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MICROALGAE AS ALTERNATIVE PROTEINS FOR THE SUSTAINABLE FOOD INDUSTRY: A REVIEW

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Abstract
This paper reviews the nutritional properties and consumer perceptions of microalgae foods through various recent studies on alternative protein sources. Food choices, including meat consumption, are a common concern for humanity. Thus, we focused on whether microalgae foods have a sufficient value as a protein source and what nutritional benefits they have. Based on existing papers, we conducted a systematic review using Web of Science, Google Scholar, and Scopus to comprehensively investigate and summarize the nutritional characteristics of microalgae, sustainable diets, and awareness of microalgae as an alternative protein source. Research has shown that microalgae have been consumed by humans as a protein source since ancient times, and contain enough protein to be used as an alternative protein source. They also have many other nutritional benefits, such as vitamins. We have found that consumers are increasingly interested in alternative protein sources, and the more they learn about microalgae, the more accepting they become. These results may suggest a need for further research to improve microalgae as an alternative protein source in the long run and develop them into a variety of foods.

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Introduction

Present-day meat consumption and manufacturing patterns are far from being sustainable and impose a heavy burden on the environment. Animal agriculture is one of the leading causes of greenhouse gas emissions and anthropogenic climate change. Enormous meat consumption also has adverse health effects. To compensate for these downsides of meat consumption, an increasing number of consumers are turning to meat alternatives [1]. A sustainable diet means reducing meat intake or using alternative protein sources [2]. Previous research on meat substitutes has primarily focused on consumer acceptance and preference of meat replacers [3]. De Boer et al. [3] found that a very limited percentage of non-vegetarian consumers reported using meat substitutes. However, acceptance and attractiveness of meat substitutes increase when they are similar to common meals with meat (for example, meals with minced meat) [4].

Compared to meat, plant-based meat alternatives are considered to be a healthier source of protein and offer a range of environmental, social and health advantages. They can play an essential role in cutting down meat consumption. However, previous studies lack information on what role plant-based meat alternatives may play. And it is not known how the characteristics of specific meat alternatives can influence consumers' ability to moderately replace meat in their diets. Carbon footprint, production methods, and brands play a secondary role in the choice of meat alternatives [5].

Compared to meat proteins, plant proteins are preferable because they have a lower environmental impact. Cereals (*e. g.*, barley, wheat) and legumes (*e. g.*, peas, soybeans, lupins, beans) are considered the most important sources of plant protein. However, there are also controversies associated with legumes, namely soybeans, and genetically modified organisms [6].

Another study involves consumer analysis of the acceptability of insects as a meat alternative. The researchers found that general acceptance was low due to food aversion, but high concern for the environment and the use of minced insects in ready-to-eat foods to reduce aversion can lead to increased consumer acceptance of insect-based meat alternatives [7].

The term microalgae refers to a wide range of photosynthetic organisms. The cell structure is eukaryotic in microalgae and prokaryotic in cyanobacteria. In the context of microorganisms such as microalgae and cyanobacteria, "eukaryotic" and "prokaryotic" refer to fundamental differences in their cellular structures. Eukaryotic microorganisms, such as microalgae, have cells with a well-defined nucleus enclosed within a membrane. This nucleus houses their genetic material (DNA) and is surrounded by various membrane-bound organelles, including mitochondria and chloroplasts. These organelles perform specialized functions within the cell. On the other hand, prokaryotic microorganisms, such as cyanobacteria, lack a distinct nucleus enclosed within a membrane. Instead, their genetic material floats freely in the cell's cytoplasm. They also lack membrane-bound organelles like mitochondria and chloroplasts. Prokaryotic cells are generally smaller and simpler in structure compared to eukaryotic cells. Thus, in summary, when discussing microalgae and cyanobacteria, the terms "eukaryotic" and "prokaryotic" refer to whether these microorganisms have cells with a defined nucleus and membrane-bound organelles (eukaryotic) or cells without a defined nucleus and such organelles (prokaryotic). Microalgae are capable of synthesizing carbohydrates, accumulating sugars, and storing other important organic substances and lipids through a process known as photosynthesis and other metabolic pathways. Regarding carbohydrate synthesis, microalgae are photosynthetic organisms, meaning they receive energy from sunlight to convert carbon dioxide and water into carbohydrates (primarily in the form of sugars such as glucose). This process occurs in the chloroplasts of microalgal cells, where chlorophyll and other pigments capture sunlight and convert it into chemical energy. The synthesized carbohydrates are used as an energy source for the microalgae. In the case of sugar accumulation, during photosynthesis, microalgae produce more sugars than they immediately need for energy. Microalgae can store these extra sugars as a reserve energy source in the form of starch or other polysaccharides. This stored energy can be mobilized when the microalgae need it, such as when light is scarce or nutrients are scarce. Microalgae also have the ability to accumulate lipids, including triglycerides and fatty acids, within their cells. This lipid accumulation can occur under certain growth conditions, such as nutrient limitation, and is often induced to enhance the production of biofuels or other valuable lipids. Lipids act as energy stores and can be harvested for a variety of uses. Finally, microalgae can synthesize and accumulate other organic materials, including proteins and pigments. Proteins are essential for the growth and maintenance of microalgal cells, and pigments such as chlorophyll, carotenoids, and phycobiliproteins are involved in photosynthesis and light harvesting [8]. The consumption of microalgae has a long history. Spanish chroniclers described local fishermen gathering blue-green masses of microalgae from the lake and preparing them into dry cakes known as "*tecuitlatl*". For centuries, Chadians have harvested spirulina (also known as "*dihé*") from Lake Kossorom on the northeastern edge of Lake Chad for daily consumption. *Nostoc* is traditionally used in South America, Mongolia, and China to make foods known as "*fa cai*" and "*lakeplum*". Another edible blue-green alga, *Aphanotheca sacrum* (formerly *Phylloderma sacrum),* is used in Japan for a special delicacy called "suizenji-nori" [9].

What makes microalgae perfect candidates for modern-day "nutraceuticals" or "functional foods" is their ability to synthesize products of useful value for human nutrition. Nutritional components of microalgae include long-chain fatty acids, vitamins, minerals, essential and non-essential amino acids, enzymes, and carotenoids [10]. As a food innovation, alternative protein foods based on microalgae are promising, because microalgae can be produced on uncultivated land and have high yields per square meter. This is eco-friendly food production. The high nutritional value and high protein content are also characteristics that make consumers regard microalgae as healthy food [1]. Against this background, this study aims to review the nutritional value and related criteria for a specific meat substitute called microalgae and analyze consumer preferences for it.

Objects and methods

Search strategy

For this review, we searched PubMed, Scopus, ResearchGate, and Google Scholar using the following search term chains: meat substitutes, alternative proteins, functions of microalgae, microalgae food standards, and microalgae food consumer preferences, following PRISMA flow guidelines. Figure 1 is a flowchart depicting the process of selecting studies for inclusion in this review.

Eligibility criteria

Articles included in this review had to meet the eligibility criteria for this review, which included selecting studies related to the function of microalgae, the nutritional properties of microalgae, and microalgae as an alternative protein.

Screening and data extraction

For inclusion criteria, we considered a variety of article types, including original articles, full-length articles, Internet articles, summary reports, and series. We did not impose restrictions on publication date or language. Exclusion criteria included inaccessible full texts, full texts not containing raw data, inappropriate topics, university theses and dissertations, and topics irrelevant to the main focus of the review.

Selecting studies and extracting data

We used a literature review approach. A total of 215 references were selected using the PRISMA flowchart from the leading journal search sites such as PubMed, Google Scholar, ResearchGate, and Scopus. As a result, a total of 51 articles from 2016 to 2023 were finally selected. The PRISMA flowchart is shown in Figure 1.

Results

Microalgae-based foods

Algae are unicellular or multicellular organisms that vary in size and shape, and are classified into microalgae (micro) and macroalgae/algae (macro) groups. Microalgae have the ability to self-organize into clusters and filaments. Colonies of microalgae are often formed by individual cells clustered together. These clusters can provide a number of benefits, including protection from predators, improved nutrient uptake, and better access to light. These clusters can vary in size from small groups to large visible colonies,

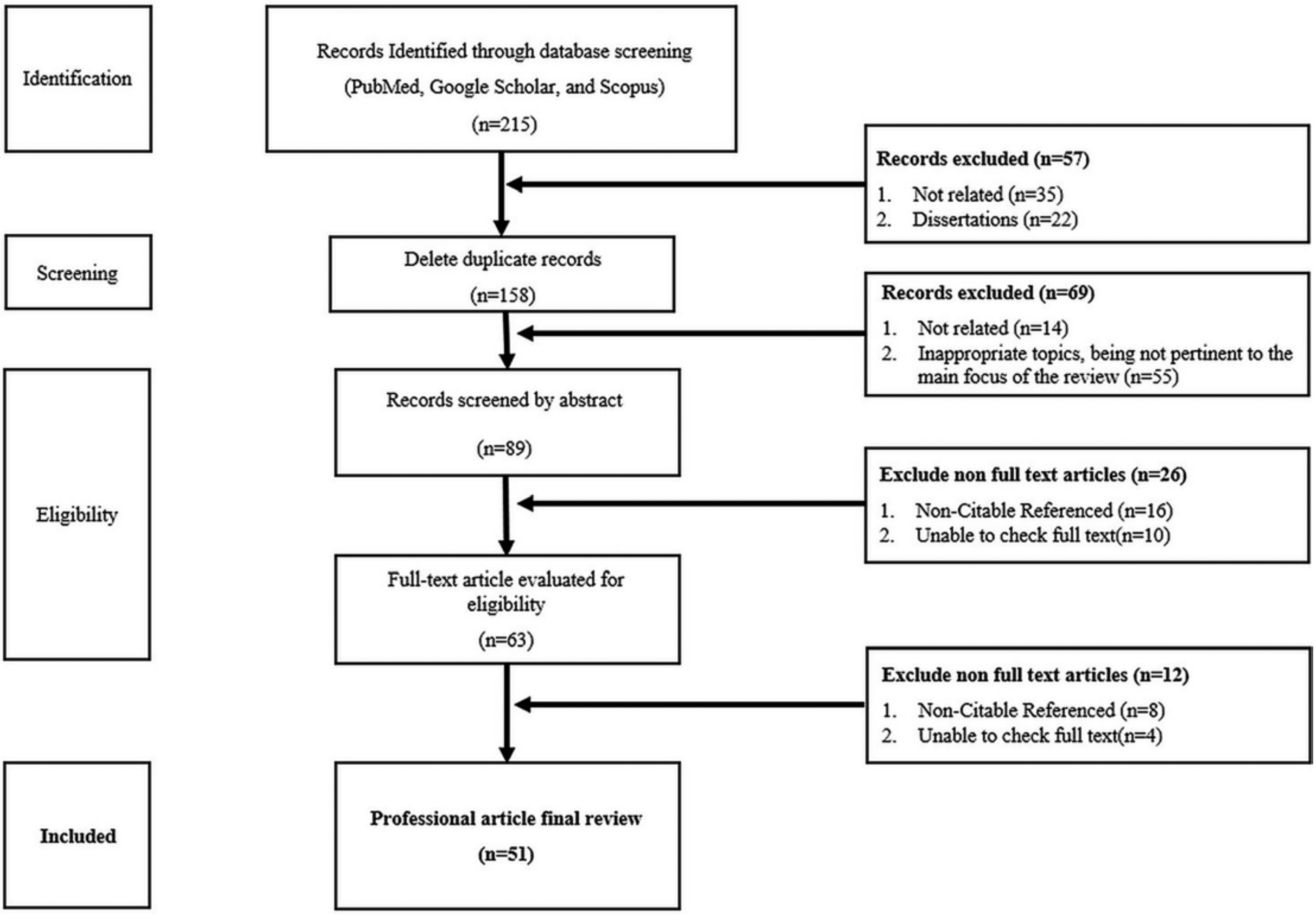

Figure 1. PRISMA flow chart for literature review search results

depending on the species and environmental conditions. Some microalgae can also be organized into filamentous structures. These filaments are chains of interconnected cells that can form elongated structures. Filaments can help microalgae effectively navigate their environment, utilize nutrients, and optimize their position for light absorption. Filamentous microalgae are adapted to life in aquatic environments because they can float on water or attach to substrates by extending their filaments. As a result, these self-organizing structures of microalgae show adaptability and resourcefulness in response to their surroundings, and contribute to their ecological success in a variety of aquatic ecosystems. If you look at microalgae, you will see clusters and filaments that allow them to organize themselves [11]. Microalgae are a smaller, more heterogeneous group with organisms ranging in size from 1 µm to 1 mm and are found primarily in freshwater or soil [12].

Increasing demands for water, food, and energy are putting pressure on sustainability. Natural resources are finite. Climate change is threatening ecosystems and societies. Biodiversity conservation is especially important. This requires planning for the efficient use of food resources [13]. Food production primarily depends on soil resources because there are limited alternative sources of sustenance. Therefore, dwindling food resources are one of the major concerns for the future [14]. In the 20th century, the need to use biomass for food spurred the use of microalgae. However, with the growth of traditional agriculture, the alternative of growing microalgae on a large scale was pushed to the back burner [11]. Nevertheless, the moment has already arrived for traditional agriculture to make the transition from the current food system to sustainable production options. By 2050, climate change is expected to increase by 50–90%. The main driver is an increase in the world population [15,16].

Microalgae may seem like the food of the future, but they are not. Microalgae are already longtime allies of human nutrition, with *Nostoc* and *Spirulina* having been used as food since before 1900 [17]. Microalgae biomass has been mainly used as foodstuff supplements and additives. In recent decades, it has been considered a foodstuff with health advantages. (Figure 2).

For example, certain microalgae species are rich in docosahexaenoic acid (DHA), which is often found in juices, drinks, or milk for infants and children [19–20].

Approximately 13,090 new products containing algae or algae-derived ingredients were developed globally from 2015 to 2019. Among these new products, 79% were foods and 21% were beverages [21]. Microalgae are an excellent source of a wide variety of compounds. They have the high protein content (*Atrosphaera platensis*) and low fiber content (*Chlorella*) [22,23]. Microalgae species such as *Pyro-*

Figure 2. Classification of products based on algae and microalgae [18]

cystis lunula, *Nannochloropsis gaditana*, and *Atrospera platensis* have the potential for the production of commercial carbohydrates such as monosaccharides, disaccharides, and polyalcohols [24].

Microalgae protein composition and nutritional value

Microalgae have become an important ingredient in recent years for a variety of applications, from the global food and beverage industry and aquaculture to human nutrition and animal feed. This is due to the following reasons: (1) long-term sustainability, as microalgae have the lowest carbon, water, and arable land footprint compared to any crop (2) high content of healthy nutrients such as protein, essential amino acids, vitamins, antioxidants, omega-3 PUFAs, and minerals; (3) high productivity compared to terrestrial crops and animal foods; and (4) environmental remediation (*e. g.*, ecosystem services) [25–27].

With an estimated 200,000 species, microalgae are as diverse as they are numerous. Among the microalgae, we are most familiar with *Cyanophyceae* (blue-green algae), *Chlorophyceae* (green algae), *Bacillariophyceae* (including the diatoms), and *Chrysophyceae* (including the golden algae) [28]. The protein content of microalgae is highly dependent on the environment, in which they live, and the protein composition varies between species and strains. However, many microalgae species typically contain high levels of protein. This can range from 40 to 60 percent on a dry matter basis [29]. Most microalgal species have been found to have a crude protein content greater than 40% based on dry mass. The distribution of the crude protein content in microalgal biomass ranges from 6 to 63% [27]. We analyzed the protein content of 16 species of microalgae and found that the protein content (% of dry cellular material) ranged from 12% (*Chaetoceros gracilis*, a diatom) to 35% (*Nannochloropsis oculata*, a eustigmatophyte).

Sustainable consumerism is driving a demand and preference for new alternative protein sources that can reduce meat consumption. As a result, plant-based alternative protein sources, such as pulses and microalgae, have received increasing attention in recent years [30,31]. When measuring protein quality, microalgae such as *Chlorella sorokiniana* and *Chlorella vulgaris* have been shown to have higher Protein Digestibility-Corrected Amino Acid Score (PDCAAS) levels than legumes such as lentils, peas, chickpeas, and soy, which are commonly used as plant-based protein alternatives [32]. Microalgae have also traditionally been consumed in a dried state, which helps to provide a rich source of nutrients and health benefits [33]. However, some species can have low digestibility, so using various techniques to disrupt the cell wall and selecting species that are inherently less recalcitrant is important for species consumed as whole cells. When consuming microalgae, the use of purified forms can significantly improve digestibility and final protein quality compared to protein concentrates or protein isolates. The downside is that they are more expensive to produce [34].

Microalgae have a variety of health benefits, including antioxidant, anti-cancer, and anti-inflammatory properties. These are all effects that can be synthesized through the metabolism of microalgae. *Spirulina*, in particular, has been found to be safe and has no toxic effects, according to reports submitted to the US Food and Drug Administration (FDA) [35]. Microalgae have a high nutritional value. They are also rich in PUFAs and have a high content of bioactive peptides [36].

Consumer attitudes toward microalgae as an alternative protein source

As a novel food, algae can contribute to healthier and more sustainable food consumption. Because of this potential, it is thought to be a promising alternative protein source. Therefore, it is very necessary to analyze the preferences and perceptions of consumers who may accept microalgae as food [37]. In addition to positive drivers for microalgae as a food, previous studies have found barriers including meat preference habits when considering microalgae-based meat substitutes [1], and an unsafe image and lack of information about microalgae as a food [38,39].

Drivers of consumers' positive acceptance of microalgae foods included perceiving microalgae as healthy food and believing that they have health benefits [40,41], knowledge of microalgae and experience with them as a food [42], and consumers' belief that they do not need meat in their diet [1]. When consumers were given enough information about how microalgae were produced, they were less averse to microalgae foods [43].

We reviewed a study that measured consumer attitudes toward microalgae as a real food (hereafter referred to as microalgae attitudes). The study developed four items to measure consumers' varying attitudes toward microalgae in terms of health, nutrients, and sustainability. The four items are: (1) Algae are a more sustainable source of protein than meat", (2) Algae contain a lot of protein", (3) Algae are very healthy", and (4) Algae contain a lot of vitamins and nutrients". Consumers participated in the study by indicating their opinions on the four items above on a 5-point Likert scale (1 = completely disagree to 5 = completely agree). The average of the four items for each consumer was used to derive a final measure of microalgae attitudes. The reliability of the multiple-item manipulation of the dependent variable was high across all responding consumers. Overall, consumers had an average microalgae attitude of 3.38 to 3.65 [37].

The time-saving aspect can be helpful when introducing meat alternatives such as microalgae-based foods to consumers. For example, compared to grilling your own meat or making your own food after work, meat alternatives are largely processed and marketed as easy to prepare, requiring only heating and eating [39]. Increasingly, consumers prefer foods that meet health and sustainability standards. In addition, economics will be a critical factor in the adoption of microalgae as a protein source alternative to meat. These findings provide food producers and marketers with insights that are critical to increasing microalgae's share of the food market. It is best to achieve low prices through economies of scale before going to market. In addition, recipes should be provided so that consumers can utilize microalgae when making familiar foods [37].

Microalgae product quality standards and legislation

Studies have shown that the risks associated with microalgae include toxins, pathogens, allergens, heavy metals, and pesticides. However, toxins have not been found in spirulina and chlorella [8]. Microalgae are considered nontraditional foods and must undergo a series of toxicity tests to prove they are harmless. Toxins are categorized into biological and abiotic. Biological toxins are algal toxins and nucleic acids, while abiotic toxins are environmental pollutants, with heavy metals being a typical example [10].

Companies that want to sell products containing microalgae must comply with regulations. Relevant safety and regulatory requirements vary by country or region. This usually means applying for a license and submitting scientific information and health and safety assessments to the appropriate authorities (Figure 3).

In Europe, three levels of regulation oversee microalgae-related ingredients and marketing: (1) general food safety regulations, (2) new food ingredients, and (3) nutrition and health claims for foods [44]. Of these, the closest regulation relevant to microalgae is Regulation (EC) No 2015/2283 on novel foods, which repealed the earlier Regulation No 258/97. One of the criteria for food to be considered a novel food continues to be the absence of use for human consumption to a significant degree within the EU before May 15, 1997. The scope of Regulation No 2015/2283 in principle remains the same as that of Regulation (EC) No 258/97. Nevertheless, it was necessary to review, clarify and update categories of foods that constitute new foods in the light of scientific and technological developments that occurred after 1997. According to Regulation (EC) No 2015/2283, these categories should include whole insects and their parts; foods with novel or intentionally modified molecular structures; foods from cell or tissue cultures derived from animals, plants, microorganisms, fungi or algae; foods from microorganisms, fungi or algae; and foods derived from mineral substances. The definition of novel foods can also include foods composed of certain micelles or liposomes [45].

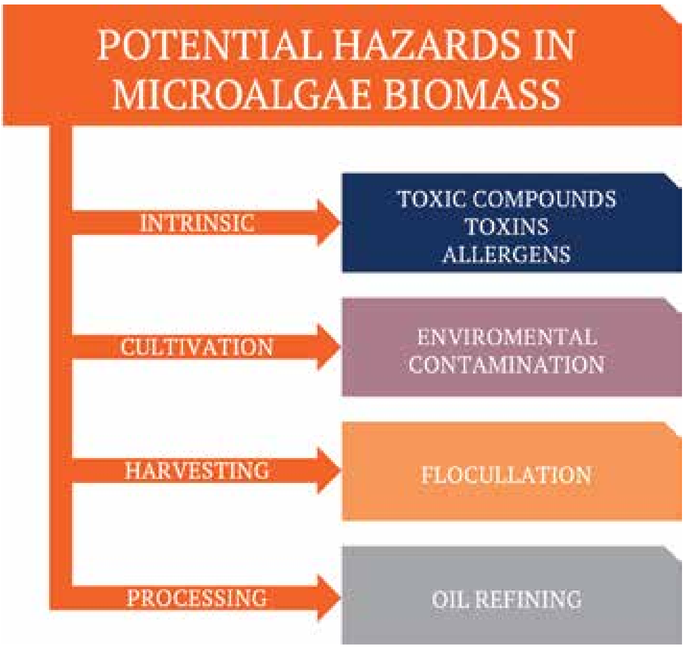

Figure 3. Classification of hazards linked to using algal biomass [18]

Even the few microalgae known so far have the potential to be used in a wide range of applications because they produce a wide range of nutrients (fatty acids, pigments, proteins, vitamins, and precursors to vitamins). Currently, the use of algae requires a permit. The strict legal provisions are intended to protect consumers from insufficiently researched or potentially unsafe foods. With the discovery and general approval of the potential of microalgae, approving additional species of algae with specific nutrient profiles will open up new opportunities for alternative proteins [46].

Discussion

Microalgae products come in a variety of flavors and colors, which is enough to maintain interest toward them [38]. However, the public still has questions about the safety of these new products; thus, there are as many challenges for microalgae and microalgae-infused food supplements as growth prospects. Therefore, food regulations need regular updates to ensure that consumers are well informed about the risks and safety of new foods. To benefit consumer health, the more ingredients included, the more stringent the maximum allowable concentration [47].

Meanwhile, policies are proposed to integrate microalgae cultivation into agricultural systems, with the aim of increasing consumption demand: good production practices, advances in production technologies, and quality monitoring. Finally, extensive knowledge of production in local growing realities is required, with a strategy designed with strong planning across the entire food chain [48,49].

The high production cost of microalgae, technical challenges associated with extraction and purification, and palatability issues have hindered their application in food despite their nutritional similarities to other plant-based proteins [50]. A cost-benefit analysis and review of production costs are important and necessary considerations for utilizing microalgae. Microalgae also need to be proven to be a functionally better alternative to animal proteins [51].

Conclusion

The negative effects of meat consumption on the environment and health have led to a growing interest in sustainable diets. Meat alternatives are being developed, and microalgae are one of them. Microalgae foods are high in protein and have many nutritional benefits that support health. The quality of their protein is also higher than that of legumes, a well-known alternative protein source. Consumer interest in new protein sources to replace animal protein has also increased. Microalgae, in particular, are generally accepted as a healthy and sustainable source of protein that contains many vitamins and nutrients. They are also less aversive to consumers than foods made from insects, another alternative protein source. As standards and regulations for microalgae foods are gradually refined, the market for alternative proteins using microalgae is expected to grow.

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