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Review article

Application and prospect of microbial food *Chlorella*

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ABSTRACT

Modern food is evolving in the direction of green, healthy, and convenient products, and developing natural products with health benefits is an important direction for the food industry. *Chlorella* is rich in nutrients, such as carotene and fatty acids, which provide it with a variety of health benefits, and therefore widely used in the food industry as a health or functional food. This study reviews the research progress and specific applications of *Chlorella* in health, functional, and other foods, and expounds on the bottlenecks faced in the use of *Chlorella* in food industry. This review provides a theoretical basis for the research, utilisation, and production of new food materials involving *Chlorella*.

1. Introduction

Growing population and rapid industrialisation have brought many changes to the society, but along with various problems, such as the high level of greenhouse gas emissions and insufficient food production. The world's population may exceed 10 billion in the near future. This population growth has resulted in insufficient domestic food and nutrient production to meet population needs in over 120 countries [1]. Concurrently, problems such as barren or restricted land, the misuse of pesticides, debate over genetically modified foods, and the addition of synthetic compounds and antibiotics to meat feed have led to an increase in food prices [2]. In this context, the United Nations proposed 17 Sustainable Development Goals in 2015 to, increased the incentive for scientific research on developing better food products, processing methods, and ingredient selection. Microbial foods are a possible solution, and microalgae are one of the most promising candidates. There are many types of microalgae, although only a few are suitable for human consumption. Commercial large-scale cultivation of microalgae for biomass and bioproducts began in the 1960s, along with the use of green microalgae and *Arthrospira platensisin* in the 1970s [3]. *Chlorella* and *Arthrospira* sp. are the earliest known microalgae used as dietary supplements [4]. *Chlorella*, which is a single-celled green alga with a fast growth rate and strong adaptability to different environments growth conditions, is one of the most studied and largest microalgal genera [5]. *Chlorella vulgaris* with a high CO_2 assimilation capacity based on the Calvin-Benson-Basham cycle (Fig. 1), can produce edible biomass through photosynthesis for use in food or dietary supplements [6].

There are approximately 10 species of *Chlorella*, that are widely used as health food additives and animal feed in many countries. *Chlorella* biomass has been used as a feed additive in the United States, Japan, and Israel for more than 30 years. In the United States,

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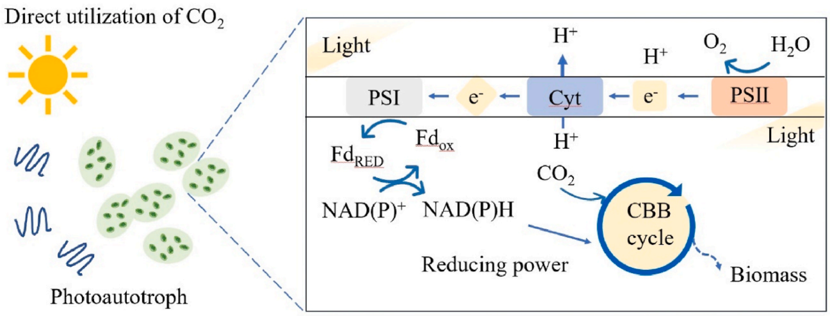

Fig. 1. Microbial foods from one-carbon compounds [7]. Photoautotrophs can generate electrons from water molecules using light energy, while aerobic hydrogenotrophs can extract electrons from molecular hydrogen (H_2) produced from water using renewable electricity. The dashed arrows represent multiple metabolic reactions. Cyt, cytochrome; e−, electron; Fd_{OX}, oxidized ferredoxin; Fd_{RED}, reduced ferredoxin; H+, proton; NAD(P)+, oxidized nicotinamide adenine dinucleotide (phosphate); NAD(P)H, reduced nicotinamide adenine dinucleotide (phosphate); PS I, photosystem I; PS II, photosystem II.

Chlorella has been classified as safe for consumption (GRAS) by the Food and Drug Administration [8]. In the European Union, *Chlorella* has been commercialised and is the most commonly consumed microalgae, that can be sold directly to consumers, moreover, its entire biomass can be used as food [1]. For example, the Dulcesol Group, a leader in the Spanish bakery and pastry industry, invested in production lines to develop healthy bakery products containing *Chlorella*. In China, the most common species are *Chlorella pyrenoidosa*,

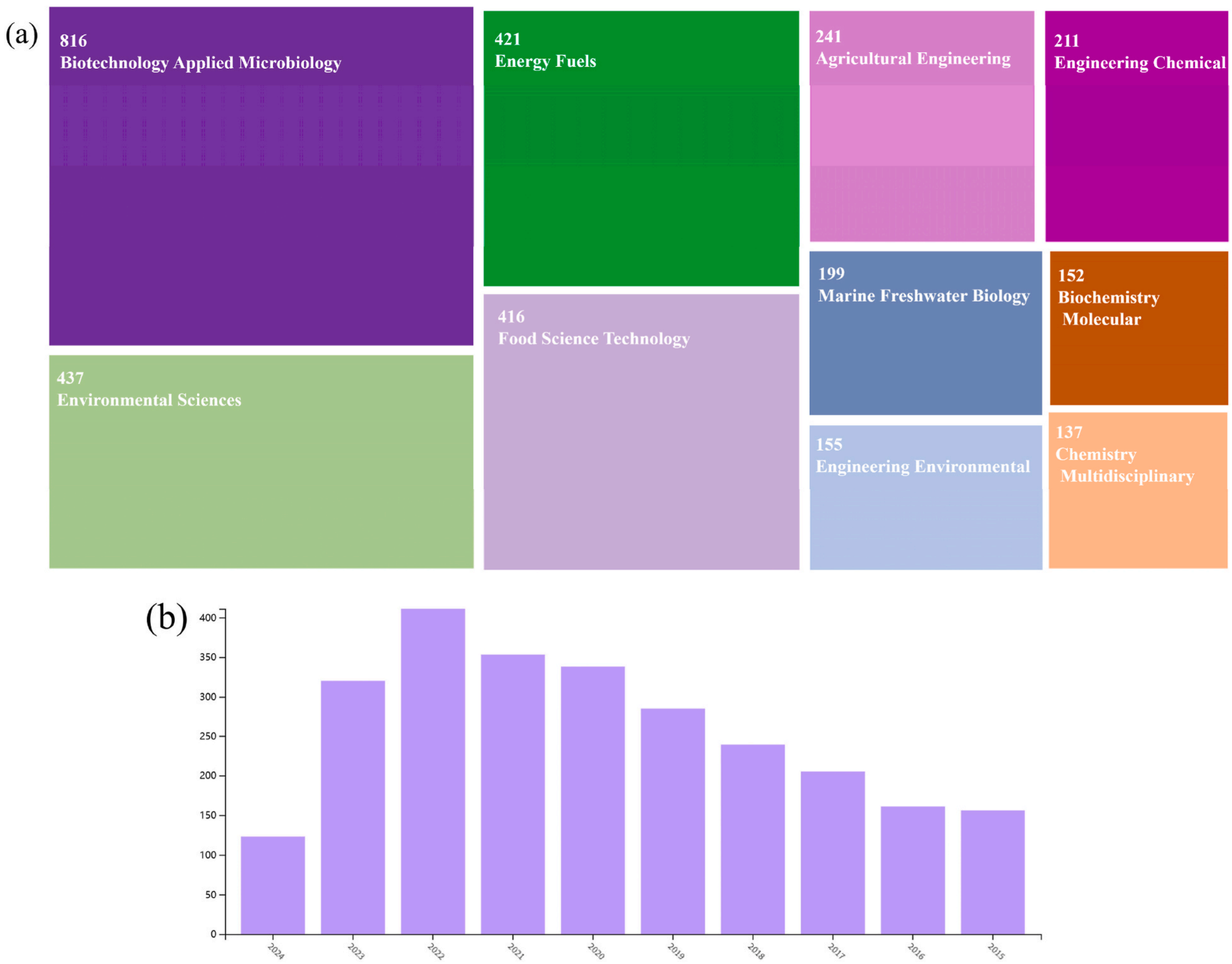

Fig. 2. *Web of Science* database surveyed the publication of papers from 2015 to 2024 Using '*Chlorella*' and 'food' as keywords (a) the number of papers published by year and (b) the field of publication.

Chlorella vulgaris and *Chlorella ellipsoidea*, of which *Chlorella pyrenoidosa* is one of the four newly approved algae. *Chlorella* is a rich source of antioxidants, which have the functions of improving immunity, lowering blood sugar and, lipids levels, and antitumor properties, and thus, it has good application prospects in the fields of food and medicine [9]. As presented in Fig. 2, the number of published studies (determined by searching using the terms '*Chlorella*' and 'food') in the Web of Science database has increased gradually. Most of the publications focus on the biotechnological applications of microbes (816 studies), while 416 studies were in the field of food science and technology. These results revealed that studies regarding the application of *Chlorella* in food technology has gradually been favoured by an increasing number of researchers.

However, owing to bottlenecks, such as scale-up, biomass acquisition, and the high cost of other downstream processes, functional staple foods based on *Chlorella* have not been widely developed. The use of *Chlorella* in food by consumers faces technical challenges owing to its limited sensory appeal. In response to these challenges, in this review we aims describe the beneficial properties of *Chlorella* and its potential applications in the food industry, furthermore, we introduce the biochemical composition and, nutritional, and functional properties of *Chlorella* biomass, and the characteristics of related food products. This study describes the latest trends in the industrial application of *Chlorella* in staple foods and it provides a theoretical basis for future applications of *Chlorella* in the food industry.

2. Nutrients in *Chlorella* and their functions

Chlorella cells are rich in protein, essential amino acids, polysaccharides, pigments, fatty acids, vitamins, minerals, and other nutrients with comprehensive and balanced nutritional value and health benefits [10]. In addition, most of the lipids in microalgae are

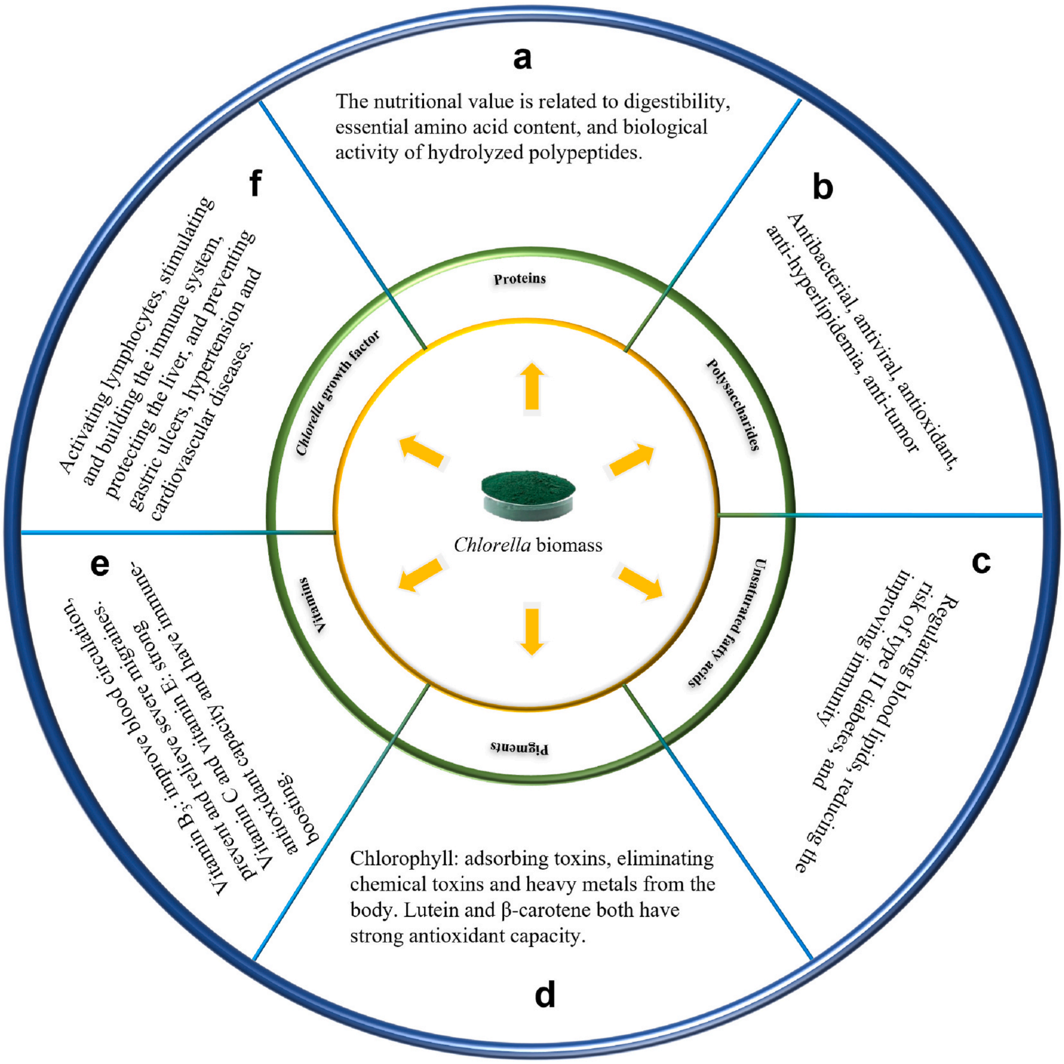

Fig. 3. The biochemical composition and functional properties of *Chlorella* biomass.

rich in unsaturated fatty acids (docosahexaenoic acid and eicosapentaenoic acid) and α-linolenic acid (ALA), which have the effects of preventing cardiovascular diseases, regulating immunity, and promoting brain development [11]. *Chlorella* has broad application prospects in healthcare products and infant foods. The biochemical composition and, nutritional, and functional properties of *Chlorella* biomass are presented in Fig. 3.

2.1. Proteins and amino acids

The land requirement for growing algae commercially to produce protein is much lower than that for animal sources, such as beef, pork, and chicken [12]. The protein content of *Chlorella* cells can reach 55%–65 %, and thus, they are rich unicellular of proteins [13]. For example, the protein in *Chlorella* can reach more than 50 % of the cell dry weight, which is much higher than common protein, sources such as soy (37 %), milk (26 %), meat (43 %), and yeast (39 %) [14]. The nutritional value of *Chlorella* protein products is attributable to their digestibility, essential amino acid content, and the biological activity of the hydrolysed polypeptides. The partial hydrolysates of *Chlorella pyrenoidosa* and *Chlorella vulgaris* proteins, with molecular masses of 10–20 kDa, have good emulsifying and foaming properties, and the active peptide, with a molecular weight of 2–5 kDa, has antioxidant, antihypertensive, and immunomodulatory effects [14]. The nutritional value of food proteins depends on the protein content, and on the type of amino acids. There are 18 types of amino acids in *Chlorella*, with glutamic acid, aspartic acid, and leucine being the most abundant. The content of essential amino acids accounts for 42 % of the total amino acid content and >20 % of the cell dry weight. The amino acids of *Chlorella pyrenoidosa* are similar to those essential amino acids, and possess good nutritional and hypoallergenic properties [15]. The umami flavour of *Chlorella* originates from the free amino acids, such as glutamic and aspartic acid [16]. In addition, *Chlorella* proteins scavenge free superoxide anion radicals and, free hydroxyl radicals, and thereby improve immunity [17]. However, more than half of the proteins in *Chlorella* are insoluble in water, making their use in food and pharmaceuticals difficult [14]. *Chlorella* proteins have potential application prospects in dairy products, especially high-concentration emulsions such as mayonnaise, salad dressings, and impregnations. For instance, *Chlorella pyrenoidosa* protein (CPP) is an emerging sustainable ingredient for nutrient-fortified baked goods, flavored fermented products, and bioactive substrate encapsulation carriers [18]. However, due to its high molecular weight and hydrophilicity of CPP, its application in complex food systems (such as biphasic systems) still presents challenges. In response to this phenomenon, Jiang et al. provided a new method for constructing a gel water-oil system using CPP, using CPP/xanthan gum-based hydrogel (HG) and beeswax-based oleogel (OG) to fabricate bigels. The opposite rotation of bigels can be adjusted by the ratio of OG and HG. The results showed that an increase in OG could enhance viscoelasticity, hardness, adhesion, chewiness, and thermal stability. OG/HG bigels have greater thixotropic recovery and oil holding capacity than HG/OG bigels. In the *in-vitro* digestion and food 3D printing, the high specific surface area and highest thixotropic recovery caused by the emulsion structure (OG = 50 %) of OG/HG bigel facilitate the release of free fatty acids and the molding of 3D printed objects, respectively (Fig. 4) [19].

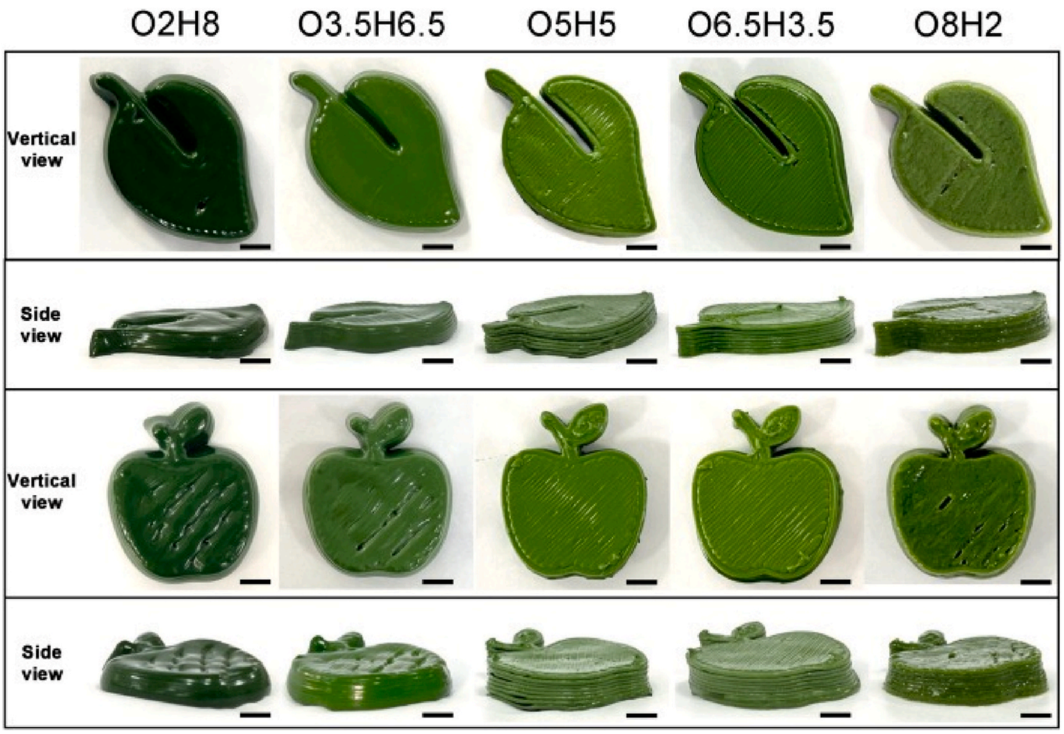

Fig. 4. Vertical and side views of 3D-printed objects using bigels [19].

2.2. Polysaccharides

The polysaccharides content of *Chlorella* is second only to the protein, at approximately 25%–35 % of the cell dry weight, in different algal strains and the use of different culture methods [20]. Polysaccharides, as the main active ingredients of *Chlorella*, have received increasing attention owing to their various health-promoting activities, including antibacterial, antiviral, antioxidant, anti-hyperlipidamia, antitumor activities [21]. *Chlorella* polysaccharides have significant antioxidant capacity, as they can scavenge OH·, DPPH·, and O_2^-· [22]. *Chlorella* polysaccharides enhance macrophage phagocytosis by promoting the production of cytokines and, increasing the expression levels of human leukocyte antigen (HLA) and macrophage co-stimulatory molecules, thereby enhancing immunity. Typically, polysaccharides isolated from *Chlorella* are divided into starch-like glucans and non-starch polysaccharides [23]. Yuan et al. analysed the molecular weight, monosaccharide composition, chemical structure and biological functions of different *Chlorella* polysaccharides, and reported that the starch-like glucans and non-starch polysaccharides of *Chlorella* can be divided into homopolysaccharides and heteropolysaccharides, and the heteropolysaccharides can further be divided into neutral, acidic, and amino heteropolysaccharides [24]. The cell wall of *Chlorella pyrenoidosa* mainly contains hemicellulose (31.0 %), which is mainly composed of galactose, mannose, arabinose, xylose, and rhamnose [25]. Although microalgal polysaccharides have potential applications in food, their low yield is one of the main limitations of industrial-scale applications, and the development of efficient microalgal polysaccharides production technology has become an urgent need. Liu et al. characterized and compared the physicochemical properties and antioxidant activity of *Chlorella* polysaccharides extracted by hot-water extraction method at different temperatures (60–180 °C) and *Chlorella* polysaccharides extracted at different alkali concentrations (0–4%). The results showed that the polysaccharides dissolution rate of the two methods reached the maximum values of 71.19 % and 58.79 % at 120 °C and 4 % alkali concentration, respectively. The molecular weight of the polysaccharides extracted by the alkaline method was larger than that of the polysaccharides extracted by the hot water method. The polysaccharides obtained at 1 % alkali concentration exhibited the highest antioxidant activity [26].

2.3. Unsaturated fatty acids

Chlorella is rich in unsaturated fatty acids [27]. Among them, polyunsaturated fatty acids, such as ALA, regulate blood lipids, reduce the risk of type II diabetes, and improve immunity [28]. The composition and content of fatty acids differ among the *Chlorella* species, moreover, they are also affected by the different culture methods [29]. Soares et al. measured the main fatty acids in *Chlorella zofingiensis*, including C16:0 (31.9 %), C18:1 (16.0 %), and C18:2 (12.3 %), and found that unsaturated fatty acids accounted for 64.2 % of the total fatty acid content [30]. A previous study identified eight fatty acids of *C. sorokiniana* (KNUA104) at different temperatures, at 10 °C, the fatty acid with the highest content type was C18:3 (31.64 %), followed by C18:2 (22.42 %). Whereas, at 25 °C, the two most abundant fatty acids were C18:3 (27.10 %) and C16:0 (24.63 %) [31]. Ördög et al. measured *Chlorella* sp. MACC-452 fatty acids and found that unsaturated fatty acids accounted for up to 64.3 % of the total fatty acids, with alpha-linoleic acid and ALA being most abundant at 13.6 % and 10.8 %, respectively [32]. ALA is an essential omega-3 unsaturated fatty acid that is obtained from the diet. However, the production cost of omega-3 fatty acids using microalgae is high, and it is necessary to reduce the production cost by increasing the biomass and consequently, the omega-3 fatty acid content [33]. Taking ALA production of *Chlorella* sp. as an example, the usual techniques used to increase biomass involve changing the culture conditions, such as adding acetylcholine or changing the culture temperature, however, radical changes in the characteristics of *Chlorella* is not feasible, as this is often at the expense of biomass. Although promote the yield of ALA in *Chlorella* can be improved via two-stage culture, high-yield strains are essential for increasing ALA production by *Chlorella* sp. The common methods of improving yield include genetic engineering and random mutagenesis. We used a low-temperature plasma technology in our previous work to induce a high-yield ALA-producing strain of *Chlorella*, and thereby the use of *Chlorella* as an excellent food source of fatty acids in food products is expected to be realised [27].

2.4. Pigments

Chlorella contains natural pigments, such as chlorophyll (≤5.5 %), lutein, and β-carotene (precursor of vitamin A) [34]. Chlorophyll has the effect of adsorbing toxins, eliminating chemical toxins and heavy metals from the body, and it can be used to remove harmful substances from the intestines, kidneys, lungs, and blood. It can also effectively protect the liver, kidneys, and other important detoxification organs [35]. Lutein and β-carotene have strong antioxidant capacity and can effectively scavenge various free radicals produced by the body's metabolism. Lutein has beneficial effects in protecting eyesight and preventing chronic diseases, such as atherosclerosis and diabetes [36]. β-carotene prevents a variety of degenerative diseases caused by ageing, and is an indirect source of vitamin A, which is of great significance in the prevention of night blindness [37]. *Chlorella* also contains macular pigment, which are composed of lutein and zeaxanthin (at a ratio of 5:1). While comparing diabetic and control groups, macular pigment supplementation significantly reduced glucose tolerance and insulin resistance in the diabetic group by 25 % and 41 %, respectively. In the diabetic group, macular pigment supplementation reduced the levels of inflammatory cytokines and serum markers for diseases, and contributed to biological efficacy by scavenging free radicals and stabilising blood glucose levels [38].

2.5. Vitamins

Vitamins are a class of organic compounds necessary for maintaining good health, among which vitamin B_1 protects the nervous system, promotes gastrointestinal peristalsis, and improves appetite [39]. Vitamin B_3 can improve blood circulation, prevent and relieve severe migraines [40]. Vitamins C and E have strong antioxidant, immune-boosting and skincare properties. *Chlorella* cells contain a variety of vitamins, such as vitamin B_1, vitamin B_{12}, and vitamin C, and thus, *Chlorella* is considered a great candidate for a plant based source of biologically active vitamin B_{12} [41]. Although *Chlorella* cannot synthesize vitamin B_{12}, it can take up vitamin B_{12} from the environment, through supplementation to the growth medium [42]. Vitamin B_{12} deficiency has been linked to human megaloblastic anaemia, peripheral neuropathy, cardiovascular disease and impaired cognitive function [25]. Processing vitamins from *Chlorella* into food or health products is of great significance in improving the health of people with vitamin deficiencies [43].

2.6. Chlorella growth factor

Chlorella growth factor (CGF), a hot water extract of *Chlorella*, is mainly composed of amino acids, water-soluble proteins, polysaccharides, DNA, RNA, vitamins, and plant hormones. It has a significant function in promoting the growth, division, and reproduction of *Chlorella* cells, and is a unique intracellular bioactive component of *Chlorella*, and is known to be 'hormone-like' [44]. CGF activates lymphocytes; stimulates and builds the immune system; protects the liver; and prevent gastric ulcers, hypertension, and cardiovascular diseases. It has great potential for application in health foods [45]. CCF has anti-tumour effects, and inhibitory effects on the proliferation of liver, breast, cervical, and other cancer cells. Kunt et al. studied the inhibitory effect of *Chlorella* extracts on human gelatinase and found that *Chlorella* protein extract could up-regulate the expression of TIMP-3 and down-regulate the expression of c-Jun, thereby inhibiting the expression of three matrix metalloproteinases (MMPs) in cancer cells. Thus, these extracts have great application value in the treatment of breast cancer [46]. Hideaki et al. in their invention patent application, reported that *Chlorella* growth extract can promote hair papilla cell proliferation [47]. Li et al. isolated an extract of *Chlorella pyrenoidosa* with a molecular weight of >100 kDa composed mainly of rhamnose, galactose, and arabinose, which promoted the proliferation of epithelial cells and the secretion of cytokines by macrophages [48]. However, CCF is a mixture of multiple substances, and its content is affected by the culture conditions and extraction process, it is not possible to quantify the component accurately [49]. Different species of microalgae have different CGF, components, such as phycocyanin for spirulina and astaxanthin for *Haematococcus pluvialis*; therefore, despite its multiple health benefits, CGF cannot be used as a measure of the merits of *Chlorella*. This is important factor limiting the development of *Chlorella* in the food industry.

3. Applications of *Chlorella* in the food industry

In the global nutrition market, microalgae are sold as dietary supplements in the form of algal powder, algal tablets, and capsules; however, their application in traditional foods is limited. There are two types of applications for *Chlorella* biomass powder in traditional foods; as a colourant, and as a agent to improve the nutritional, physicochemical, and organoleptic properties of the final product. *Chlorella* is used in bread, pasta, noodles, biscuits, puffed foods, yoghurt, sherbet, juice, cheeses, sausages, burgers, and mayonnaise, etc. Some products manufactured by well-known companies include orange and green algal biscuits from Grupo Dulcesol in Spain, gummies from Majami in Poland, wafer biscuits and dressing juices from Evasis Edibles in Australia, thick juice from Frecious in New Zealand, chew sticks from Greenic in Germany, and puffs from Honest Fields in Romania (Table 1).

Table 1
Current consumer products containing microalgae.

Type of food product	Algae type	Brand	Company	Website or references
Drink	*Chlorella*	Die Stille Helga	Helga, Austria	https://hellohelga.com/helga_english/
Cracker	*Chlorella*	Helga Bio Algen cracker	Helga, Austria	https://hellohelga.com/helga_english/
Super Gigg bar	*Chlorella*	Greenic	Greenic, Germany	https://www.greenic-bio.de/
Orange and *Chlorella* bites	*Chlorella*	Grupo Dulcesol	Grupo Dulcesol, Spain	
Green fruit smoothie	*Spirulina* and *Chlorella*	Happy Planet	Happy Planet Foods, Canada	https://www.happyplanet.com/
Vichyssoise	*Chlorella*	Vesana Superfoods	Vesana Superfoods, Spain	http://www.vesana.es/
Vegetable juice	*Chlorella*	Frecious Slow Juice	Frecious, the Netherlands	https://www.frecious.bio/nl/
Organic fudge	*Chlorella*	Earth of Eco	Majami, Poland	http://www.majami.pl/en
Smoked seaweed and sea salt organic puffs	*Chlorella*	SC Honest Fields Europe, Romania	Romania	http://www.honestfields.com/
Crispy matcha biscuits	*Chlorella*	Tohato Harvest	Tohato, Japan	[50]
Baked bean crackers in the shape of edamame beans	*Chlorella*	Ginbis	Ginbis, Japan	[50]
Sunflower seeds sprouted with Himalayan salt, spices, spinach and microalgae	*Spirulina and Chlorella*	Wickedly Prime	Amazon.com Services, USA	https://www.amazon.com/
Nori and wasabi coated peanuts	*Chlorella*	Tomy'z/Tomizawa/Tomiz	Tomizawa, China	[50]
Greens organic bar	*Chlorella* and *Spirulina*	Raw Sun Bite	Lavica Food, Poland	[50]

3.1. Application of Chlorella in health foods

Chlorella can be used as a food additive, which can add colour and flavour to food and improve nutrition. The addition of fermented food such as *Chlorella* can improve the quality and flavour of food, while producing high-value-added products by the incorporation of nutrients such as proteins. In 2003 in Japan, food additives with *Chlorella* hot water extract as the main raw material were developed. Concurrently, the rapid development of biotechnology has promoted the industrialisation of *Chlorella*, and the study of the nutritional and health-promoting properties of *Chlorella* is of great significance for the development of functional foods. Currently, the main forms of *Chlorella*-containing health foods are *Chlorella* tablets, powder, capsules, granules and health drinks.

3.2. Research and development and application of Chlorella in functional foods

With improvements in consumption levels and awareness of healthy diet and nutritional balance, *Chlorella*, a natural, healthy, and nutritious raw material, meets the requirements of a healthy dietary supplement. The use of *Chlorella* in functional foods is primarily owing to its high nutritional value. Cellular nutrients, such as protein, fat, and carbohydrates, make *Chlorella* a high-quality nutrient source, with the advantages of high protein, low fat, low sugar, and low calorie content [7]. *Chlorella* can be added to foods as a nutrient enhancer to produce *Chlorella*-based functional foods with a seaweed flavour. Sensory characteristics, such as flavour, may be more important for functional foods than for regular foods, as functional foods are often more expensive and consumers need an excellent flavour to compensate for the increased cost.

3.3. Application of Chlorella in food

Traditional bakery products have shortcomings such as short shelf life and low nutritional value. The water-retention effect and rich and diverse nutrients of *Chlorella* can complement and strengthen these products, which improves their nutritional value, and gives them a unique colour and flavour, resulting in a variety of snacks with high nutritional value.

3.3.1. Chlorella bread

Chlorella biomass is one of the most promising sources of new products and applications that can be used to enhance the nutritional and technical value of food. The enrichment of bread with microalgal biomass is a great challenge, as it affects the development of the gluten structure. Studies have evaluated the effect of *Chlorella* on dough rheology and bread texture by changing the amount of *Chlorella* added to wheat dough. The results revealed that *Chlorella* biomass at 3.0 g/100 g of dough had a positive effect on the rheological and viscoelastic properties of the dough and strengthened the gluten network. Greater biomass additions had a negative effect on dough rheology, bread texture and flavour. Biomass addition had no effect on yeast fermentation kinetics or the time required for fermentation [51]. Incorporating *Chlorella* biomass into dough matrix is a significant technical challenge. Bread-making properties are primarily attributed to the ability of gluten proteins to form viscoelastic networks when mixed with water. The weakening of this network owing to the addition of *Chlorella* to dough, result in a reduction in bread volume and, subsequently, had a negative impact on other quality attributes. Therefore, the amount of *Chlorella* biomass added has a markable influence on bread properties. Weakening the viscoelastic network is the result of the structural dilution of gluten, resulting in a decrease in bread volume owing to the presence of foreign proteins (e.g., *Chlorella*, considering its composition, viz. a high protein content) in wheat flour dough, which then negatively affect other quality attributes, such as crumb size and tenderness [52]. However, preserving the textural and rheological characteristics remains a challenge.

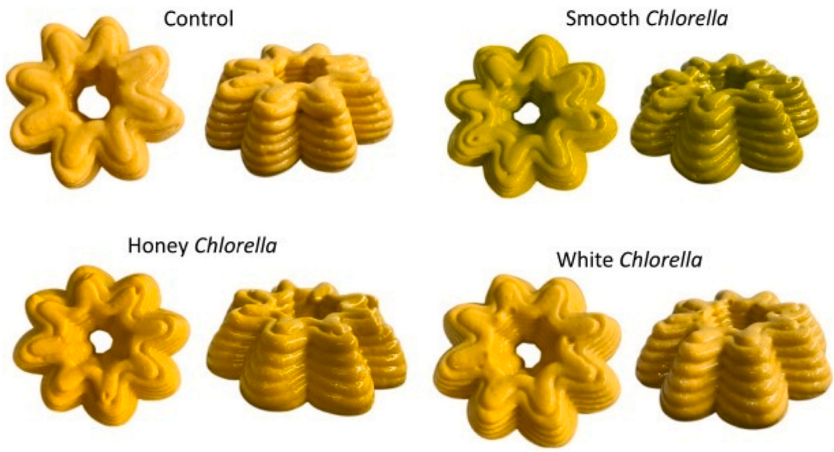

Fig. 5. 3D-printed samples of the control puree and purees with 3 % smooth, honey, and white Chlorella [54].

3.3.2. Chlorella cookies

Adding microalgae to cookies is a novel approach in the design of new food products as these microalgae, provide functional properties and specific health benefits beyond traditional nutrients. Traditional cookies have similar nutrition profiles consisting, of mainly sugar and oil, that are high in calories. The development of a new type of nutritional cookie containing *Chlorella* can make up for the nutritional deficiencies of traditional products, as the nutrients in the traditional products can be complemented and strengthened. In the 1980s, *Chlorella* cookies were successfully developed in Japan, with *Chlorella* alone, or vegetable juice or kelp. The dosage was controlled between 0.2% and 2%. Zaida Natalia Uribe-Wandurraga et al. added *Chlorella* biomass to the dough for 3D printing of cookies, thereby improving dimensional characteristics and achieving a more stable and resistant 3D structure (Fig. 5) [53].

3.3.3. Application of Chlorella in other foods

Considering the use of *Chlorella* as an adjunct to produce innovative and healthy foods, some researchers envision the creation of new foods rich using different microalgal biomass. In 2007, Empis et al. developed a butter biscuit containing *Chlorella* biomass, and in 2010, some researchers developed pasta with *Chlorella* and *Spirulina* [55]. Table 2 lists the studies of *Chlorella* biomass as a food ingredient, showing that *Chlorella* biomass has a diverse range of applications in different food matrices [50].

Macroalgae are widely used in traditional dishes in some geographical regions (such as Asia), and the addition of microalgae to food a topic of interest among researchers. The main challenge faced in adding microalgae to food products is sensory acceptance, mainly regarding the flavour and appearance when added at higher concentrations. However, despite the low sensory acceptance scores for some products with microalgae, microalgal biomasses has high potential for food nutrient enrichment and applications in the food technology field. Therefore, the reduction of the undesirable flavour of *Chlorella* is an important challenge that needs to be solved. In addition to deodorisation and embedding methods, this issue can be solved using simple and practical addition strategies, such as algal cuisine or flavour masking methods [56]. *Chlorella* has good prospects for application in healthy food, functional, and staple foods.

4. Challenges in the use of *Chlorella* in food industry

Currently, *Chlorella* is used commercially as a food additive and is sold as a dietary supplement as well. However, only a limited number of *Chlorella* species are available in the market. Several challenges remain to be overcome, including (1) high production costs, (2) thick cell walls, (3) green colour and fishy odour, and (4) low consumer acceptance. Research should be conducted on reducing the production cost of *Chlorella*, developing a more energy-saving technology for gentle cell wall breaking, removing the fishy odour, and improving consumer acceptance of *Chlorella*-supplemented foods. The following is a detailed explanation of the challenges faced in the use of *Chlorella* in the food industry.

4.1. High production cost

The supply of high-value *Chlorella* products is challenged by the total cost of production, including the costs of cultivation systems, maintenance, and limited cultivation productivity [66]. Providing uniform and intense light during *Chlorella* cell photo culture is a challenge because it is an expensive process. Moreover, the percentage of contamination is high, and harvesting and extracting algae require various equipments, such as centrifuges [67]. Additionally, the high operating and maintenance costs of photobioreactors increase the total cost of producing microbial food products via photosynthesis [68]. There are two types of *Chlorella* culture reactors: an open and a closed culture system. The open culture systems include natural ponds, artificial ponds, and artificial open photobioreactors (single-layer runway type, multi-layer runway type, and membrane type), and the closed culture systems mainly include vertical columns, type, discs, tubes, plates, and stirred photobioreactors. The advantages of an open culture system are that the culture system is easy to build, cost-effective, and easy to operate. However, the utilisation efficiency of this type of system is low, the culture conditions are difficult to control, and there is a high dependence on the environment. A closed culture system has a high microalgae biomass yield, the culture conditions can be accurately controlled, and the adaptation range is wide; however, the cost of this type of system is high, the energy consumption is high, and it is difficult to achieve large-scale utilisation. In view of the advantages and disadvantages of these two systems, researchers have developed a hybrid culture system and a phased culture mode (closed first and then open) to combine the advantages of the two culture systems and achieve high efficiency, high-yield, and large-scale cultivation of microalgae. Recent studies have promoted the utilisation of wastewater from various industries, because it contains high concentrations of minerals and nutrients that are beneficial for *Chlorella* growth. However, researchers believe that wastewater bioremediation is often used for non-human-consumption applications as the large quantities heavy metals in wastewater can be toxic to humans, which creates a conflict between waste conversion and food safety. The production cost of *Chlorella* biomass can be reduced by using food wastewater for *Chlorella* production. Therefore, in the face of high production costs, the search for high-quality breeding technology and efficient and low-cost harvesting technology are solutions.

4.2. Thick cell wall

Most applications of microalgae in food use whole-cell biomass owing to its low cost and technical feasibility. However, the microalgal cell walls can impair nutrient utilisation and processing, and the odour and pigments may have negative sensory effects, thereby limiting their use in food products. There are two types of *Chlorella* cell walls: glucose-mannose and glucosamine [69]. Most of the nutrients exist within algal cells, which are not easily absorbed by the body, and *Chlorella* usually needs to be powdered during

Table 2
Case studies of *Chlorella* in different food matrices in recent years.

Type of food product	*Chlorella*	Ratio	Characteristic	References
Vegetable purees	smooth *Chlorella*, honey *Chlorella* and white *Chlorella*	10 % zucchini, 10 % carrots, 3 % *Chlorella*, 77 % others	purees showed a shear thinning behavior and a weak gel structure.	[54]
Cookies	*Chlorella vulgaris*	2 % *Chlorella*, 47 % wheat flour, sugar 20 %, 31 % others	The cookies showed high color and texture stability, higher protein content.	[57]
Fresh green smoothies	*Chlorella* and *Spirulina*	56.5 % white seedless grapes, 15.5 % broccoli, 25.8 % cucumber and 2.2 % alga.	Showed the highest vitamin B_{12} content.	[58]
Broccoli soups	*Chlorella* sp.	At concentrations ranging from 0.5 to 2.0 %	lower L* values, increased content of polyphenols and to a higher antioxidant capacity.	[59]
Wheat bread dough	*Chlorella vulgaris*	Doughs and breads were prepared with the fresh *C. vulgaris* biomass (1.0 g of Cv/100 g of flour + Cv).	Higher antioxidant capacity.	[60]
Breadsticks	*Chlorella vulgaris* and *Arthrospira platensis*	*Chlorella* dough comprised 1.5 % of *Chlorella vulgaris* and 98.5 % wheat flour.	*Chlorella* enriched breadsticks showed the highest studied pigments content.	[61]
Glutinous-rice cake	*Chlorella vulgaris*	1 % corn starch + C. vulgaris extract	Low peroxide number, low risk of rancidity, and longer shelf-life time of dodol.	[62]
Meat substitutes	*Chlorella vulgaris*	10 % Yellow *Chlorella vulgaris*,90 % pea protein	The product wet or dry, to pea meat substitutes did not lead to significant differences in hardness, visual appearance or anisotropy index.	[63]
Soya drink	*Chlorella vulgaris*	1.5 % Powdered *Chlorella vulgaris*	*Chlorella vulgaris* can be added as a natural ingredient to produce fermented soya drinks and increase the survival of LAB.	[64]
Cookies	*Chlorella* sp.	Microalgae meal at level of incorporation of 6 %	Without affecting the quality attributes of the cookies.	[65]

processing process to improve the body's absorption and utilisation of nutrients [70]. Currently, *Chlorella* used in the food industry often undergoes grinding, high-pressure homogenisation, ultrasonic crushing, and other mild and non-toxic physical wall-breaking methods, which are environmentally friendly extraction techniques [71]. There are also studies on breaking the wall by adding enzymes, such as carbohydrate-active enzymes and proteases [72]. The cell wall can also be broken down through microbial fermentation [73]. Many pretreatment techniques have been developed to improve the wall-breaking effect, including laser treatment, cell disruption technology, microfluidic high-pressure homogenisation, pulsed arcs, high-frequency focused ultrasound, and cationic polymer coating of membranes (Fig. 6) [74,75]. Taoukis et al. used a pulsed electric field to assist with the extraction of chlorophyll from *Chlorella*, and the results showed that the pulsed electric field has a low energy consumption and a short processing time. They explored the kinetics of chlorophyll extraction, which can be used to reliably predict and estimate the extraction efficiency under different process conditions [76]. Future research on *Chlorella* wall-breaking technology development should occur in two directions: the continuous development of new physical and chemical wall-breaking methods to compensate for the shortcomings of the original methods, and use of a variety of traditional physical or chemical wall-breaking methods simultaneously. Each strategy has its advantages and disadvantages, and complement each other, on the premise of maintaining activity, multiple components are separated and purified.

4.3. Green colour and fishy odour

Chlorella is high in chlorophyll and it may reduce the quality of food products as the chlorophyll content affects the colour and taste (bitterness) of food and is best removed before consumption. To reduce the *Chlorella* chlorophyll content, high-pressure homogenisation (HPH) has been combined with methanol and ethanol treatment to destroy cell wall and decolourise *Chlorella*. The effectiveness of HPH combined with alcohol treatment for the removal of chlorophyll from *Chlorella pyrenoidosa* and its effect on the quality and technical function of the resulting depigmented *Chlorella pyrenoidosa* in food products were evaluated. This method effectively removed chlorophyll and increased the whiteness of *Chlorella* from 43.5 to 86.6. The results showed that the combination of HPH and alcohol treatment enhanced the sensory characteristics and function of *Chlorella*, thereby promoting its application in the food industry [78]. To reduce the chlorophyll content during *Chlorella* culture, antagonistic heterotrophic culture and targeted mutation strategies have been employed to obtain microalgae with little or no chlorophyll [79]. However, these methods often have a negative impact on microalgal growth and target product yields (e.g. proteins).

The flavour of *Chlorella* is mainly derived from volatile organic compounds (VOCs), and the colloidal properties of heat-sensitive compounds (such as proteins) may be affected by the drying method, resulting in changes in protein quality [3]. Heat treatment leads to the degradation of existing VOCs and the formation of new VOCs, resulting in changes in the organoleptic profile. Evaluating the effects of the drying process is essential for increasing the use of microalgal functional ingredients in novel foods products. Simon Van De Walle et al. reported that the drying methods used affects the quality *Chlorella* powder obtained, thereby making it an important feature that determines its incorporation potential in foods. Heterotrophic *Chorella vulgaris* was dried using agitated thin-film drying (ATFD), pulse combustion drying (PCD), and other methods. ATFD was found to improve the gelling, water-holding ability, and solubility of *Chlorella* proteins as well as protein digestibility in vitro, whereas high-temperature PCD led to an increase in the cocoa-like odour [80].

4.4. Low consumer acceptance

Consumer acceptance is critical to the success of new foods; despite the positive effects of *Chlorella* on human health, there are some potential challenges associated with microalgal foods. Moreover, the development of microalgal related products is limited by low sensory acceptance among consumers [6]. Although the addition of *Chlorella* improves the nutritional properties of the product, it can

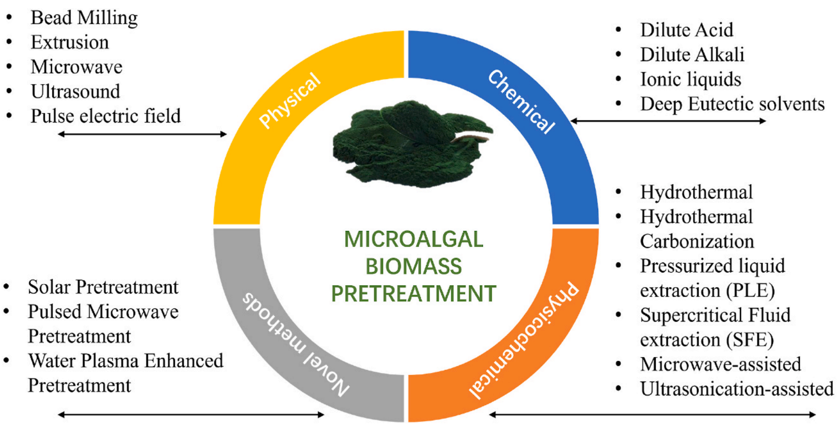

Fig. 6. Different pretreatment techniques for microalgal biomass [77].

also change its colour and taste. *Chlorella* contains sulfur compounds and fat-derived volatiles will produce a strong smell of the sea, dark green algae, used in food is not easy to be accepted by consumers. As a result, consumers may be sceptical about *Chlorella*-based foods. In addition, consumers' knowledge of microalgae is limited, and they are not fully aware of its health and environmental benefits.

These challenges are faced with *Chlorella*-based functional foods, as consumers are often unaware about the positive health effects of functional foods owing to a lack of understanding of this concept. Consumers tend to be more receptive to new foods if they provide tangible benefits. They are reluctant to sacrifice key product qualities (e.g. good taste) for more socially acceptable attributes (e.g. greater sustainability). Therefore, there is a need to understand the determinants of consumer behaviour towards microalgal foods and improve their acceptance of *Chlorella*-based foods. For example, as consumers demand a high level of acceptable food appearance, 3D printing technology can play a role in helping to providing attractive microalgal foods and promote the sales of *Chlorella*-based foods.

5. Practical applications of *Chlorella*-based foods

Owing to the issues in the application of *Chlorella*, in the previous section, it is important to explore the production of novel *Chlorella*-based food products and enrich the applications of *Chlorella*. The following discussion may facilitate the use of *Chlorella* in food applications. *Chlorella* is usually applied as whole-cell biomass, whereas the purpose of adding *Chlorella* to food is to improve its nutritional value. Therefore, *Chlorella* can be considered as a substitute for dietary fibre to provide nutritional value and promote gastrointestinal peristalsis. For example, *Chlorella* can be combined with roses, bitter melon, and other ingredients to make tea bags to mask its odour. Of course, the green and fishy smell of microalgae can be enhanced as a feature in green vegetable juices and seafood-flavored foods. It can also be combined with matcha and other materials of similar colour to make cakes or dairy products. This cannot be achieved by simply adding a certain amount of *Chlorella* during the traditional process. The species of *Chlorella*, the number of raw materials added, the baking temperature, and time are all factors that need to be considered. *Chlorella* may affect the nutritional, functional, organoleptic, and processing properties (colour, water activity, stability, and texture) of a product during its shelf life. In order to use *Chlorella* with disrupted cell walls as the raw material, effective wall-breaking technology, as introduced in part 4.2, such as ultrasonic wall breaking and, microwave wall breaking, should first be employed, *Chlorella* with disrupted cell walls can be added to a variety of foods, including fruit and vegetable juices, salads, biscuits, and bread, and its application in staple foods extensively explored. Dietary habits and nutritional awareness are important factors that influence consumer choices. Staple foods are foods that people consume often, and hence traditional foods may become potential substrates for *Chlorella*. For example, noodles and steamed stuffed buns are traditional Chinese foods, and Chinese consumers may prefer steamed buns to Italian noodles. Most staple foods and crops are carbohydrate-based products, which can lead to a deficiency in several essential nutrients needed for a healthy body, including macro- and micronutrients such as essential amino acids and, fatty acids. Therefore, staple foods are important carriers for enriching and fortifying the diet using *Chlorella* as a raw material. This is a developing trend in the field of food technology. Although some products containing microalgae are available in the international market, further research is required, and the successful development of new products depends on the early active participation of consumers.

6. Conclusion

Although *Chlorella* is rich in nutrients, and is becoming popular as a green, natural nutritional enhancer in food products, unprocessed *Chlorella* has characteristic odour that not conform to mainstream consumer preferences. This limits the application of *Chlorella* in food products. In the production of food containing *Chlorella*, the general need is to add appropriate flavour, using herbal, or other ingredients to mask its undesirable flavour; however, the effect is often unsatisfactory. In addition, the original *Chlorella* powder is blue-green, which affects the food's appearance. Removing the odour and improving the color of *Chlorella* have become the focus of research and development for the promotion of *Chlorella*-based food.

However, there are some challenges in incorporating *Chlorella* biomass into traditional products. With the increasing popularity of the healthy eating and the development of related technology, the way *Chlorella* is consumed is evolving from its initial form of tablets, capsules, or powders used as nutritional supplements. Here, we discuss several commercial products containing *Chlorella* and innovative studies on *Chlorella* biomass in the past few years. *Chlorella* is a novel ingredient that is gaining popularity, and is a valuable resource with the potential to be an important nutritional source for consumers worldwide. To promote the healthy development of *Chlorella* industry, more research and development should be put into breeding, harvesting, cell wall breaking, extraction, purification, and food application.

Ethics statement

Not applicable.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Xuechao Zheng: Writing – original draft, Funding acquisition, Data curation. **Lin Chen:** Writing – original draft, Data curation. **Lei Yin:** Writing – original draft, Software. **Huan Rao:** Writing – review & editing, Supervision. **Haowang Zheng:** Formal analysis, Conceptualization. **Chetian Xun:** Writing – original draft, Investigation. **Jianxiong Hao:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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