

Chlorella sorokiniana biomass as an alternative ingredient in food (Review)

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Abstract. *Chlorella sorokiniana* (*C. sorokiniana*) is a freshwater green microalga belonging to the Chlorophyta division. The analysis of the dry biomass of microalgae *C. sorokiniana* has revealed a typical physicochemical composition of 48% protein, 13-22% lipid content, 30-38% carbohydrates and 80% unsaturated fatty acid content. The biomass is also high in physiologically active phytochemicals, including chlorophylls and carotenoids. Due to its considerable potential, *C. sorokiniana* has emerged as a desirable and innovative resource for developing novel food and functional products. Furthermore, using *C. sorokiniana* microalgae as functional components can effectively enhance the nutritional composition of food products. It can positively impact human health by improving overall well-being and quality of life, while reducing the potential risks associated with illness and diseases. The present review discusses the biomass characteristics, bioactive substances, functional components, biomass-based food products and health advantages linked to the consumption of *C. sorokiniana* microalgae.

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1. Introduction

The escalating issue of meat inflation, the increasing global population and the need for a nutritionally balanced diet, while minimizing greenhouse gas emissions have all contributed to a surge in demand for protein from plant sources. Since the primary protein sources are derived from animals and the strain on current animal sources is deemed unsustainable, there is an urgent need for novel, sustainable protein sources that are safe and of high nutritional quality. Therefore, the food industry and researchers are tasked with finding alternative protein sources and production methods to satisfy consumer demand and anticipated global protein needs.

While plant-based proteins are nutritionally beneficial, they require land and water, which are becoming increasingly scarce. Moreover, the conversion of plant proteins into meat proteins is inefficient, requiring 6 kg of plant proteins to produce 1 kg of meat proteins (1). Single-cell proteins, mainly composed of dried cells (biomass) produced by algae, yeast, bacteria and fungi, present a viable alternative to traditional protein sources in this context (2). Microalgae, unicellular photosynthetic organisms, are considered promising alternative protein sources (3). They can produce biomass and oxygen by harnessing sunlight as an energy source, CO₂ as a carbon source and inorganic salts as a carbon supply (4).

Microalgae exist in freshwater, marine and edaphic habitats, with some species also found in humid soils (5). Unlike conventional food crops, microalgae can thrive in various conditions without requiring arable land, thus not competing with crops, such as soybeans and cereal grains (6).

The broad spectrum of microalgae includes >100,000 species divided into four distinct groups: Chlorophyceae (green algae), Bacillariophyceae (eukaryotic diatoms), Chrysophyceae (golden algae) and Cyanophyceae (blue-green algae) (7). Despite their vast diversity, microalgae remain under-researched and poorly understood (4). Researchers have found that microalgae are an attractive source for developing beneficial products with significant value in the cosmetics, food and nutrition, medicinal and pollution-prevention sectors (8).

Chlorella spp. was the first algae discovered and isolated in culture by Beijerinck (9). Of note >20 *Chlorella* species have been identified, with >100 strains documented (10). The exploration of the dietary benefit of *Chlorella* on human health began in the early 1950s when *Chlorella* was introduced as a

food source during a global food crisis (11). It was first grown and consumed in Asia, mainly in Japan, before gaining popularity as a nutritional supplement worldwide (12).

Chlorella spp. contains protein levels similar to traditional protein sources such as meat, egg, soybean and milk (3). Research has shown that it contains 55-67% protein, 1-4% chlorophyll, 9-18% dietary fiber, and various minerals and vitamins on a dry matter basis (13). In particular, *Chlorella vulgaris* is known to contain up to 58% protein on a dry weight basis (14) and is considered safe for human consumption by the Food and Drug Administration (FDA) in the USA, along with other microalgae species, such as *Arthrospira platensis* (*Spirulina*) due to their long history of human consumption and nutrient profile (15). However, limited reports are available on the protein content and quality of *Chlorella sorokiniana* (*C. sorokiniana*) and its application in food. Thus, the present review aimed to evaluate *C. sorokiniana* biomass as an alternative ingredient for food. The present review aimed to enhance the understanding of the biomass characteristics, bioactive substances, functional components, biomass-based food products, and health advantages linked to the consumption of *C. sorokiniana* microalgae.

2. Review methodology and literature selection

A systematic approach was utilized to select pertinent literature from databases such as Scopus, Web of Science, CrossRef, PubMed and Google Scholar to ensure a comprehensive review. The search for articles included in the present review involved utilizing specific key terms, such as '*C. sorokiniana* Characteristics', 'Macronutrients in *C. sorokiniana*', 'Protein Quality of *C. sorokiniana*', 'Micronutrients in *C. sorokiniana*', 'Bioactive compounds in *C. sorokiniana*', and '*C. sorokiniana* Food Products'. Subsequently, the databases were used in conjunction with VOSviewer software to conduct a bibliometric analysis (16). Articles were selected based on various criteria, including their relevance to the topic and the presence of substantial reviews or experimental data on *C. sorokiniana* microalgae.

3. *C. sorokiniana* biomass characteristics

C. sorokiniana, a freshwater green microalga, is part of the Chlorophyta division. Initially, it was considered to be a thermotolerant mutant of *Chlorella pyrenoidosa* (17). However, chloroplast 16S rDNA and 18S rRNA profiling in the late 1980s and early 1990s identified *C. sorokiniana* as a separate species (10). This tiny, single-celled alga, ranging in size from 2 to 4.5 μm in diameter, can grow mixotrophically on various carbon and nitrogen sources, rendering it ideal for waste feedstock cultivation (18).

This chlorophyte has several advantages over other microalgae species. Previous studies have demonstrated that *C. sorokiniana* can achieve optimal growth at temperatures ranging from 35 to 40°C (19), with phototrophic doubling times as short as 4-6 h (20). It can also grow under harsh conditions, including wastewater or heavy metal wastes (21). It has been reported to have a more rapid growth in mixotrophic and even heterotrophic conditions, with a preference for sugars, such as glucose or simple organic acids, such as acetate (22).

Table I. Physicochemical characteristics of *Chlorella sorokiniana* on a dry basis.

Components	Content
Appearance, dry	Free-flowing powder
Color, dry	Green
Taste and smell	Fishy, characteristic of algae
External admixtures	Absent/none
Moisture content, %	4%
Protein, g/100 g dry biomass	47.82±2.30
Lipids, g/100 g dry biomass	17.50±4.50
Carbohydrates, g/100 g dry biomass	30.00±4.00
Minerals, mg	4.36±0.56

The information presented in this table is derived from previous studies (23,25,26,29).

The typical physicochemical composition and biological value (BV) indicators of the dry biomass of *C. sorokiniana*, obtained by cultivation in a pilot bioreactor, are presented in Tables I and II. The dry biomass of *C. sorokiniana* contains an average of 48% protein, which is markedly higher than that of soy, peanut, wheat germ and other animal sources such as beef, fish and chicken (23,24). The lipid content of the biomass sample is 13-22%, comparable to or slightly higher than that of other aquacultures, such as *Spirulina spp* (25). The biomass is rich in physiologically active phytochemicals, the most abundant of which are chlorophylls (22.13 mg/g dry biomass) and carotenoids (6.04 mg/g dry biomass) (23) (Table III). *C. sorokiniana* consists of 30-38% carbohydrates on a dry weight basis (26), which is higher than that *C. vulgaris*, with ~15% carbohydrate content on a dry basis (27).

Factors such as temperature, nutritional composition and light availability can influence the quantities of biomass, macro- and micronutrients, and other useful bioactive substances, including antioxidants, in *Chlorella* cells (28).

4. Macronutrients in *C. sorokiniana* biomass

The average macronutrient content of *C. sorokiniana* biomass based on previous studies (23,31,32) is summarized in Table IV. The analysis of *C. sorokiniana* dry biomass has revealed that it contains an average of 45% protein, which is much greater than the protein content of foods such as peanut (26%), wheat germ (27%) and other animal sources, such as beef (22%), fish (22%) and chicken (24%) (23,24,33).

The essential amino acid composition of *C. sorokiniana* biomass is presented in Table V. Notably, these amino acid profiles, which mammals do not synthesize, are present in notable concentrations in *C. sorokiniana* microalgae; thus, when compared to the recommendations of the World Health Organization for the use of essential amino acids in food, this microalga could be used in the food industry (32). The high protein content and favorable amino acid composition of *C. sorokiniana* microalgae suggest that this microalga may serve as a potential protein source.

Table II. Indicators of the biological value (BV) of the dry biomass of *Chlorella sorokiniana* microalgae.

Polyunsaturated fatty acids (PUFAs)	Concentration (mg/g)	Carbohydrates (sugars)	Concentration (mg/g)	Phytochemicals	Concentration (mg/g)
ω3	26.6±1.16	Sucrose	204.0±2.00	Chlorophyll	22.13±2.20
Eicosapentanoic acid	0.53±0.02	Glucose	133.0±1.20	Phenolic compounds	0.05±0.02
α-linolenic acid	16.1±0.60				
Docosahexaenic acid	10.0±0.20	Xylose	58.0±0.50	Carotenoids	6.04±0.60
ω6	25.7±1.00				
E-linoleic acid	3.3±0.05	Fructose	20.0±0.30	Organic acids	2.70±0.40
Z-linoleic acid	14.8±0.50				
Octadecatrienoic acid	7.5±0.10				

The information presented in this table is derived from previous studies (23,30).

Table III. PER, BV, NPU and DC of various protein sources.

Protein sources	PER ^a	BV ^a	NPU ^a	DC ^a
Beef	116.0	103.9	96.1	100.0
Soy protein	88.0	96.1	80.3	99.0
Wheat gluten/cereal	32.0	83.1	88.2	91.9
<i>Chlorella</i> spp.	80.0	87.2	81.5	93.6

The information presented in this table is derived from previous research (14). ^aParameters are presented as % casein values. PER, protein efficiency ratio; BV, biological value; NPU, net protein utilization; DC, digestibility coefficient.

Table IV. Macronutrient content of *Chlorella sorokiniana* biomass on a dry basis.

Macronutrients	Content (g/100 g dry weight)
Proteins	44.75±1.88
Lipids	16.42±5.18
Carbohydrates	37.61±1.81

The information presented in this table is derived from previous studies (23,31,32).

According to Gouveia and Oliveira (25), the lipid content of *C. sorokiniana* biomass on a dry basis ranges from 11.24-21.6% dry matter. It is equivalent to or slightly higher than other aquacultures, such as *Spirulina* spp. (4-9% dry matter). As shown in Table VI, ~80% of the total fatty acid content of *C. sorokiniana* lipids is polyunsaturated fatty acids (PUFAs) (23). Since this microalga biomass contains sufficient amounts of omega-3 PUFAs (~25-28% of total fatty acids) and omega-6 PUFAs (~25-27% of total fatty acids), it can effectively prevent and treat several diseases. Indeed, omega-3 PUFAs, such as alpha-linolenic acid (ALA, C18:3), eicosapentaenoic acid (EPA, C20:5), docosapentaenoic acid (DPA, C22:5) and docosahexaenoic acid (DHA, C22:6) are effective in

Table V. Essential amino acid composition of *Chlorella sorokiniana* microalgae.

Essential amino acid	Content (mg/g)
Histidine	6.1±0.60
Threonine	19.0±2.20
Isoleucine	4.53±0.04
Valine	20.0±1.20
Methionine	1.8±0.20
Tryptophan	0.2±0.02
Phenylalanine	18.0±1.00
Leucine	30.0±2.10
Lysine	21.0±1.60

The information presented in this table is derived from previous studies (23,32).

preventing or treating cardiovascular disorders (34), lowering hypertension (35) as well as preventing cancer (36,37), type 2 diabetes (38), inflammatory bowel disorder (39), asthma (13), arthritis (40), kidney and skin disorders (38,39), depression (13) and schizophrenia (41). Furthermore, due to its lipid composition, *C. sorokiniana* may also play an essential function in preventing atherosclerosis, hypercholesterolemia and tumors (40).

Another key class of macronutrient substances found in the hydrophilic fraction of *C. sorokiniana* microalgae biomass comprises carbohydrates. Effectively, 36-39% dry weight of carbohydrates found in *C. sorokiniana* microalgae biomass are found in the form of sucrose, glucose, xylose and fructose (23,31,42). Moreover, the cell wall of *C. sorokiniana* is also composed of many insoluble polysaccharides, primarily mannose and glucose (43). Polysaccharides are polymeric carbohydrate structures commonly employed in the food industry (44). Owing to their high polysaccharide concentration, microorganisms such as *Chlorella* microalgae have been actively investigated in the search for novel natural antioxidants (44,45). In the study by Pugh *et al* (46), immurella polysaccharides, a high molecular weight polysaccharide, were

Table VI. PUFAs and carbohydrates in *Chlorella sorokiniana* microalgae dry biomass.

Components	Content (mg/g dry weight)
PUFAs	
Alpha-linolenic acid (ALA)	16.1±0.60
Eicosapentanoic acid (EPA)	0.53±0.02
Docosahexaenoic acid (DHA)	10.0±0.20
Total omega-3	26.6±1.16
E-linoleic acid (LA)	3.3±0.05
Z-linoleic (LA)	14.8±0.50
Octadecatrienoic acid	7.5±0.10
Total omega-6	25.7±1.00
Carbohydrates	
Sucrose	204.0±2.00
Glucose	133.0±1.20
Xylose	58.0±0.50
Fructose	20.0±0.30

The information presented in this table is derived from previous studies (23,30). PUFAs, polyunsaturated fatty acids.

isolated from *C. sorokiniana*, which presented a higher activity against cancer. Polysaccharides derived from *C. sorokiniana* microalgae have also been shown to exhibit substantial antioxidant, tumor-fighting, immunomodulatory characteristics, and the ability to remove superoxide and hydroxyl peroxide radicals (47).

5. Protein quality of *C. sorokiniana* biomass

The considerable protein content of *C. sorokiniana* has positioned it as a promising alternative protein source. The protein content in microalgae is typically determined by measuring the total nitrogen and multiplying it by the factor $N \times 6.25$ (48). Experimentally determined N-protein factors for specific microalgal species range from $N \times 3.06$ to 5.95 (49,50). This variation in the N-protein factor is attributed to non-protein nitrogen, which comprises up to ~10% of the nitrogen in microalgae and includes substances, such as amines, glucosamines, nucleic acids and cell wall components (48).

Of note, four important quality parameters are considered to assess the nutritional value of algal protein: Protein efficiency ratio (PER), BV, digestibility coefficient (DC) or true digestibility, and net protein utilization (NPU) (14). PER is a fundamental method for evaluating protein quality, involving animal feeding trials with weanling rats throughout 3 to 4 weeks (48). It measures the weight gain per unit of protein consumed, expressed as $PER = \text{weight gain (g)} / \text{protein intake (g)}$ (48).

BV, another metric for assessing protein quality, is based on the proportion of retained nitrogen to absorbed nitrogen (48). The calculation for BV is $BV = [I - (F - F_0) - (U - U_0)] / [I - (F - F_0)]$, where I represents nitrogen intake, F is fecal nitrogen, U is urinary nitrogen, and F₀ and U₀ are the amounts of fecal and

urinary nitrogen excreted when animals are fed a nitrogen-free or low-nitrogen diet (51).

DC, also known as real digestibility, is another indicator of protein quality. It reflects the proportion of meal nitrogen absorbed by the animal and is calculated using the same parameters as BV: $DC = [I - (F - F_0)] / I$ (48). NPU, on the other hand, is determined by dividing nitrogen retained by nitrogen intake. The measure of both digestibility and BV is provided by the NPU, which is calculated using the formula $NPU = (B - B_k) / I$. Herein, B represents the body nitrogen assessed at the end of the test period in animals fed the test food. B_k represents the body nitrogen measured in another group of animals fed a protein-free or low-protein diet (48).

A comparison between *Chlorella spp.* microalgae and traditional protein sources based on four quality parameters (PER, BV, DC and NPU) is presented in Table III. The values in the table were compared to casein, the primary protein found in milk, which serves as a reference for scoring. Overall, the values presented in Table III indicate that the nutritional value of *Chlorella spp.* algal protein is lower than that of beef and casein. However, it is comparable to that of soy protein and superior to that of wheat gluten (52).

Amino acid composition. Protein quality can vary significantly based on the digestion and the availability of essential amino acids (EAAs) (53). Animal protein sources are often considered complete proteins as they contain a high concentration of EAAs that the human body cannot synthesize (3). By contrast, plant proteins are frequently observed as incomplete protein sources as they may lack one or more EAAs, such as histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (54). For example, soy protein, which is increasingly popular in the human diet, is deficient in methionine, an essential antioxidant in the body (55). However, the specific EAAs acids lacking in plant-based proteins can vary. Therefore, consuming a varied diet of plant proteins from fruits, vegetables, grains and legumes can provide an adequate quantity of all EAAs (56).

Plant-based proteins are often more challenging to digest than animal proteins due to differences in their protein structure (57). Plant-based proteins have a higher proportion of β -sheet conformation and a relatively lower α -helix conformation than animal proteins (58). The high concentration of β -sheet conformation in plant proteins renders them more resistant to proteolysis in the gastrointestinal system, reducing their digestibility (58). Additionally, plant-based sources may contain non-starch polysaccharides or fibers that hinder enzyme access to proteins and can further reduce protein digestion (59).

Despite these differences, there is an increasing concern about the high levels of saturated fats and cholesterol present in animal-derived foods, which have been linked to cardiovascular disease and diabetes. As a result, nutritionists and organizations such as the Food and Agriculture Organization (FAO) recommend a diverse diet rich in plant-based proteins (60).

Analyzing the amino acid composition is crucial for understanding the value of food raw materials. Algae, including microalgae such as *Chlorella spp.* and *Spirulina spp.*, are widely recognized as viable protein sources with

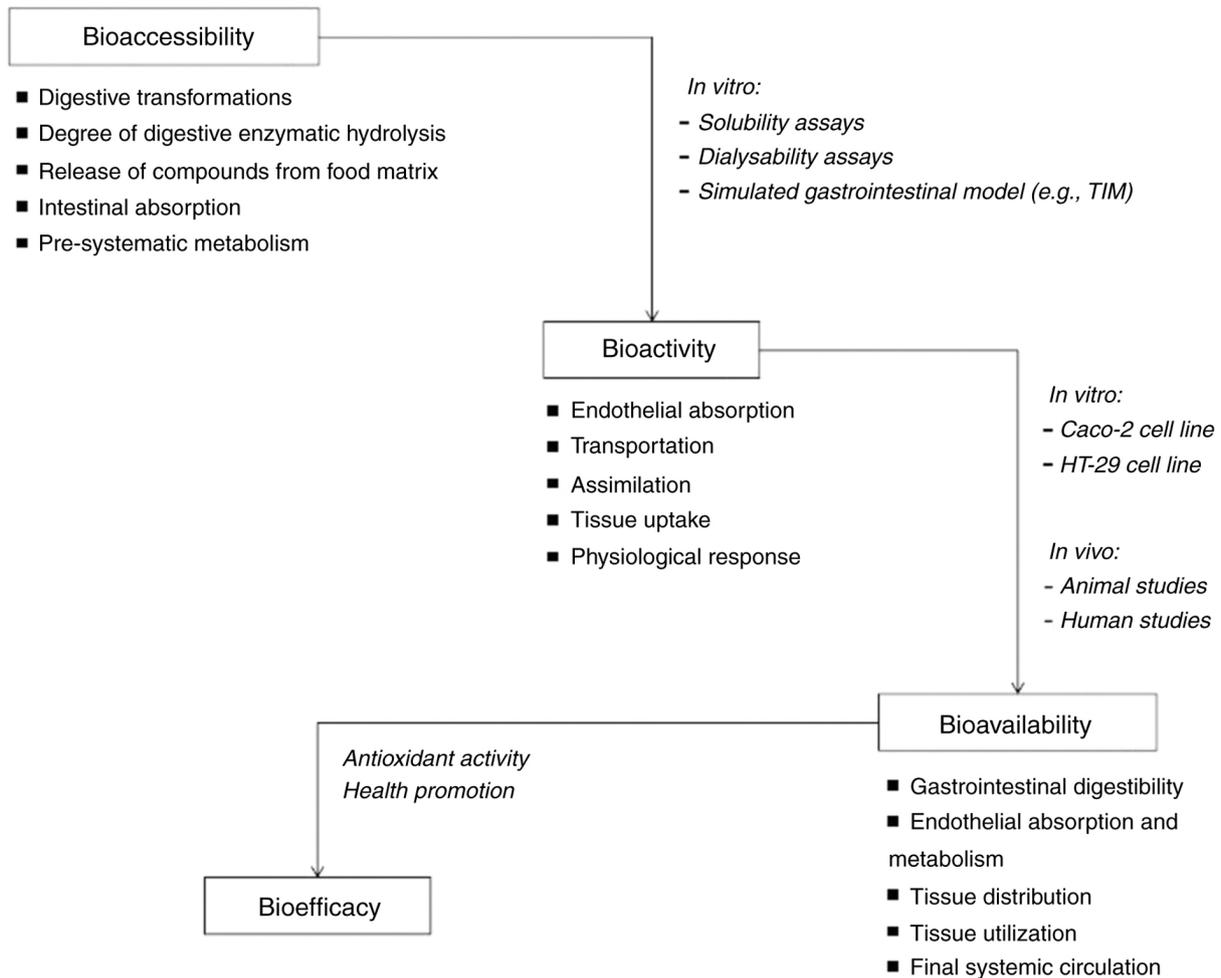


Figure 1. The path of chemicals to bioefficacy, including a schematic representation of methodologies for determining algal compounds' bioaccessibility, bioactivity and bioavailability, as previously demonstrated (3).

EAA compositions that meet the FAO standards (61,62). WHO/FAO/UNU has recommended these microalgae species for their adequate EAA content for human consumption (62). Microalgae, including *Chlorella spp.*, have been found to contain amino acids, such as isoleucine, valine, lysine, tryptophan, methionine, threonine and histidine in amounts equivalent to or greater than protein-rich sources, such as eggs and soybeans (33). Notably, the dry biomass of *C. sorokiniana* microalgae provides all the amino acids necessary for the growth and development of living organisms (Table III). Research has shown that the quality of *Chlorella* products, including *C. sorokiniana* (Table V), is high based on the essential amino acid index, with values higher than that of soybean protein (63). These findings suggest that *Chlorella* proteins are of high quality.

Microalgae protein digestibility. The FDA defines bioavailability as the rate and extent to which the active ingredient is absorbed and becomes accessible at the site of action. It encompasses the entire process after consuming a food element, including its digestibility and solubility in the gastrointestinal tract, absorption into the circulatory system through intestinal epithelial cells, and eventual incorporation into the target site of utilization (Fig. 1). The bioavailability of food

nutrients is considered crucial for maintaining optimal health. It also plays a crucial role in developing functional foods and health claims based on food components, as understanding the circulating metabolites and mechanisms of action is essential.

Bioavailability is further divided into two stages: Bioaccessibility and bioactivity. Bioaccessibility refers to the proportion of a component in a meal that is released from the food matrix during digestion and becomes available for intestinal absorption or undergoes biotransformation by gut bacteria (64). On the other hand, bioactivity involves the assimilation of a food element across intestinal cells, transport to the target site, interaction with the target site, any necessary biotransformation, and the resulting physiological response (65). The activity of ingested substances or their metabolites in metabolic pathways leads to biological effects on the body.

Several factors influence digestibility, rendering *in vitro* research challenging. These factors include the composition of macronutrients, enzyme specificity, anti-nutritional substances, fiber content and variations in absorptive capabilities along the gastrointestinal tract. Additionally, unraveling the bioavailability of food constituents is more complex than pharmaceutical drugs due to the diverse nature of food compounds, the various factors affecting their transition during digestion,

and the different absorption mechanisms for water-soluble and lipid-soluble molecules (66). *In vitro* research can be an initial screening technique to identify potential food matrices, growing conditions, and processing methods.

In addition to understanding the definition of the two stages of bioavailability, it is crucial to define digestibility to comprehend the metabolism dynamics of different dietary matrices. Digestibility refers to the amount of nutrients an individual absorbs and is typically calculated as the difference between the nutrients ingested and the amount retained in feces.

Assessing the potential of novel food sources involves determining their biochemical composition. *In vitro* and *in vivo* studies on digestibility, bioaccessibility and bioavailability are crucial in gaining comprehensive knowledge about nutrient interactions with food components. Such studies also investigate the effects of pH and enzymes on absorbability, providing insight into the potential nutrients that can be absorbed (Fig. 1) (67). Therefore, understanding the digestibility and bioaccessibility of algal compounds is essential to maximize the potential of algal food products and co-products.

Researching algal digestibility is particularly critical, considering the complexity of the polysaccharide cell wall. The cell wall composition of some algae, including microalgae, typically consists of cellulose, hemicellulose, pectin compounds and glycoproteins (68). These robust wall structures can restrict the accessibility of digestive enzymes to intracellular algae compounds, limiting nutrient and algae component bioaccessibility or bioavailability. Extraction or pre-treatment procedures, such as enzymatic hydrolysis or chemical, mechanical and physical approaches, can promote cell disruption, enhancing algal nutrient digestibility and bioaccessibility (69). Approaches can encourage disruption cells, thereby enhancing algal nutrient digestibility and bioaccessibility.

There are limited studies available in the literature on microalgae digestibility, including investigations on *in vitro* bioaccessibility, primarily due to the aforementioned factors. However, certain microalgae species exhibit distinct characteristics regarding digestibility. For instance, *Arthrospira platensis* has a fragile cell wall, indicating easily digestible biomass. On the other hand, Chlorophyta species such as *Chlorella vulgaris* and *Haematococcus lacustris* are tiny microorganisms (5-10 μm) with rigid cell walls that require prior disruption to enhance the digestibility and bioaccessibility of microalgal compounds (70).

A recent study conducted by Gómez-Jacinto *et al* (71) focused on the *in vitro* selenium bioaccessibility, *in vivo* bioavailability and bioactivity of the Se-enriched microalga *C. sorokiniana* for potential use as a functional food. The *in vitro* gastrointestinal digestion of the selenized microalga demonstrated 81% bioaccessibility. *In vivo* experiments on mice treated with Se-enriched *C. sorokiniana* revealed significant selenium concentrations in the kidney, indicating a potential excretion pathway through urine (71). This suggests that Se-enriched *C. sorokiniana* can serve as a nutraceutical ingredient and a dietary selenium supplement for humans.

When comparing the digestibility of traditional protein sources, milk and eggs have the highest true digestibility values,

Table VII. Mineral composition of *Chlorella sorokiniana* dry biomass.

Metals	Content (mg/100 g dry weight)
Iron (Fe)	18.00 \pm 2.00
Copper (Cu)	3.00 \pm 0.07
Zinc (Zn)	20.20 \pm 2.02
Manganese (Mn)	8.00 \pm 0.15
Cobalt (Co)	1.00 \pm 0.01
Magnesium (Mg)	91.00 \pm 5.00
Calcium (Ca)	31.00 \pm 2.50

The information presented in this table is derived from previous studies (23,81).

~97% (72). They are followed by meats, fish and poultry (73). By contrast, microalgae species such as *Scenedesmus obliquus*, *Arthrospira platensis* (Cyanobacteria), *Spirulina sp.* and *Chlorella sp.* exhibit apparent DC values of 88.0, 86, 77.6 and 76.6%, respectively (14,68).

6. Micronutrients in *C. sorokiniana* biomass

Chlorella is an excellent source of vitamins and minerals that adults and children can easily consume to meet their daily vitamin requirements (12). As demonstrated in Table VII, *C. sorokiniana* microalgae biomass contains the balanced mineral content required in humans. In acceptable amounts, iron, copper and zinc are trace elements necessary for the human body to function normally (74). It is important to note that *C. sorokiniana* biomass contains substantial amounts of iron (18 mg/100 g dry weight) and zinc (20.2 mg/100 g dry weight), of which adequate intake prevents anemia (75) and muscular weakness (76), respectively. Iron is required for respiration, energy production, DNA synthesis and cell proliferation (77). In previous a study conducted by Nakano *et al* (78), oral *Chlorella* supplementation (6 g/day) for 12-18 weeks lowered markers of anemia in a cohort of 32 women in their second and third trimesters of pregnancy compared to the control group. This suggests that *Chlorella* supplementation considerably reduces the risk of developing pregnancy-associated anemia (79).

Moreover, some of the vitamins found in notable quantities in *Chlorella* are vitamin A (in the form of β -carotene), vitamin C, vitamin E, Vitamins K and vitamin B, such as thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), folic acid (B9) and cobalamin (B12) (12,44). These vitamins fuel the body, detoxify and restore digestive function, activate the immune system and renew cells (44). Moreover, the vitamin E content of green microalgae *Chlorella* contributes to its high antioxidant activity (80,81).

7. Bioactive compounds in *C. sorokiniana* biomass

Bioactive compounds are nutrients and non-nutrients found in food (both plant and animal sources) that have physiological effects in addition to their nutritional qualities (82). Foods high

Table VIII. Indicators of the biological value of *Chlorella sorokiniana* dry biomass.

Phyto-chemicals	Content (mg/g dry weight)
Chlorophyll	22.13±2.20
Carotenoids	6.04±0.60
Total phenolic compounds (GAE)	12.27±2.31
Total flavonoid content (QE)	15.05±3.83

The information presented in this table is derived from previous studies (23,87). GAE, gallic acid equivalent; QE, quercetin equivalent.

in bioactive chemicals that are routinely consumed positively impact human health by performing antioxidant activity, reducing the risk of developing diseases such as heart disease, diabetes, cancer, cataracts and stroke (83,84). Microalgae are highly diverse and contain significant bioactive compounds, including polyphenols, carotenoids and organic acids, as presented in Table VIII.

Pigments are essential for the metabolism of photosynthetic microalgae and have antioxidant, anti-carcinogenic, anti-inflammatory, anti-obesity, anti-angiogenic and neuro-protective properties in different metabolisms, including human metabolism (44). *Chlorella* contains substantial levels of chlorophyll, similar to higher plants, although in markedly higher concentrations than terrestrial plants, with the potential to collect chlorophyll to a concentration of 2.5% (86). Chlorophyll is the principal natural green pigment found in microalgae and plants, where it is needed for oxygenic photosynthesis (87). As presented in Table VIII, *C. sorokiniana* biomass contains a notable amount of biologically active phytochemicals, with chlorophylls (22.13 mg/g dry biomass) dominating (23). Azaman *et al* (88) also discovered that under photoautotrophic conditions, *C. sorokiniana* exhibited a total chlorophyll concentration of 24.37 µg/mg dry weight of the sample, which was more significant than that of *Chromochloris zofingiensis*.

Carotenoid is a pigment in algae, plants, fungi and bacteria that ranges in hue from yellow to red (84). It has health-promoting qualities are mostly linked to its antioxidant activity that scavenges singlet molecular oxygen and radicals (84). Previous research has shown that carotenoids significantly contribute to the total antioxidant capacity of microalgae (89,90). In particular, Diprat *et al* (91) identified the carotenoid types present in *C. sorokiniana* microalgae powder, which includes violaxanthin, lutein, zeaxanthin, β-carotene and α-carotene. Using high-performance liquid chromatography coupled to diode array and mass spectrometry detectors, Fernandes *et al* (92) also identified 11 carotenoids in *C. sorokiniana*, with the greatest significant quantities of all-*trans*-lutein (831.18±1.18 g/g dry weight), all-*trans*-β-carotene (156.21±0.22 g/g dry weight) and all-*trans*-α-carotene (71.47±0.10 g/g dry weight). Indeed, including *C. sorokiniana* biomass as a raw material in functional foods will allow goods to be enriched with essential nutritional components while obtaining the appropriate color range without synthetic dyes (23).

Phenolic compounds, or polyphenolics, are abundant secondary metabolites with antioxidant activity and numerous biological functions that can be derived from microalgae (93). Due to their importance as one of the most significant classes of natural antioxidants, phenolic compounds are gaining popularity among consumers and food manufacturers. They function as reducing agents and hydrogen donors, capable of scavenging free radicals (94). Phenolic compounds have two basic chemical structures formed by one or more aromatic rings with hydroxyl (-OH) groups (93). They are chemically classified into several classes, including phenolic acids (hydroxybenzoic acids, hydroxycinnamic acids), flavonoids (flavones, flavonols, flavanones, flavanonols, flavonols and anthocyanins), isoflavonoids (isoflavones and coumestans), stilbenes, lignans and phenolic polymers (proanthocyanidin) (95).

Based on the Folin-Ciocalteu method, Olasehinde *et al* (85) reported that *C. sorokiniana* dry biomass contains a total phenolic content of 12.27±2.31 gallic acid equivalent (GAE) mg/g, which is higher compared to *C. minutissima* (10.53±2.82 GAE mg/g dry weight) (Table VIII). Likewise, the same authors revealed that *C. sorokiniana* dry biomass contains a total flavonoid content of 15.05±3.83 quercetin equivalent (QE) mg/g dry weight (85). Furthermore, Safafar *et al* (96) also presented that *C. sorokiniana* dry biomass contains 5.84±0.04 GAE mg/g total phenolics and 2.45±0.04 QE mg/g total flavonoid content. Furthermore, they found that *C. sorokiniana*, grown in different light intensities, had the same phenolic acid profile (96). Still, the total detected phenolic acids in the sample grown in average light intensity was somewhat higher. Thus, generating phenolics and other antioxidant chemicals in microalgae depends on growth circumstances and challenges such as oxidative stress (96).

Apart from having antioxidant properties, phenolic compounds have therapeutic qualities such as anti-tumor and antibacterial activities (84). *Chlorella* phenolic chemicals may also prevent liver cell carcinogenesis by blocking lipid peroxidation on the cell membrane, neutralizing cellular free radicals and preventing DNA damage (84).

8. *C. sorokiniana* biomass-based food products

Given the abundance of nutrients and bioactive substances found in *C. sorokiniana*, it is no surprise that it can be regarded as one of the most promising new food and functional goods sources. *C. sorokiniana* has a well-balanced chemical composition and provides a source of beneficial bioactive chemicals (13). As a result of its potential, *C. sorokiniana* can represent an alternative and novel source of natural components that can be used as functional ingredients to improve the nutritional content of foods.

Chlorella sp. has been extensively utilized as a dietary supplement for an extended period of time. The microalgal market offers *Chlorella* sp. in powder and tablet forms, primarily due to its ability to effectively sequester heavy metals, such as mercury, recognized as toxic substances, and facilitate their elimination from the body (84). In recent times, there has been a surge in research efforts focused on advancing food products derived from *C. sorokiniana* biomass. Pasta (23) and gluten-free bread (91) have been incorporated with *C. sorokiniana* biomass for baked food products.

Bazarnova *et al* (23) examined the effects of incorporating the dry biomass of microalgae *C. sorokiniana* as a replacement for flour mixture to enrich pasta effectively. In their study, it was shown that flour replacement with *C. sorokiniana* should not be >5% due to the formation of a distinct fish flavor of the product. Moreover, substituting 5% *C. sorokiniana* biomass to pasta flour increased proteins to 15.7±0.50% and lipids to 4.1±0.06%. Furthermore, the addition of *C. sorokiniana* dry biomass to pasta has helped improve the polyunsaturated fatty acids, chlorophyll and carotenoid content of the product (23).

Diprat *et al* (91) also examined the partial replacement of pea flour with *C. sorokiniana* powder at 2.5 and 5% to increase the nutritional quality of gluten-free bread. The replacement of 5% *C. sorokiniana* powder vs. the control with pea flour increased the protein content (67 to 85 mg/g), lutein content (1.6 to 57.5 µg/g) and omega-3 content in the fatty acids (5.0-6.1%) of the gluten-free bread. Furthermore, it is interesting to note that compared with the pasta food product studied by Bazarnova *et al* (23), the sensory analysis of the gluten-free bread with 5% replacement of *C. sorokiniana* powder had an acceptance rate >70%, with no observed distinct fishy flavor being identified. Furthermore, the addition of *C. sorokiniana* powder had no effect on the texture and specific volume of the bread product.

The expanding number of studies on *Chlorella* as a food ingredient have revealed that *Chlorella sp.* has a significant potential to be an alternative protein source and future food. However, further product application of *C. sorokiniana* biomass in food needs to be conducted in order to assess its effects on the physicochemical, sensory, techno-functional and antioxidant properties as a food ingredient. Consumers also strongly demand food to prevent illness, improve mental health and increase life quality, as well as a desire to consume alternative proteins to be healthy and more sustainable. As a result, the worldwide market for *Chlorella* ingredients is predicted to expand and meet human nutritional requirements (84).

9. Conclusions and future perspectives

C. sorokiniana microalgae has shown promise as an alternative protein ingredient to meet the increasing demand for sustainable food products. There is a growing consumer interest and awareness regarding alternative protein sources, rendering the use of microalgal protein in developing healthier food products highly relevant. The high protein content, balanced amino acid profiles and bioactive substances in *C. sorokiniana* make it a potential dietary source with various health benefits. As numerous high-value microalgae products are intended for human consumption or other applications, they are subject to food laws and regulatory standards set by organizations such as the FDA. Collaborative efforts from researchers across disciplines are necessary to expand the market for microalgal food products further. These efforts should focus on identifying novel microalgae species and their unique characteristics, developing more cost-effective cultivation and biorefinery systems, improving the sensory properties of microalgal food products and assessing the availability of algal compounds.

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JLZF and MSA performed the literature review. JLZF and MSA wrote the manuscript. Furthermore, both authors have thoroughly reviewed, and have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

Use of artificial intelligence tools

During the preparation of this work, AI tools were used to improve the readability and language of the manuscript or to generate images, and subsequently, the authors revised and edited the content produced by the AI tools as necessary, taking full responsibility for the ultimate content of the present manuscript.

References

1. Hertzler SR, Lieblein-Boff JC, Weiler M and Allgeier C: Plant proteins: Assessing their nutritional quality and effects on health and physical function. *Nutrients* 12: 3704, 2020.
2. Sharif M, Zafar MH, Aqib AI, Saeed M, Farag MR and Alagawany M: Single cell protein: Sources, mechanism of production, nutritional value and its uses in aquaculture nutrition. *Aquac* 531: 735885, 2021.
3. Bleakley S and Hayes M: Algal proteins: Extraction, application, and challenges concerning production. *Foods* 6: 33, 2017.
4. Bahi A, Ramos-Vega A, Angulo C, Monreal-Escalante E and Guardiola FA: Microalgae with immunomodulatory effects on fish. *Rev Aquac* 15: 1522-1539, 2023.
5. Schenk PM: Phycology: Algae for food, feed, fuel and the planet. *Phycol* 1: 76-78, 2021.
6. Martins CF, Ribeiro DM, Costa M, Coelho D, Alfaia CM, Lordelo M, Almeida AM, Freire JPB and Prates JAM: Using microalgae as a sustainable feed resource to enhance quality and nutritional value of pork and poultry meat. *Foods* 10: 2933, 2021.

7. Madeira MS, Cardoso C, Lopes PA, Coelho D, Afonso C, Bandarra NM and Prates JAM: Microalgae as feed ingredients for livestock production and meat quality: A review. *Livest Sci* 205: 111-121, 2017.
8. Sathasivam R, Radhakrishnan R, Hashem A and Abd Allah EF: Microalgae metabolites: A rich source for food and medicine. *Saudi J Biol Sci* 26: 709-722, 2019.
9. Beyerinck MW: Cultivation experiments with *Zoochlorella*, *Lichengonidia*, and other lower algae. *Bot Ztg* 47: 725-739, 741-754, 757-768, 781-785, 1890. <http://img.algaebase.org/pdf/AC100CF003338161AAoHt43C4207/35049.pdf>.
10. Wu HL, Hseu RS and Lin LP: Identification of *Chlorella* spp. isolates using ribosomal DNA sequences. *Bot Bull Acad Sin* 42: 115-121, 2001.
11. Ortiz Montoya EY, Casazza AA, Aliakbarian B, Perego P, Converti A and de Carvalho JC: Production of *Chlorella vulgaris* as a source of essential fatty acids in a tubular photobioreactor continuously fed with air enriched with CO₂ at different concentrations. *Biotechnol Prog* 30: 916-922, 2014.
12. Rani K, Sandal N and Sahoo PK: A comprehensive review on chlorella-its composition, health benefits, market and regulatory scenario. *Pharma Innovation* 7: 584-589, 2018.
13. Matos J, Cardoso C, Bandarra NM and Afonso C: Microalgae as healthy ingredients for functional food: A review. *Food Funct* 8: 2672-2685, 2017.
14. Becker EW: Microalgae as a source of protein. *Biotechnol Adv* 25: 207-210, 2007.
15. Ferdous UT, Nurdin A, Ismail S and Balia Yusof ZN: Evaluation of the antioxidant and cytotoxic activities of crude extracts from marine *Chlorella* sp. *Biocatal Agric Biotechnol* 47: 102551, 2023.
16. van Eck NJ and Waltman L: Software survey: VOSviewer, a computer program for bibliometric mapping. *Scientometrics* 84: 523-538, 2010.
17. Kunz WF: Response of the alga *Chlorella sorokiniana* to 60 Co gamma radiation. *Nature* 236: 178-179, 1972.
18. Ramanna L, Guldhe A, Rawat I and Bux F: The optimization of biomass and lipid yields of *Chlorella sorokiniana* when using wastewater supplemented with different nitrogen sources. *Bioresour Technol* 168: 127-135, 2014.
19. de-Bashan LE, Trejo A, Huss VA, Hernandez JP and Bashan Y: *Chlorella sorokiniana* UTEX 2805, a heat and intense, sunlight-tolerant microalga with potential for removing ammonium from wastewater. *Bioresour Technol* 99: 4980-4989, 2008.
20. Janssen M, Kuijpers TC, Veldhoen B, Ternbach MB, Tramper J, Mur LR and Wijffels RH: Specific growth rate of *Chlamydomonas reinhardtii* and *Chlorella sorokiniana* under medium duration light/dark cycles: 13-87 s. *J Biotechnol* 70: 323-333, 1999.
21. León-Vaz A, León R, Díaz-Santos E, Vígara J and Raposo S: Using agro-industrial wastes for mixotrophic growth and lipids production by the green microalga *Chlorella sorokiniana*. *N Biotechnol* 51: 31-38, 2019.
22. Wan MX, Wang RM, Xia JL, Rosenberg JN, Nie NY, Kobayashi N, Oyler GA and Betenbaugh MJ: Physiological evaluation of a new *Chlorella sorokiniana* isolate for its biomass production and lipid accumulation in photoautotrophic and heterotrophic cultures. *Biotechnol Bioeng* 109: 1958-1964, 2012.
23. Bazarnova J, Nilova L, Trukhina E, Bernavskaya M, Smyatskaya Y and Aktar T: Use of microalgae biomass for fortification of food products from grain. *Foods* 10: 3018, 2021.
24. Koyande AK, Chew KW, Rambabu K, Tao Y, Chu D and Show P: Microalgae: A potential alternative to health supplementation for humans. *Food Sci Hum Wellness* 8: 16-24, 2019.
25. Gouveia L and Oliveira AC: Microalgae as a raw material for biofuels production. *J Ind Microbiol Biotechnol* 36: 269-274, 2009.
26. Lizzul AM, Lekuona-Amundarain A, Purton S and Campos LC: Characterization of *Chlorella sorokiniana*, UTEX 1230. *Biology (Basel)* 7: 25, 2018.
27. Andreeva A, Budenkova E, Babich O, Sukhikh S, Dolganyuk V, Michaud P and Ivanova S: Influence of carbohydrate additives on the growth rate of microalgae biomass with an increased carbohydrate content. *Mar Drugs* 19: 381, 2021.
28. Panahi Y, Yari Khosroushahi A, Sahebkar A and Heidari HR: Impact of cultivation condition and media content on *chlorella vulgaris* composition. *Adv Pharm Bull* 9: 182-194, 2019.
29. Petkov G and Garcia G: Which are fatty acids of the green alga *chlorella*? *Biochem Syst Ecol* 35: 281-285, 2007.
30. Otles S (Ed.): *Methods of analysis of food components and additives*. CRC Press, 2011.
31. Illman AM, Scragg AH and Shales SW: Increase in *Chlorella* strains calorific values when grown in low nitrogen medium. *Enzyme Microb Technol* 27: 631-635, 2000.
32. Smyatskaya Y, Toumi A, Atamaniuk I, Vladimirov I, Donaev FK and Akhmetova IG: Influence of the drying method on the sorption properties the biomass of *chlorella sorokiniana* microalgae. *E3S Web Conf* 124: 01051, 2019.
33. Christaki E, Florou-Paneri P and Bonos E: Microalgae: A novel ingredient in nutrition. *Int J Food Sci Nutr* 62: 794-799, 2011.
34. Hamed I, Özogul F, Özogul Y and Regenstein JM: Marine bioactive compounds and their health benefits: A review. *Compr Rev Food Sci Food Saf* 14: 446-465, 2015.
35. Bao DQ, Mori TA, Burke V, Puddey IB and Beilin LJ: Effects of dietary fish and weight reduction on ambulatory blood pressure in overweight hypertensives. *Hypertension* 32: 710-717, 1998.
36. Amaro HM, Barros R, Guedes AC, Sousa-Pinto I and Malcata FX: Microalgal compounds modulate carcinogenesis in the gastrointestinal tract. *Trends Biotechnol* 31: 92-98, 2013.
37. Kagan ML, Levy A and Leikin-Frenkel A: Comparative study of tissue deposition of omega-3 fatty acids from polar-lipid rich oil of the microalgae *Nannochloropsis oculata* with krill oil in rats. *Food Funct* 6: 186-192, 2015.
38. Gouveia L, Batista AP, Raymundo A and Bandarra N, Blades M and Blades M: *Spirulina maxima* and *Diacronema vlkianum* microalgae in vegetable gelled desserts. *Nutr Food Sci* 38: 492-501, 2008.
39. Mata TM, Martins AA and Caetano NS: Microalgae for biodiesel production and other applications: A review. *Renew Sustain Energy Rev* 14: 217-232, 2010.
40. Gouveia L, Marques AE, Sousa JM, Moura P and Bandarra NM: Microalgae - source of natural bioactive molecules as functional ingredients. *Food Sci Technol Bull Funct Foods* 7: 21-37, 2010.
41. Priyadarshani I and Rath B: Commercial and industrial applications of micro algae-A review. *J Algal Biomass Utln* 3: 89-100, 2012.
42. Lizzul AM, Lekuona-Amundarain A, Purton S and Campos LC: Characterization of *chlorella sorokiniana*, UTEX 1230. *Biology (Basel)* 7: 25, 2018.
43. Ishiguro S, Robben N, Burghart R, Cote P, Greenway S, Thakkar R, Upreti D, Nakashima A, Suzuki K, Comer J and Tamura M: Cell wall membrane fraction of *Chlorella sorokiniana* enhances host anti-tumor immunity and inhibits colon carcinoma growth in mice. *Integr Cancer Ther* 19: 153473541990055, 2020.
44. Andrade LM, Andrade CJ, Das M, Nascimento CAO and Mendes MA: *Chlorella* and *Spirulina* microalgae as sources of functional foods, nutraceuticals, and food supplements; an overview. *MOJ Food Process Technol* 6: 00144, 2018.
45. Spolaore P, Joannis-Cassan C, Duran E and Isambert A: Commercial applications of microalgae. *J Biosci Bioeng* 101: 87-96, 2006.
46. Pugh N, Ross SA, ElSohly HN, ElSohly MA and Pasco DS: Isolation of three high molecular weight polysaccharide preparations with potent immunostimulatory activity from *Spirulina platensis*, *Aphanizomenon flos-aquae*, and *Chlorella pyrenoidosa*. *Planta Med* 67: 737-742, 2001.
47. Sheng J, Yu F, Xin Z, Zhao L, Zhu X and Hu Q: Preparation, identification and their anti-tumor activities in vitro of polysaccharides from *Chlorella pyrenoidosa*. *Food Chem* 105: 533-539, 2007.
48. Matos ÂP: Microalgae as a potential source of proteins. In: *Proteins: Sustainable Source, Processing and Applications*. Academic Press, Brookline, MA, pp63-96, 2019.
49. López CV, García Mdel C, Fernández FG, Bustos CS, Chisti Y and Sevilla JM: Protein measurements of microalgal and cyanobacterial biomass. *Bioresour Technol* 101: 7587-7591, 2010.
50. Lourenço SO, Barbarino E, Lavín PL, Lanfer Marquez UM and Aida E: Distribution of intracellular nitrogen in marine microalgae: calculation of new nitrogen-to-protein conversion factors. *Eur J Phycol* 39: 17-32, 2004.
51. Becker EW: Microalgae for human and animal nutrition. In: *Handbook of Microalgal Culture: Applied Phycology and Biotechnology*. Richmond A (ed). Blackwell Science, Oxford, pp461-503, 2013.
52. Fu Y, Chen T, Chen SHY, Liu B, Sun P, Sun H and Chen F: The potentials and challenges of using microalgae as an ingredient to produce meat analogues. *Trends Food Sci Technol* 112: 188-200, 2021.
53. Boisen S and Eggum BO: Critical evaluation of in vitro methods for estimating digestibility in simple-stomach animals. *Nutr Res Rev* 4: 141-162, 1991.
54. Young VR and Pellett PL: Plant proteins in relation to human protein and amino acid nutrition. *Am J Clin Nutr* 59 (Suppl 5): 1203S-1212S, 1994.

55. Friedman M and Brandon DL: Nutritional and health benefits of soy proteins. *J Agric Food Chem* 49: 1069-1086, 2001.
56. Hoffman JR and Falvo MJ: Protein-which is best?. *J Sports Sci Med* 3: 118-130, 2004.
57. Berrazaga I, Micard V, Gueugneau M and Walrand S: The role of the anabolic properties of plant-versus animal-based protein sources in supporting muscle mass maintenance: A critical review. *Nutrients* 11: 1825, 2019.
58. Nguyen TTP, Bhandari B, Cichero J and Prakash S: Gastrointestinal digestion of dairy and soy proteins in infant formulas: An in vitro study. *Food Res Int* 76: 348-358, 2015.
59. Duodu KG, Taylor JRN, Belton PS and Hamaker BR: Factors affecting sorghum protein digestibility. *J Cereal Sci* 38: 117-131, 2003.
60. Food and Agriculture Organization (FAO): Dietary protein quality evaluation in human nutrition: report of an FAO Expert Consultation. Food and nutrition paper 92. FAO: Rome, Italy, 2013.
61. Fleurence J: Seaweed proteins: Biochemical, nutritional aspects and potential uses. *Trends Food Sci Technol* 10: 25-28, 1999.
62. Chronakis IS and Madsen M: Algal proteins. In *Handbook of food proteins*. Woodhead Publishing 353-394, 2011.
63. Waghmare AG, Salve MK, LeBlanc JG and Arya SS: Concentration and characterization of microalgae proteins from *Chlorella pyrenoidosa*. *Bioresour Bioprocess* 3: 16, 2016.
64. Rodrigues DB, Marques MC, Hacke A, Loubet Filho PS, Cazarin CBB and Mariutti LRB: Trust your gut: Bioavailability and bioaccessibility of dietary compounds. *Curr Res Food Sci* 5: 228-233, 2022.
65. Alegria A, Garcia-Llatas G and Cilla A: Static digestion models: General introduction. In: *The Impact of Food Bioactives on Health: In vitro and ex vivo models*. Verhoeckx K, Cotter P and López-Expósito I (eds). Springer, New York, NY, pp3-12, 2015.
66. Rein MJ, Renouf M, Cruz-Hernandez C, Actis-Goretta L, Thakkar SK and da Silva Pinto M: Bioavailability of bioactive food compounds: A challenging journey to bioefficacy. *Br J Clin Pharmacol* 75: 588-602, 2013.
67. Dima C, Assadpour E, Dima S and Jafari SM: Bioavailability and bioaccessibility of food bioactive compounds; overview and assessment by in vitro methods. *Compr Rev Food Sci Food Saf* 19: 2862-2884, 2020.
68. Niccolai A, Zittelli GC, Rodolfi L, Biondi N and Tredici MR: Microalgae of interest as food source: Biochemical composition and digestibility. *Algal Res* 42: 101617, 2019.
69. Maehre HK, Edvinsen GK, Eilertsen KE and Elvevoll EO: Heat treatment increases the protein bioaccessibility in the red seaweed dulse (*Palmaria palmata*), but not in the brown seaweed winged kelp (*Alaria esculenta*). *J Appl Phycol* 28, 581-590, 2016.
70. Bernaerts TMM, Verstrecken H, Dejonghe C, Gheysen L, Foubert I, Grauwet T and Van Loey AM: Cell disruption of *Nannochloropsis* sp. improves in vitro bioaccessibility of carotenoids and ω 3-LC-PUFA. *J Funct Foods* 65: 103770, 2020.
71. Gómez-Jacinto V, Navarro-Roldán F, Garbayo-Nores I, Vílchez-Lobato C, Borrego AA and García-Barrera T: In vitro selenium bioaccessibility combined with in vivo bioavailability and bioactivity in Se-enriched microalga (*Chlorella sorokiniana*) to be used as functional food. *J Funct Foods* 66: 103817, 2020.
72. Demarco M, Oliveira de Moraes J, Matos ÂP, Derner RB, de Farias Neves F and Tribuzi G: Digestibility, bioaccessibility and bioactivity of compounds from algae. *Trends Food Sci Technol* 121: 114-128, 2022.
73. Moughan P: Digestion and absorption of proteins and peptides. *Designing Funct Foods* 148-170, 2008.
74. World Health Organization: Trace Elements in Human Nutrition and Health. 1996. Available from: <https://www.who.int/publications/i/item/9241561734>.
75. Camaschella C: Iron-deficiency anemia. *N Engl J Med* 372: 1832-1843, 2025.
76. Ozturk H, Niazi P, Mansoor M, Monib AW, Alikhail M and Azizi A: The function of zinc in animal, plant, and human nutrition. *J Res Appl Sci Biotechnol* 2: 35-43, 2023.
77. Hentze MW, Muckenthaler MU, Galy B and Camaschella C: Two to tango: Regulation of mammalian iron metabolism. *Cell* 142: 24-38, 2010.
78. Nakano S, Takekoshi H and Nakano M: *Chlorella pyrenoidosa* supplementation reduces the risk of anemia, proteinuria and edema in pregnant women. *Plant Foods Hum Nutri* 65: 25-30, 2010.
79. Bito T, Okumura E, Fujishima M and Watanabe F: Potential of *Chlorella* as a dietary supplement to promote human health. *Nutrients* 12: 2524, 2020.
80. Batista AP, Niccolai A, Fradinho P, Fragoso S, Bursic I, Rodolfi L, Biondi N, Tredici MR, Sousa I and Raymundo A: Microalgae biomass as an alternative ingredient in cookies: Sensory, physical and chemical properties, antioxidant activity and in vitro digestibility. *Algal Res* 26: 161-171, 2017.
81. Chini Zittelli G, Rodolfi L, Biondi N and Tredici MR: Productivity and photosynthetic efficiency of outdoor cultures of *Tetraselmis suecica* in annular columns. *Aquac* 261: 932-943, 2006.
82. Cazarin CBB, Bicas JL, Pastore GM and Marostica Junior MR: Bioactive Food Components Activity in Mechanistic Approach. Elsevier, Amsterdam, pp1-3, 2022.
83. Carbonell-Capella JM, Buniowska M, Barba FJ, Esteve MJ and Frígola A: Analytical methods for determining bioavailability and bioaccessibility of bioactive compounds from fruits and vegetables: A review. *Compr Rev Food Sci Food Saf* 13: 155-171, 2014.
84. Widyaningrum D and Prianto AD: *Chlorella* as a source of functional food ingredients: Short review. *IOP Conf Ser Earth Environ Sci* 794: 012148, 2021.
85. Olasehinde TA, Odjadjare EC, Mabinya LV, Olaniran AO and Okoh AI: *Chlorella sorokiniana* and *Chlorella minutissima* exhibit antioxidant potentials, inhibit cholinesterases and modulate disaggregation of β -amyloid fibrils. *Electron J Biotechnol* 40: 1-9, 2019.
86. Beale SI and Appleman D: Chlorophyll synthesis in *Chlorella*: Regulation by degree of light limitation of growth. *Plant Physiol* 47: 230-235, 1971.
87. da Silva JC and Lombardi AT: Chlorophylls in microalgae: occurrence, distribution, and biosynthesis. In: *Pigments from Microalgae Handbook*. Springer International Publishing, Switzerland, pp1-18, 2020.
88. Azaman SNA, Nagao N, Yusoff FM, Tan SW and Yeap SK: A comparison of the morphological and biochemical characteristics of *Chlorella sorokiniana* and *Chlorella zofingiensis* cultured under photoautotrophic and mixotrophic conditions. *PeerJ* 5: e3473, 2017.
89. Goiris K, Muylaert K, Fraeye I, Foubert I, De Brabanter J and De Cooman L: Antioxidant potential of microalgae in relation to their phenolic and carotenoid content. *J Appl Phycol* 24: 1477-1486, 2012.
90. Takaichi S: Carotenoids in algae: Distributions, biosyntheses and functions. *Mar Drugs* 9: 1101-1118, 2011.
91. Diprat AB, Silveira Thys RC, Rodrigues E and Rech R: *Chlorella sorokiniana*: A new alternative source of carotenoids and proteins for gluten-free bread. *LWT* 134: 109974, 2020.
92. Fernandes AS, Petry FC, Mercadante AZ, Jacob-Lopes E and Zepka LQ: HPLC-PDA-MS/MS as a strategy to characterize and quantify natural pigments from microalgae. *Curr Res Food Sci* 3: 100-112, 2020.
93. Machu L, Misurcova L, Ambrozova JV, Orsavova J, Mlcek J, Sochor J and Jurikova T: Phenolic content and antioxidant capacity in algal food products. *Molecules* 20: 1118-1133, 2015.
94. Wojdylo A, Oszmianski J and Czemerys R: Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chem* 105: 940-949, 2007.
95. Manach C, Scalbert A, Morand C, Rémésy C and Jiménez L: Polyphenols: food sources and bioavailability. *Am J Clin Nutr* 79: 727-747, 2004.
96. Safafar H, van Wagenen J, Møller P and Jacobsen C: Carotenoids, phenolic compounds and tocopherols contribute to the antioxidative properties of some microalgae species grown on industrial wastewater. *Mar Drugs* 13: 7339-7356, 2015.

