Additionally, changes in environmental conditions can also change the profiles of sterols. Sterols can be used as antioxidant, anticarcinogenic, and anti-inflammatory compounds, reducing the effects of neurological diseases such as Parkinson's and Alzheimer's diseases and providing anti-hypercholesterolemia and anti-diabetic effects [113, 114].

Martin-Creuzburg and Merkel [115] showed that freshwater microalgae of Chlorophyceae, Trebouxiophyceae, Eustigmatophyceae, Cryptophyceae, and Bacillariophyceae were capable of producing sterols. In addition, the researchers found that seventeen freshwater microalgae produced sterols. The highest producer of sterols was *Monoraphidium minutum* ($15.3 \pm 1.1 \mu\text{g mg L}^{-1}$); in contrast, the lowest sterol was found in *Gomphonema parvulum* ($2.1 \pm 0.2 \mu\text{g mg L}^{-1}$).

Table 5. Pigments produced by freshwater microalgae.

Species	Pigments	References
<i>Dunaliella salina</i>	β -carotene, astaxanthin, zeaxanthin, lutein, cryptoxanthin	Dufosse <i>et al.</i> [90]; Rabbani <i>et al.</i> [91]; Hana <i>et al.</i> [92]; Dufosse <i>et al.</i> [90]
<i>H. pluvisialis</i>	Astaxanthin, canthaxanthin, lutein	Buono <i>et al.</i> [93]; Dufosse <i>et al.</i> [90]
<i>C. vulgaris</i>	Canthaxanthin, astaxanthin, β -carotene, echinenone	Patias <i>et al.</i> [94]
<i>C. sorokiniana</i>	Chlorophyll, carotenoids	Miazek <i>et al.</i> [95]; Ogbonna <i>et al.</i> [96]
<i>S. obliquus</i>	Astaxanthin, Lutein, β -carotene, echinenone	Sallehudin <i>et al.</i> [97]; Patias <i>et al.</i> [94]; Qin <i>et al.</i> [98]
<i>Scenedesmus dimorphus</i>	Astaxanthin, chlorophyll, lutein, zeaxanthin,	Sallehudin <i>et al.</i> [97]; Ahmad <i>et al.</i> [99]
<i>Ankistrodesmus</i>	Lutein, chlorophyll, β -carotene	Sallehudin <i>et al.</i> [97]; Ogbonna <i>et al.</i> [96]
• <i>B. braunii</i>	Neoxanthin, loroxanthin, violaxanthin, lutein, α -carotene, and β -carotene, echinenone	Tonegawa <i>et al.</i> [100]; Ambati <i>et al.</i> [101]

The sterol composition of freshwater green algae (Chlorophyta) is very diverse. In addition, many microalgae have been shown to contain unusual and rare sterols of the C30 sterol group, such as 24-propylidenecholesterols (IVs,t) [116]. Several microalgal sterols have not been found in terrestrial higher plants. However, substantial differences in sterol chemical structure, especially in side chain configurations, have been reported even within a single species. For example, the C-24 alkyl group of the sterol moiety in green algae is attached in the 2,4- β orientation [117]. The two main sterols of *C. reinhardtii* are ergosterol and 7-dehydroerigerosterol but also small amounts of fungisterol, 22-dihydroerigerosterol, and 22-dihydrochondrillasterol have been found in this algae [118].

5. FUNCTIONAL PROPERTIES

The great potential of microalgal applications encompasses human nutrition, feedstuffs, bio fertilizer, and waste treatment. In addition, it extends to the field of health care in terms of new anti-inflammatory, antiallergic, and analgesic drugs [119]. For example, *C. vulgaris* and *Spirulina* produce sulfated polysaccharides, which are considered nutraceuticals recommended in cancer prevention a/o treatment [120, 121].

5.1. Antioxidants

Antioxidants can help optimize human physiology and prevent diseases [122, 123]. The ability of microalgae to act as antioxidants is sometimes more remarkable than that of plants or fruits. Vitamins, carotenoids, and polyphenols such as flavonoids are responsible for the antioxidant activity of microalgae [124]. High amounts of phycobiliprotein in microalgae lead to an increase in antioxidants in several Cyanobacteria [125]. The antioxidant activity of the extracted-freshwater microalgae is rarely reported.

In a study conducted by Shanab *et al.* [125], freshwater microalgal extracts (*Anabaena oryzae*, *Nostoc humifusum*, *Nostoc muscorum*, *Oscillatoria* sp., *Spirulina platensis*, *Phormidium fragile*, *Wolleea saccata*, and *C. vulgaris*) were tested for antioxidant activity. The result showed that *Spirulina* has high antioxidant activity compared to other species, with values of 69.3% and 75.9%, respectively. Cyanobacteria has also been reported as potential antioxidants. The ethanolic extract of *Euglena cantabrica* has a radical scavenging inhibition of 71-100%, which is even higher than that of butylated hydroxytoluene (BHT) used as control (26%). It is postulated that catechin and chlorogenic acid are the phenolic compounds responsible for the antioxidant activity [126]. Nevertheless, there are other antioxidants compounds such as flavonoids and carotenoids. The extract from *Scenedesmus* sp., *C. vulgaris*, *C. reinhardtii*, contain polyphenols, flavonoid, and carotenoids [127].

Moreover, sterols such as Desmosterol, Sitosterol, Ergosterol, Ocellasterol, Cholesterol, and Clionasterol can act as antioxidants [128]. Seventeen freshwater microalgae were analyzed for the sterol producer. *Monoraphidium minutum* and *Ankistrodesmus fusiformis* are the highest producers of total sterol [115]. Unfortunately, the investigation of sterol from freshwater microalgae is still rare. Hence, further exploration of freshwater microalgae-sterol as an antioxidant is very open.

5.2. Anti-inflammatory

Inflammation is an initial immune reaction when foreign pathogens disrupt cellular homeostasis. Various inflammatory mediators, including cytokines, chemokines, cyclooxygenase-2 (COX-2), prostaglandins (PGs), and nitric oxide synthase (NOS), can cause various diseases [129, 130]. Compounds from the ethanolic extract of freshwater microalgae, *Micractinium* spp., can decrease the expression of TNF- α and IL-6 [131]. Furthermore, *Chloromonas reticulata* compounds extracted using ethanol can reduce the expression of major regulatory inflammatory factors, such as NOS and COX-2. In addition, containing active substances, these extracts can reduce mRNA levels associated with inflammation [132].

Lipid components influence the anti-inflammatory effects shown by some species of freshwater microalgae. For example, lipid extracts can inhibit the activity of COX enzymes, which are inflammatory mediators that induce the conversion of arachidonic acid into prostaglandins and decrease cytokine (TNF- α , IL-1 β , and IL-6) production [133]. In addition, phytosterols extracted from *Nannochloropsis oculata* exert anti-inflammatory effects by lowering NOS and COX-2 [134]. Furthermore, fucoxanthin, PUFA, EPA, and DHA, such as the compound of microalgae fatty acids oxylipins, act as an anti-inflammatory [135]. Moreover, diet PUFAs from microalgae reduce inflammatory bowel disease symptoms [136].

5.3. Antibacterial

Freshwater microalgae such as *E. viridis*, *Microcystis aeruginosa*, *C. vulgaris* and *S. platensis* have demonstrated antibacterial activity in trials involving pathogenic bacteria such as *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi* [137]. Additionally, *Planktochlorella nurekis* at a concentration of 0.75–6 mg/mL inhibits a group of pathogenic bacteria including *Salmonella enterica* var. *Enteritidis*, *S. enterica* var. *Infantis*, *Campylobacter jejuni*, *E. coli* [138]. Furthermore, PUFA-extracted from *P. nurekis* at a concentration of 100 $\mu\text{g}\cdot\text{mL}^{-1}$ inhibits growth of *S. aureus* and a mixed culture of *Enterococcus faecalis* and *Pseudomonas aeruginosa* [139]. Fatty acids, SAFAs, MUFAs, and PUFAs, can act as antibacterial compounds [135], as shown with algae containing lauric acid (C12:0), myristic acid (C14:0), pentadecanoic acid (C15:0), and stearic acid (C18:0) which decrease the metabolic activity of Gram-negative bacteria such as *P. aeruginosa* and *E. coli* PCM 2209 [140].

There are various ways to apply microalgae to combat pathogenic bacteria, using extracts, homogenates, pure compounds or whole cells [140, 141] thus promoting the antibacterial activity exerted by specific compounds such as fatty acids, glycolipids, phenolics, terpenes, diketones, or alkaloid indoles [142]. For example, an organic extract of *Spirogyra* sp. showed inhibition on *S. aureus*, *E. coli*, *St. xylosum* and *P. aeruginosa* at a concentration of 0.1 $\text{g}\cdot\text{mL}^{-1}$ [143].

H. pluvialis, *Scenedesmus* sp., *Chlorella* sp., and *Spirulina* sp. were challenged against Gram-positive and Gram-negative bacteria. Vigorous antibacterial activity was found on the 70% methanolic extract of *Scenedesmus* sp. NT8c and *Chlorella* sp.

with a minimum inhibitory concentration (MIC) value of 1 mg.mL⁻¹. Among all tested microorganisms, *E. coli*, *S. typhoid*, and *P. syringae* failed to be inhibited by the extract [144].

5.4. Antiviral

Several freshwater microalgae have demonstrated antiviral activity. For example, extracts of *Anabaena sphaerica*, *Chroococcus turgidus*, *Oscillatoria limnetica*, *Cosmarium* sp., and *S. platensis* inhibit adenovirus type 40. Methanol extract of *S. platensis* showed high antiviral activity with IC₅₀ viral titer values of 2 mg.mL⁻¹ [145]. The best antiviral components were found in *S. platensis* extracts. *S. platensis* extracts also have good antiviral capacity against human immunodeficiency virus type 1. This ability is possibly due to the sulfoquinovosyl diacylglycerol (SQDG) compound found in *Spirulina* [146]. SQDG isolated from *S. platensis* was reported to have antiviral activity against herpes simplex virus type 1 (HSV-1) with an IC₅₀ value of 6.8 g.mL⁻¹ [147].

Pigments, pheophorbide a, carotenoids, astaxanthin and phycobiliproteins (allophycocyanin, phycocyanin) have antiviral activity. Pheophorbide a (PPba) has antiproliferative activity because it exhibits several antiviral effects, particularly against enveloped viruses [118]. This pigment can bind to viral cell receptors and impact after entering the virus. Carotenoids can reduce the harmful effects of some viruses, such as those associated with cytokine storms [148]. Pressurized liquid extraction (PLE) of carotenoids from the ethanol extract of *H. pluvialis* has been reported to inhibit HSV-1 growth by 85% [149]. Polysaccharides in microalgae have antiviral potential by preventing viruses from reaching their host cells. In addition, various freshwater microalgae produce glycoproteins with antiviral potential. For instance, microalgae lectins can attach to carbohydrates involved in HIV glycosylation to bind to CD4 cellular receptor target cells [150]. The water soluble *S. platensis* polysaccharide showed HSV-1 inhibitory activity at IC₅₀ value of 21.32 µg.mL⁻¹ [151].

5.5. Anticancer

The bioactive compounds of microalgae can be used as anticancer agents. Protein hydrolysates of *C. vulgaris* show anticancer activity [152]. The peptide fraction isolated from *C. vulgaris* inhibits the growth of the hyperdiploid human cell, (AGS cell line) with a range of IC₅₀ values of 70.7 ± 1.2 µg.mL⁻¹ to 1.74 ± 0.3 g ml⁻¹ [153]. *Granulocystopsis* sp. extracts show high cytotoxicity against prostate, breast, colorectal, melanoma and lung cancer cells (<20 mg.ml⁻¹) but the positive control using doxorubicin showed higher anticancer activity than the microalgae extract on Vero (normal) cells [154]. However, microalgae extract has an apoptotic mechanism in the tumor pathway positively associated with doxorubicin's anticancer effect [155]. Furthermore, prostate cancer showed a sensitivity effect during the administration of the methanol extract of *Granulocystopsis* spp. The values of IC₅₀ of the *Granulocystopsis* spp methanolic extract were lower than 20 µg.mL⁻¹ [154]. The US National Center Institute (NCI) determines the level of cytotoxicity: IC₅₀ > 100 mg/ml = inactive, IC₅₀ 20-100 mg/ml = moderately active and IC₅₀ < 20 mg/ml = active [156].

Anticancer bioactive compounds include polysaccharides, glycoproteins, astaxanthin, lycopene, β-carotene, EPA, DHA, polyphenols and flavonoids [157]. Carotenoids, astaxanthin, β-carotene, lutein, lycopene, and canthaxanthin inhibit the proliferation of human lung cancer cells (NCI-H226) and suppress the growth factors of breast and endometrial cancer cells [158]. Several freshwater microalgae inhibit cancer cells such as breast, colon, prostate, pancreatic, and endometrial cancers, namely *Nannochloropsis gaditana*, *Isochrysis galbana*, *A. flos-aquae* and *S. platensis* [6, 7]. Aqueous extract from *Geitlerinema carotinosum*, *Nostoc linckia* can inhibit rat glioma cell (C6 cell lines) with IC₅₀ levels of 112.69 and 121.48 µg.mL⁻¹ [159]. Phytochemical from *C. vulgaris* inhibit MCF7 breast cancer cell with IC₅₀ value of 100 µg.mL⁻¹ [8]. Moreover, Coibamide-A from *Leptolyngbya* sp has a small value of IC₅₀ (300 ng.mL⁻¹) toward human lung cancer NCIH 460 [160].

CONCLUSION AND DEVELOPMENTAL PROSPECTS

The demand for food supply in the future will be greater than it is now, so it is necessary to increase food production by more than 60% to meet the global population's needs. Based on reviews on nutritional and functional properties, freshwater microalgae have the potential to continue to develop as food alternatives.

Countries in tropical regions rich in sunlight have better opportunities to explore and utilize microalgae. Therefore, countries that do not have beaches or adjacent seas can switch to the cultivation of freshwater microalgae, which have been proven to have excellent nutritional contents. Microalgae production must be modified to the desired characteristics and factors that affect each species to obtain optimal results. Each species has different prerequisites for the growth and production of metabolites.

Apart from *Chlorella* sp., information regarding the safety issues and the large scale production of freshwater microalgae is still limited. This limitation due to inadequate data regarding the toxic compounds, nutrition value, growth parameters, and economic feasibility of freshwater microalgae. Therefore, in the future, the exploration of freshwater microalgae as foods, should be focused on those topics.

Researchers can also develop various processed foods from freshwater microalgae. Proper product development, especially in terms of production and processing, will help each country achieve its goal of addressing food security successfully.

The prospect of good development of the use of freshwater microalgae needs to be considered because, in general, the development of food alternatives requires adjustments of consumers. Therefore, freshwater microalgal food alternatives need to receive a good reception from the public and are expected to compete in the market. The challenge to overcome is to convert freshwater microalgal biomass into nutritious foods, good taste, and good functional properties. This is because the public tends to judge foods based on the nutritional content and functional properties and the taste and appearance of a product. We hope that freshwater microalgae will soon be implemented to help solve the global food crisis.

LIST OF ABBREVIATIONS

ABTS	= 2,2'-azino-bis (3-ethylbenzothiazoline6-sulphonic acid)
CD4	= cluster of differentiation 4
DHA	= Docosahexaenoic Acid
DPPH	= 2,2-diphenyl-1-picrylhydrazyl
EPA	= Eicosapentaenoic Acid
FAO	= Food and Agriculture Organization
HIV	= Human Immunodeficiency Virus
IC₅₀	= Inhibitory Concentration where 50% of cell is inhibited
IL	= Inter Leukin
mRNA	= messenger Ribonucleic Acid
MIC	= Minimum Inhibitory Concentration
MUFAs	= Mono Unsaturated Fatty Acids
PUFAs	= Poly Unsaturated Fatty Acids
SAFAs	= Saturated fatty Acids
TNF	= Tumour Necrosis Factor
UNU	= United Nation University
WHO	= World Health Organization

AUTHOR CONTRIBUTIONS

A.A.P., M.W., and Y.D.J. conducted a literature review and contributed to discussions, A.M., and R.N provided a Table data. M.W. provided guidance, led discussions, and approval of the complete version. A.A.P. Y.D.J., A.M. revised the paper.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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