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Review

Bioactive Compounds from *Spirulina* spp.—Nutritional Value, Extraction, and Application in Food Industry

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Abstract: The surging popularity of plant-based diets and the growing emphasis on clean-label products have intensified interest in *Spirulina* within the food industry. As more people adopt vegetarian, vegan, or flexitarian lifestyles, demand for plant-based protein sources has escalated. *Spirulina*'s high protein content and complete amino acid profile make it an ideal candidate to meet this demand. However, incorporating *Spirulina* into food products is not without its challenges. Its strong, earthy, or fishy taste can be off-putting to consumers and difficult to mask in food formulations. Furthermore, isolating *Spirulina's* bioactive compounds while preserving their integrity is complex, especially considering the heat sensitivity of many of these components. Traditional extraction methods often employ high temperatures, which can degrade these valuable compounds. Consequently, there is a growing preference for non-thermal extraction techniques. This paper provides an overview of recent advancements in *Spirulina* cultivation, bioactive extraction, and their application in food products.

Keywords: *Spirulina* spp.; bioactive molecules' extraction; nutritional value; food products' fortification

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1. Introduction

The food industry is increasingly oriented toward using *Spirulina* due to its remarkable nutritional benefits, functional properties, and alignment with contemporary consumer trends emphasizing health, sustainability, and natural ingredients [1–4]. *Spirulina*, a blue-green alga, is celebrated as a superfood because it is incredibly nutrient-dense. It comprises about 60–70% protein by dry weight, making it one of the most protein-rich foods available [5–7]. *Spirulina* is a complete protein, containing all essential amino acids, which is particularly beneficial for vegetarians, vegans, and those seeking to increase their protein intake. Additionally, it is a rich source of vitamins and minerals, including B vitamins, iron, calcium, magnesium, and potassium, which are crucial for various bodily functions such as energy production and immune support [8–10].

The rise of plant-based diets and the clean-label movement has further fueled the food industry's interest in *Spirulina*. As more consumers adopt vegetarian, vegan, or flexitarian lifestyles, there is a growing demand for plant-based protein sources [11–14]. *Spirulina* fits this demand perfectly due to its high protein content and complete amino acid profile. Furthermore, its use as a natural colorant aligns with the increasing preference for products free from synthetic additives. The blue-green pigments, particularly phycocyanin, not only enhance the visual appeal of food products but also add nutritional value due to their antioxidant properties [15–17]. The environmental sustainability of *Spirulina* cultivation is another significant driver. *Spirulina* requires less land, water, and energy compared to traditional livestock farming and generates lower greenhouse gas emissions [18,19]. This makes it a sustainable alternative that can help reduce the ecological footprint of food production, appealing to eco-conscious consumers and companies.

Despite its numerous benefits, incorporating *Spirulina* into food products presents several challenges and limitations. One of the primary challenges is its strong, distinctive flavor, often described as earthy or fishy, which can be off-putting to some consumers and difficult to mask in food formulations [20,21]. This strong flavor can limit the versatility of *Spirulina* in various culinary applications. Additionally, the vibrant blue-green color of *Spirulina*, while appealing in some contexts, may not be suitable for all types of food products, especially those where a natural appearance is desired [22]. Stability is another concern. Some of *Spirulina*'s bioactive compounds, such as phycocyanin, are sensitive to heat and light, leading to degradation during processing and storage [23]. This degradation can reduce the nutritional and functional benefits of the final product. Ensuring consistent quality and safety of *Spirulina* can also be challenging due to variations in cultivation and harvesting practices. Compliance with regulatory standards and food safety measures is crucial to prevent contamination and ensure consumer safety [24–29]. Isolating bioactive compounds from *Spirulina* involves several challenges, including maintaining the integrity and functionality of heat-sensitive compounds [30]. Traditional extraction methods often involve high temperatures, which can degrade these sensitive bioactives. Non-thermal extraction techniques are increasingly preferred to address this issue. In this work overview of the latest finding regarding *Spirulina* spp. cultivation, the extraction of bioactives and implementation of the *Spirulina* in food products are given.

2. *Spirulina* spp. Cultivation

Representatives of the genus *Arthrospira* (*Spirulina*) cyanobacteriae, such as *Spirulina platensis*, *Spirulina maxima*, *Spirulina pacifica*, and *Spirulina fusiformis*, are widely used in photobiotechnology as a source of protein, essential amino acids, vitamins (especially B vitamins), β-carotene, and other vital compounds. *Spirulina*, renowned for its impressive nutritional profile, was cultivated by the Aztecs in the 16th century in the salty waters of Lake Texcoco. They dried the harvested algae and commercialized it as dehydrated cakes, recognizing its value as a nutritious food source [31]. Since the 1960s, *Spirulina* has been produced in industrial-scale cultivation systems and marketed globally. In 2003, the US Food and Drug Administration (FDA) granted *Spirulina* "GRAS" (Generally Recognized as Safe) status. Interestingly, a phenomenon known as "spiruliners" has emerged in southern France. Some farmers have transitioned from traditional agricultural practices to producing *Spirulina* biomass. The French Federation of *Spirulina* Producers (Fédération des Spiruliniers de France) now has around 150 members (http://www.spiruliniersdefrance.fr, accessed on 15 January 2024). This trend could also be feasible for many African countries, which have a favorable climate for *Spirulina* cultivation. Moreover, the Food and Agriculture Organization (FAO) recommends *Spirulina* cultivation as one of the viable solutions during humanitarian crises [32]. The cultivation of *Spirulina* requires significantly less space than conventional farming, such as poultry and vegetable farms, using around 49 to 132 times less area [33].

Spirulina thrives in alkaline conditions (pH 8.5–11), making its culture resistant to contamination by bacteria and other microalgae. The two most important species of *Spirulina* are *Spirulina maxima* and *Spirulina platensis*. It forms trichomes (helixes) about 0.5 mm in length, which are sufficiently large to allow for simple and cost-effective separation from the culture media through filtration [32]. *Spirulina* is cultivated in numerous countries, including Israel, the United States, India, China, Japan, Taiwan, Italy, Germany, Thailand, France, and Egypt. The cultivation methods involve open cultivation in artificial ponds, advanced open photobioreactors using sunlight, and closed photobioreactors with artificial lighting and controlled temperatures. The advantages of these cultivation methods include the ease of harvesting and drying the biomass, the efficient extraction of cell contents, and high biological value [34].

Currently, two main technologies are utilized for cultivating *Spirulina*: closed photobioreactors (PBRs) and open ponds. Both approaches are commercially employed to produce high-value products [35]. Another classification criterion is the source of illumination—artificial or solar. However, for sustainable large-scale microalgae production, only solar cultivation systems are considered feasible. Branyikova and Lucakova [32] reported that the most important requirements for a cultivation system are the following:

1. Suitable illumination: Both low and excessive light can limit microalgal growth. The PBR's geometry and location determine the amount and distribution of light throughout the day and year, affecting the cultivation season length.
2. Adequate carbon dioxide supply: Atmospheric CO_2 levels are too low for optimal microalgae growth. Depending on the system, CO_2 concentrations from 1 to 100% in the aeration gas are used.
3. Efficient mixing: This prevents microalgae from settling and biofilm formation, ensuring uniform light distribution and promoting photosynthesis through short light/dark cycles.
4. Appropriate construction material: It should prevent biofilm formation, be durable, resistant to solar radiation, and suitable for saline water.
5. Effective oxygen release: Excess oxygen from photosynthesis can lower productivity by reducing photosynthetic activity. Managing oxygen levels is crucial.
6. Suitable temperature: Overheating from sunlight can damage microalgae. In open systems, water evaporation helps control temperature, while closed systems require thermostatic regulation or surface spraying.
7. Ease of cleaning and operation: The PBR should be easy to clean, sanitize, and operate effectively [36].

A significant drawback of *Spirulina* cultivation is the high cost of chemical-based culture media. Currently, many companies use chemical-based media such as Zarrouk, Conway, and Kosaric for *Spirulina* cultivation. Zarrouk's medium has long been recognized as the standard and optimal medium for various *Spirulina* species. In large-scale industrial production, it remains the sole conventional medium used for *Spirulina* cultivation [37]. Comprising primarily sodium bicarbonate, along with sodium nitrate, potassium sulfate, magnesium sulfate, calcium chloride, and dipotassium hydrogen phosphate, Zarrouk's medium supports efficient biomass growth by providing essential nutritional supplements [38]. However, the Zarrouk culture medium is not sustainable in the long run due to its high cost [39]. At approximately USD 0.08 per liter, it represents about 35% of the total cost of algal biomass production [40]. Therefore, the scientific community has been exploring various alternative nutrient sources, such as seawater, vermicompost, and wastewater, to reduce the cost of chemical-based culture media. Among these, wastewater shows promise as an alternative nutrient source [41]. An overview of the current challenges and appropriate solutions in *Spirulina* spp. cultivation is presented in Table 1.

Table 1. The challenges and potential solutions in *Spirulina* spp. cultivation.

	Challenges	Solutions
Environmental Control	Temperature:*Spirulina* requires temperatures between 35 °C and 37 °C [42]. Light: Needs optimal light conditions for photosynthesis [42]. pH Levels: Requires a highly alkaline environment (pH 8.5–10.5) [1]. Water Quality: High-quality water is essential.	Temperature Control: Use of greenhouses or temperature-regulated systems can help maintain optimal temperatures. Light Management: Implementing artificial lighting or shading systems can control light intensity. pH Regulation: Regular monitoring and adjustment of pH using buffers can maintain the required alkalinity. Water Treatment: Utilizing filtration and water treatment systems to ensure water quality and prevent contamination.

Table 1. *Cont.*

	Challenges	Solutions
Contamination	Competing Algae and Microorganisms: Invasion by other microorganisms. Predators: Protozoa and zooplankton feeding on *Spirulina*.	Sterile Techniques: Implementing sterile techniques during inoculation and handling. Closed Systems: Using closed photobioreactors reduces the risk of contamination. Biocontrol Agents: Employing beneficial microorganisms or biocides to control unwanted species.
Nutrient Supply Challenges	Nutrient Requirements: Consistent supply of nutrients. Cost of Nutrients: High costs of nutrients like nitrogen, phosphorus, and trace elements [43].	Nutrient Recycling: Implementing nutrient recycling systems can reduce costs. Alternative Sources: Using cheaper or more sustainable nutrient sources, such as agricultural byproducts. Optimized Formulations: Developing optimized nutrient formulations to minimize waste.
Production System Challenges	Open vs. Closed Systems: Balancing contamination risk and cost [44,45]. Scalability: Maintaining efficiency and consistency at larger scales.	Hybrid Systems: Combining the advantages of open and closed systems. Modular Designs: Using modular photobioreactors for easier scalability. Automation: Implementing automated monitoring and control systems to maintain optimal conditions.
Harvesting and Processing Challenges	Efficient Harvesting: Need for efficient and cost-effective harvesting methods [46]. Post-Harvest Processing: Energy-intensive drying and processing methods [46].	Innovative Harvesting Techniques: Using methods like flocculation, electroflotation, or membrane filtration. Energy-Efficient Drying: Implementing solar drying or low-energy dehydration techniques. Integrated Processing: Developing integrated processing systems that combine harvesting and drying to reduce energy use.
Economic and Market Factors Challenges	Production Costs: High costs of cultivation and processing [47]. Market Demand: Fluctuations in demand and prices [48]. Regulatory Issues: Compliance with food safety and quality regulations.	Cost Reduction: Streamlining operations and using cost-effective technologies. Market Diversification: Exploring new markets and applications for *Spirulina*. Regulatory Compliance: Staying updated with regulations and implementing quality control systems to ensure compliance.

3. Nutritional Value of *Spirulina* (Macronutrients and Micronutrients)

Spirulina (Arthrospira) is the most nutritious and concentrated food known to mankind, rich in antioxidants, phytonutrients, probiotics, and nutraceuticals [49–51]. Its impressive nutrient composition makes it a promising solution to various dietary demands and suitable for therapeutic uses. These species have a high content of micro- and macronutrients. Their cell wall is composed of polysaccharides with a digestibility of 86%, making them easily absorbed by the human body. *Spirulina* is indeed a nutrient-rich source, containing a significant amount of protein (60–70% of its dry weight) and essential amino acids (47% of the total protein weight) which makes them complete proteins [35]. Remarkably, the protein proportion of *Spirulina* is higher than that of commonly used plant or animal protein sources, such as soybeans (35%), peanuts (25%), cereals (8–14%), meat and fish (15–25%), eggs (12%), milk powder (35%), and whole milk (3%). The protein content of *Spirulina* can vary by 50–75% depending on the time of harvest, with the highest protein values typically obtained from those harvested at early daylight [51]. In addition to its high protein content, *Spirulina* is rich in carbohydrates (15–25%), primarily polysaccharides (glucosans and rhamnosans) and mono- or disaccharides (glucose, fructose, and sucrose). It also contains lipids (6–8%), with a significant portion being essential fatty acids (1.3–15%), predominantly palmitic acid, γ-linolenic acid (GLA), linoleic acid, and oleic acid. *Spirulina* provides all essential minerals (7–13%), including potassium, calcium, chromium, copper, iron, magnesium, manganese, phosphorus, selenium, sodium, and zinc. It is also rich in vitamins, particularly several B vitamins (B1, B2, B3, B6, B9, and B12), as well as provitamin A and vitamins C, D, and E. Additionally, *Spirulina* contains natural photosynthetic pigments, serving as the main source of phycocyanin (14–20%), along with chlorophylls (1%) and carotenoids (0.5%). The average amounts of the most important groups of nutrients in *Spirulina* are presented in Figure 1a. Phycocyanin is a blue, water-soluble phycobiliprotein that remains stable at pH levels ranging from 5 to 8 and is found in blue algae. Other significant phycobiliproteins, such as phycoerythrin (red) and allophycocyanin (blue), are also present in microalgae from the *Spirulina* genus. Chlorophyll is a phytochemical responsible for the green color of this microalgae and plays a crucial role in the photosynthesis process. Flavonoids and phenolic acids are the primary classes of phenolic compounds found in *Spirulina* [3,35,52,53]. Unlike its protein content, *Spirulina* contains less fat, which makes it less susceptible to lipid oxidation and rancidity. Generally, *Spirulina* contains 6–8% lipids by dry weight, but this can reach up to 11%. The fatty acids composition and profile of a particular *Spirulina* species, which range from 12 to 22 carbons in length, are influenced by various factors such as the composition of the growth medium, aeration rate, temperature, light/dark cycle ratio, and illumination intensity. The antioxidant compounds present in *Spirulina* include polyunsaturated fatty acids, phycocyanin, phenolics, β-carotene (about 30 times higher than in carrots), other carotenoids, and vitamin E. These components are believed to be responsible for *Spirulina's* therapeutic properties. The application of carotenoids in foods includes their use as additives for coloring and flavoring, as well as for vitamin A supplementation [54]. The main carotenoids found in *Spirulina* are β-carotene, canthaxanthin, astaxanthin, lutein, and zeaxanthin. The extraction of polyunsaturated fatty acids (especially GLA) from *Spirulina* is costly, making direct consumption of *Spirulina* as a nutritional supplement the most cost-effective way to obtain GLA [37,55]. It is important to mention that there are differences in nutrient status among the various species of *Spirulina*, including *Spirulina platensis*, *Spirulina maxima*, *Spirulina pacifica*, and *Spirulina fusiformis*. While all these species share a similar nutritional profile, there are variations in the concentrations of specific nutrients due to differences in their growth environments, cultivation conditions, and genetic makeup. The comparison of the key nutritional aspects among these species is given in Figure 1b.

(a)

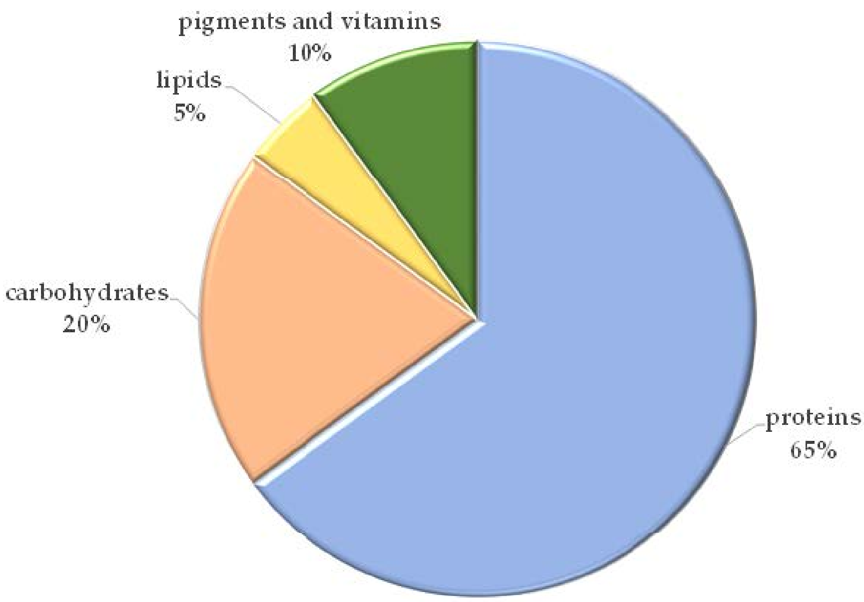

Figure 1. *Cont.*

(b)

	Protein content	Vitamins and minerals	Antioxidants	Fatty acids	Other components
Spirulina platensis	55–70% of dry weight	Rich in B vitamins, vitamin E and minerals (Fe, Ca, Mg, and K)	Phycocyanin, Beta-carotene, Chlorophyll	Gamma-linoleic acid	Polysaccharides and sulfolipids
Spirulina maxima	60–70% of dry weight	Higher levels of Fe and Zn than in *S. platensis*	Phycocyanin, Beta-carotene	Essential fatty acids	Polysaccharides and sulfolipids
Spirulina pacifica	60–70% of dry weight	Rich in B vitamins, vitamin K and minerals (Fe and Ca)	Phycocyanin, Beta-carotene	Essential fatty acids	Polysaccharides and sulfolipids
Spirulina fufiformis	50–60% of dry weight	Similar to other species	Phycocyanin, Beta-carotene, Chlorophyll	Essential fatty acids	Polysaccharides and sulfolipids

Figure 1. (**a**) The average amounts of the most important groups of nutrients in *Spirulina*. (**b**) Differences in nutrient status among the various species of *Spirulina*, including *Spirulina platensis*, *Spirulina maxima*, *Spirulina pacifica*, and *Spirulina fusiformis* [7,37,56–61].

4. Bioactives' Extraction from *Spirulina*

The extraction of bioactive compounds from *Spirulina* is a promising field that enhances the potential health benefits of this microalgae. Traditional extraction methods for *Spirulina* focus on simplicity and cost-effectiveness, often prioritizing basic mechanical and chemical processes. These methods include the following: (i) Mechanical Disruption: This involves physically breaking down *Spirulina* cells using grinding, milling, or bead beating. While effective for extracting bulk components like proteins and lipids, this method can result in the loss of sensitive bioactive compounds due to heat and oxidative stress; (ii) Solvent extraction: Organic solvents such as hexane, ethanol, or acetone have traditionally been used to extract lipids, pigments, and other hydrophobic compounds from *Spirulina*. This method is straightforward but can be less selective, leading to the co-extraction of unwanted materials and potential solvent residues in the final product; and (iii) Alkaline and Acidic Extraction: Proteins and polysaccharides are often extracted using alkaline or acidic conditions to solubilize the components, followed by precipitation and purification [61–64]. This method can degrade sensitive molecules and reduce the overall bioactivity of the extracts.

Spirulina, particularly species like *Spirulina platensis*, *Spirulina maxima*, *Spirulina pacifica*, and *Spirulina fusiformis*, is rich in a variety of bioactive compounds including phycocyanin, polysaccharides, phenolic acids, tocopherols, and polyunsaturated fatty acids. The list of key components of *Spirulina* that contribute to their therapeutic potential, along with their mechanisms of action are given in Table 2.

Table 2. Key components of *Spirulina* that contribute to their therapeutic potential, along with their mechanisms of action.

Spirulina Component	Mechanism of Action
Phycocyanin	Antioxidant Activity: Phycocyanin scavenges reactive oxygen species (ROS) and reactive nitrogen species (RNS), reducing oxidative stress at the cellular level. It protects cells from damage by neutralizing free radicals, which can otherwise lead to chronic inflammation and various diseases, including cancer [62–65].
	Anti-Inflammatory Effects: Phycocyanin inhibits the enzyme cyclooxygenase-2 (COX-2), which is involved in the synthesis of pro-inflammatory prostaglandins. It also downregulates the expression of pro-inflammatory cytokines like tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6). This dual action helps in reducing inflammation and providing relief from inflammatory conditions such as arthritis [62–65].

Table 2. *Cont.*

Spirulina Component	Mechanism of Action
Gamma-Linolenic Acid	Anti-Inflammatory Pathway: Once ingested, GLA is converted in the body to dihomo-γ-linolenic acid (DGLA), which competes with arachidonic acid for the same enzymatic pathways. This competition results in the production of anti-inflammatory prostaglandins (PGE1) instead of pro-inflammatory ones (PGE2). This shift helps to reduce inflammation, making GLA effective in managing conditions such as rheumatoid arthritis and eczema [66–70].
	Skin Health: GLA's role in maintaining skin barrier integrity and reducing transepidermal water loss makes it beneficial in treating skin conditions like atopic dermatitis [66–70].
Polysaccharides	Immune Modulation: These polysaccharides enhance the activity of macrophages, natural killer (NK) cells, and T-lymphocytes. They stimulate the production of cytokines such as interferon-gamma (IFN-γ) and interleukin-1 (IL-1), which boost the immune response against infections and tumors [71–76].
	Antiviral Activity: The sulfated polysaccharides in *Spirulina* can inhibit the replication of viruses by mimicking heparan sulfate, a molecule that viruses typically bind to for entry into host cells. By blocking this interaction, these polysaccharides prevent viruses like HIV and herpes simplex virus from attaching to and entering human cells, thereby reducing infection rates [71–76].
Vitamins and Minerals	B-Complex Vitamins: These are essential for energy metabolism, particularly in converting carbohydrates, fats, and proteins into energy. They also play a key role in maintaining nerve function and red blood cell formation [5,77–83].
	Vitamin E: As a potent antioxidant, vitamin E protects cells from oxidative damage by neutralizing free radicals. This vitamin is particularly important for skin health and immune function [5,77–83].
	Iron: The bioavailable iron in *Spirulina* helps in the formation of hemoglobin and myoglobin, which are crucial for oxygen transport in the blood and muscle tissues. This makes *Spirulina* an excellent supplement for preventing or treating iron-deficiency anemia [5,77–83].
	Calcium and Magnesium: These minerals are vital for bone health, muscle function, and nerve transmission. They also play a role in cardiovascular health by regulating blood pressure and heart rhythm [5,77–83].
	Selenium Antioxidant Defense: Selenium's role in the formation of selenoproteins enhances the body's antioxidant defense system, reducing the risk of chronic diseases linked to oxidative stress, including cancer and cardiovascular diseases [5,77–83]. Selenium Thyroid Function: Selenium is crucial for the synthesis of thyroid hormones, which regulate metabolism, growth, and development. Adequate selenium intake can help prevent thyroid-related disorders [5,77–83].
Phenolic Compounds	Antioxidant Action: Phenolic compounds scavenge free radicals and chelate metal ions, reducing oxidative stress. This action protects cells from DNA damage, which can lead to mutations and cancer development [50,80,81,83–86].
	Anti-Cancer Potential: Some phenolic compounds in *Spirulina* can induce apoptosis (programmed cell death) in cancer cells and inhibit their proliferation by interfering with cell cycle progression. This makes *Spirulina* a potential adjunct in cancer therapy [50,80,81,83–86].

Polysaccharides, a significant group of bioactive compounds in *Spirulina*, exhibit immunomodulatory and anti-cancer properties. These can be extracted through hot water extraction methods, followed by alcohol precipitation and dialysis [75,87,88] (Table 3). The extracted polysaccharides are used in various health supplements to boost immune function and improve gut health. Phenolic acids and tocopherols, known for their antioxidant activities, can be extracted using organic solvents like ethanol or methanol. These compounds contribute to a reduction in oxidative stress and the prevention of chronic diseases. The solvent extraction method is often followed by techniques such as solid-phase extraction or high-performance liquid chromatography (HPLC) for further purification and quantification. Polyunsaturated fatty acids, including gamma-linolenic acid (GLA), are extracted using supercritical fluid extraction or solvent extraction techniques. These fatty acids are essential for cardiovascular health, inflammation reduction, and overall metabolic function.

Table 3. Limitations in bioactive extraction form of *Spirulina*.

Challenges	Possible Solutions
Complex Matrix: *Spirulina* has a complex matrix with various components like proteins, polysaccharides, lipids, and pigments, which can interfere with the extraction of specific bioactive compounds [89–91].	Use selective extraction methods such as supercritical fluid extraction or enzyme-assisted extraction to target specific bioactive compounds while minimizing interference from other components [92–95].
Thermal Sensitivity: Many bioactive compounds in *Spirulina*, such as phycocyanin and vitamins, are sensitive to heat, which can lead to their degradation during extraction [96–98].	Employ non-thermal extraction techniques like cold press extraction, ultrasonic-assisted extraction, or supercritical CO_2 extraction to preserve heat-sensitive bioactives.
Solubility Issues: Some bioactive compounds have poor solubility in conventional solvents, making their extraction challenging [3,99,100].	Utilize a combination of solvents or surfactants, or explore solvent-free techniques like supercritical fluid extraction to enhance the solubility and extraction efficiency of these compounds.
Yield and Purity: Achieving high yield and purity of specific bioactive compounds can be difficult due to the presence of multiple compounds with similar properties [101–103].	Optimize extraction conditions (e.g., solvent type, pH, temperature, and time) and implement purification techniques such as chromatography or membrane filtration to improve yield and purity.
Environmental and Health Concerns: The use of organic solvents in extraction processes can pose environmental and health risks [18,104,105].	Adopt green extraction methods such as supercritical fluid extraction, pressurized liquid extraction, or ionic liquid-based extraction, which are more environmentally friendly and safer.
Scalability: Scaling up laboratory-scale extraction processes to industrial levels can be challenging due to differences in efficiency and cost [105,106].	Conduct pilot-scale studies to optimize and validate extraction processes before full-scale production. Utilize scalable and cost-effective technologies.

Phycocyanin, a vibrant blue pigment with potent antioxidant and anti-inflammatory properties, is one of the most studied bioactive compounds in *Spirulina*. It can be efficiently extracted using aqueous buffers, often followed by purification steps like ammonium sulfate precipitation and chromatography. This pigment is not only valued for its health benefits but also as a natural colorant in the food industry. Methods for extracting and purifying phycocyanin from *Spirulina* are being developed in applied research laboratories [107]. Several methods for extracting phycocyanin have been described in the literature, including freeze/thaw, mixing/homogenization, bead milling, ultrasonic, moderate electric field, pulsed electric fields, high-pressure homogenization, microwaves, high-pressure processing, and enzymatic extraction (Figure 2).

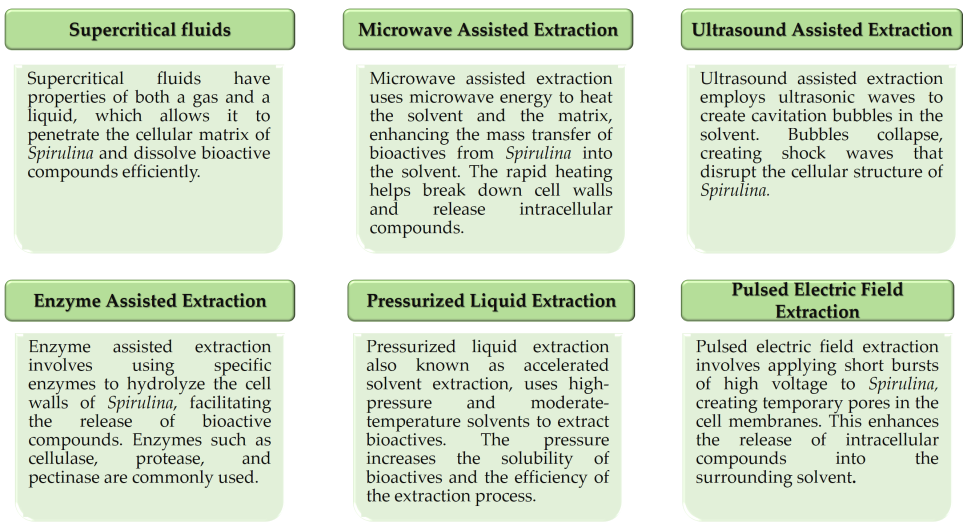

Figure 2. Advantages of using the most-used non-thermal extraction methods for extracting the bioactive form of *Spirulina*.

However, only some of these methods provide reliable purity values and have detailed comparisons of optimal conditions and parameters (Table 4). The choice of the best method depends on factors such as time, cost, and yield, as well as the intended amount of phycocyanin, whether the production is small or large scale, and the final application of the product [52]. The extraction of C-phycocyanin (C-PC) is primarily influenced by several physical and chemical variables: temperature, pH, solvent type, biomass-to-solvent ratio, and the form of the biomass (dried or fresh) [16]. C-PC extraction can be carried out at moderate temperatures (up to 50 °C), neutral pH values (6–8), and with precautions to prevent light exposure. At the laboratory scale, *Spirulina* cells are typically disrupted using freezing and thawing cycles, which often yield relatively high-purity extracts. Despite its common use, this method lacks comprehensive optimization studies. Future research should focus on optimizing parameters such as the number of cycles, duration, temperature, solvent type, and biomass-to-solvent ratio [108]. Mechanical cell disruption methods, such as bead milling and ultrasound, are more amenable to scaling up. However, these methods often produce extracts with lower purity due to the intense cell disruption, necessitating a subsequent purification step after extraction [52,109]. Microwave extraction has the disadvantage of requiring relatively high temperatures, which are not ideal for C-PC extraction [107]. While moderate electric fields (MEFs) offer potential, further research is needed to fully understand their effects on *Spirulina* cells [16]. Other methods, such as mixing and homogenization, tend to be time-consuming and often result in extracts with lower purity [110]. Regarding high-pressure processing (HPP), further studies are needed to optimize the extraction process and prevent C-phycocyanin degradation [111]. Among the reviewed extraction methods, pulsed electric fields (PEFs) appear to be the most promising technology for C-PC extraction, as they yield highly concentrated extracts with relatively high purity [112]. Enzymatic extraction is increasingly recognized for its potential as a more efficient and eco-friendlier alternative to traditional methods. This technique can be conducted at lower temperatures and pressures, which helps to preserve the stability and quality of phycocyanin. In one study, effective extraction of phycocyanin was achieved using Collupulin protease in combination with the application of a pulsed electric field. This combination of a pulsed electric field with enzymes presents a promising technology for phycocyanin extraction [113].

Table 4. Overview of some examples of extraction processes of bioactive form of *Spirulina* spp.

Extractable Component	Extraction Method and Extraction Solvent	Extraction Yield	Reference
Proteins	Osborne sequential extraction procedure.	Extracted three fractions of albumins (51.5%), globulins 2.4%), and prolamins (46.1%).	[114]
	Agitation, bead milling, ultrasound, and protein isolation via precipitation using ethanol.	The most successful protein extraction method involved bead milling for 24 h, the addition of 1 M NaCl, and pH adjustment to 7, followed by precipitation with 75% ethanol, as shown in Figure 2. This method yielded a protein content of 58.19% ± 6.23, with an extraction yield of 23.66%.	[115]
	Ultrasound-assisted process followed by isoelectric precipitation.	Extraction yield of 40.0 ± 1.91 wt%, and a protein yield of 43.6 ± 0.93 wt%.	[116]
	Ultrasound-assisted extraction using methanol–ethanol mixtures as solvent.	Protein recovery from dry *Spirulina* was 42.55 ± 0.43%, obtained by using 20 mL of the mixture of methanol and ethanol at 50 min of extraction time.	[117]

Table 4. *Cont.*

Extractable Component	Extraction Method and Extraction Solvent	Extraction Yield	Reference
Proteins	Cellulase enzyme supported the recovery process.	The highest protein recovery efficiency (40.13 ± 2.87%) was 50 UI/g dry algae for enzyme activity, 1:20 for the ratio of dry algae/solvent, and 7.0 for pH value with a process temperature of 50 °C for 90 min.	[118]
	Ultrasonic-assisted extraction.	UAE working at 80% amplitude for 30 min under a duty cycle of 60% could significantly improve the protein yield to 76.83% compared with conventional extraction without ultrasound (32.48%).	[119]
	Ultrasonic aqueous extraction at acidic, alkali, and neutral conditions was studied. *S. platensis* was soaked in the solvent for 12 h prior to extraction.	Neutral conditions showed a higher protein release of 18.6 mg/mL at optimum conditions (solid–liquid ratio 1:20, 80% ultrasound amplitude, 100% ultrasound duty cycle, and 4 °C).	[120]
	High-pressure homogenization and subsequent protein isolation by solubilization at alkaline pH followed by precipitation at acidic pH.	The optimized process conditions were found to be pH 11.38, solubilization time of 35 min, and biomass concentration of 3.6% (*w*/*w*) solids for the solubilization step, and pH 4.01 and precipitation time of 60 min for the precipitation step. At the optimized conditions, a protein yield of 60.7% (*w*/*w*) was obtained.	[121]
	Solubilization and precipitation.	A sonication pre-treatment (400 W, 24 kHz, 2 min) resulted in the solubilization of 96.2% of total proteins. The optimized precipitation conditions, which were pH 3.89 over 45 min, resulted in a protein recovery of 75.2%.	[122]
Polysaccharides	Maceration with a hot alkali solution and further fractionated by DEAE-52 cellulose and Sephadex G-100 chromatography into two purified fractions		[75]
	Hot-water extraction, alkali extraction, ultrasonic-assisted extraction, and freeze/thaw extraction	The alkali extraction method was determined as the optimal method, with the optimized extraction process consisting of a solid–liquid ratio of 1:50, a pH value of 10.25, a temperature of 89.24 °C, and a time of 9.99 h. The final extract contained 71.65% of polysaccharide and 8.54% of protein.	[93]
	Solid-liquid aqueous extraction	A polysaccharide yield of around 8.3% dry weight was obtained under the following optimized conditions: solid-to-liquid ratio of 1:45, temperature of 90 °C, and time of 120 min.	[123]
	Hot water, ultrasonic-assisted, lye and freeze/thaw extraction	The efficiency of four extraction methods was 8.35%, 65%, 76.9%, and 85.1% for hot water, lye, and ultrasound-assisted and freeze/thaw extraction methods, respectively.	[124]
	Hot water extraction	Total carbohydrate contents record 325 ± 9.5 mg/g in the soluble polysaccharide fraction.	[125]

Table 4. *Cont.*

Extractable Component	Extraction Method and Extraction Solvent	Extraction Yield	Reference
Polysaccharides	Microwave-assisted process, using water as a solvent	To achieve the best extraction conditions, the biomass was treated for 20 min to eliminate pigments and lipids. Then, the biomass was extracted for 1 min at a power of 434 W, with a biomass/solvent ratio of 1:30 *w*/*v* and no additional contact. This resulted in a carbohydrate content of 127 ± 5 mg/g of biomass.	[17]
Lipids	Ultrasound-assisted extraction and mechanical stirring	The lipid content of the extract at optimal conditions was 8.7%. The predominant fatty acids were palmitic acid (44.5%), linoleic acid (14.9%), and gamma-linolenic acid (13.4%).	[90]
	Maceration, osmotic, and Soxhlet extraction methods	Lipids obtained from maceration, osmotic, and Soxhlet extraction methods were 5.5%, 0.6%, and 9%.	[126]
	Extractions using green solvents: supercritical CO_2 (scCO_2), high-pressure (HP) ethanol, and scCO_2 with ethanol as a co-solvent (60 °C, 400 bar); compared to conventional lab-scale extractions: Soxhlet, Bligh and Dyer (B&D), and ultrasound-assisted extraction	Total extract yield using B&D showed the highest value (11.6%), followed by HP ethanol (11.4%); nevertheless, scCO_2 resulted in better selectivity for fatty acid content (0.57 g/g lipids).	[127]
	Solvent extraction, Soxhlet extraction, and ultrasonic-assisted extraction	The highest percentage oil yield was obtained by ultrasonication (6.6% for *Spirulina* sp.) followed by the Soxhlet and solvent extraction processes.	[128]
	Ultrasound-assisted osmotic shock method	Ultrasound irradiation increased lipid yields to 6.65% in the presence of 11.9% NaCl, a solvent/biomass ratio of 12:1 *v*/*w*, and a 22 min extraction time.	[129]
	Supercritical carbon dioxide (CO_2) extraction	Extract analyses showed that oil extracts contained chlorophylls a and b, and beta-carotene.	[130]
Pigments	Pulsed electric fields pre-treatment combined with the binary mixture EtOH/H_2O, 50:50, *v*/*v*, for 60–120 min	Recovery of 55–60%, 85–90%, and 60–70% was obtained for chlorophylls, carotenoids, and total phenolic compounds	[100]
	Ultrasonic- and microwave-assisted extraction	When phycocyanin was extracted using ultrasound rather than microwave, the yield and purity index of the product were higher. The maximum yield of 14.88 mg/g and a purity index of 1.60 was attained after 1 h at 40 °C and 40 kHz ultrasonic wave frequency.	[92]
	Ultrasonication, centrifugation, freezing/thawing cycle, and water extraction	This approach succeeded in producing three main types of photosynthetic pigments: carotenoids, chlorophylls, and phycocyanin from the same batch of *Spirulina*.	[131]
	Ultrasound-assisted extraction using deep eutectic solvents	Optimal extraction parameters were temperature: 60 °C; extraction cycle time: 20 min; and a solvent-to-biomass ratio of 70 mL/mg. Under optimal conditions, the experimental value for total pigment yield was 165.19 ± 1.01 mg/g dry matter.	[132]

Table 4. *Cont.*

Extractable Component	Extraction Method and Extraction Solvent	Extraction Yield	Reference
Pigments	Supercritical CO_2 extraction	The total carotenoid, chlorophyll a, and chlorophyll b contents in the extracts were equal to 3.5 ± 0.2 mg/g, 5.7 ± 0.2 mg/g, and 3.4 ± 0.3 mg/g, respectively (by dry *Spirulina* weight).	[133]
	Four-step process including cell lysis through freezing/thawing, maceration, centrifugation, and tangential microfiltration	A phycocyanin purity index of 1.32 was achieved.	[134]
	Freezing/thawing extraction with 0.01 M phosphate buffer pH 7	A phycocyanin purity index of 1.729 was achieved.	[135]

5. *Spirulina* spp. as Functional Food

Spirulina boasts an excellent nutritional and bioactive composition, making it valuable to both the food and pharmaceutical industries. The bioactive compounds it contains enable its incorporation into various food formulations. Consequently, *Spirulina* can be highlighted for diverse applications, such as a protein supplement for vegans, a blue dye for infant formulas, a source of pro-vitamin A for the general population, and for creating potentially functional foods [3]. One of the most compelling aspects of *Spirulina* in functional foods is its potential to combat malnutrition [136]. In regions where protein and micronutrient deficiencies are prevalent, incorporating *Spirulina* into staple foods could significantly improve the nutritional status of the population [10,85,137]. Its ability to thrive in harsh environmental conditions and its rapid growth rate make it a sustainable and efficient option for large-scale cultivation, offering a reliable source of nutrition in food-insecure areas [59,138,139].

Spirulina's antioxidant properties, primarily derived from compounds like phycocyanin, chlorophyll, and carotenoids, contribute to its appeal as a functional food ingredient [84,140–142]. These antioxidants play a crucial role in neutralizing free radicals, reducing oxidative stress, and preventing chronic diseases such as cardiovascular diseases, diabetes, and cancer. By incorporating *Spirulina* into everyday foods such as smoothies, energy bars, pasta, and even baked goods, manufacturers can create products that support health and wellness while appealing to health-conscious consumers [3] (Table 5). Currently, due to a greater demand for natural compounds, the large-scale cultivation of *Spirulina* is focused on the production of high-value proteins, mainly phycocyanin (blue pigment), which, according to its purity grade, can be used in food and other industries [143]. Like any food or dietary supplement, commercialized *Spirulina* is regulated to ensure its safety and quality. This includes adhering to requirements for the production process to prevent contaminants (such as microcystins, toxic metals, and pathogenic bacteria), as well as regulations for labeling and packaging [52]. *Spirulina* is an excellent complete nutritional food source, providing protein, beta-carotene, GLA, B vitamins, minerals, chlorophyll, sulfolipids, glycolipids, superoxide dismutase, phycocyanin, enzymes, RNA, and DNA. It supplies many nutrients that are often lacking in most people's diets. The lipid profile of *Spirulina* consists of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs). Notably, the polyunsaturated fatty acids in *Spirulina* have the potential to be utilized in specialized diets for managing lipid metabolism disorders [144]. Furthermore, *Spirulina* contains all nine essential amino acids, as well as other amino acids that form the proteins in these microalgae. This nutritional matrix includes essential amino acids like tryptophan, threonine, leucine, lysine, methionine, phenylalanine, histidine, and valine, which the human body cannot produce on its own. Therefore, *Spirulina* is a valuable source of both essential and non-essential amino acids, with the potential to enhance foods that have low protein content [3]. *Spirulina* is gaining attention due to its potent antiviral, anti-cancer, hypocholesterolemic, and health-improving properties. *Spirulina* offers a range of health benefits. It helps athletes maintain long-lasting energy and vitality, supports digestion, assimilation, and elimination, and aids in preventing diabetes [77,145–149]. It also helps reduce stress and depression while contributing to weight loss due to its concentrated nutrients. Additionally, it promotes tissue repair in wounds and burns, possesses anti-infectious properties, decreases cholesterol levels, lowers cardiovascular disease risk, acts as an anti-inflammatory agent, and reduces arthritis inflammation [35].

Table 5. Examples of *Spirulina*-enriched food products.

Food Product	Amount of Added *Spirulina*	Enriched Food Product Properties	Reference
Pasta	From 2 to 15% of *Spirulina* sp. CCC 540 powder	Green color pasta with nutritional and functional fortification resulting in an increase in its protein, total phenols, flavonoids, iron, and calcium content by up to 77.47%, 76.62%, 162.88%, 296.99%, and 57.27%, respectively.	[20]
Pasta	Pasta formulations included free *Spirulina* or microencapsulated *Spirulina*	The microencapsulation protected the antioxidant potential of *Spirulina* in 37.8% of the conditions of pasta cooking.	[150]
Bread	1.5 and 2.5% of *Arthrospira* sp. to flour weight	*Spirulina* addition significantly affected bread weight and volume.	[151]
Extruded snacks	From 0.4 to 2.6% of *Spirulina* sp. LEB 18	Higher concentrations of microalga produced snacks with higher protein content, total color difference (ΔE), and compact structure.	[152]
Broccoli soup	0.5 to 2.0% (*w*/*v*) of *Spirulina* sp., *Chlorella* sp., or *Tetraselmis* sp.	Incorporation of freeze-dried microalgae biomass into the broccoli soup resulted in lower L^* values, increased content of polyphenols, and higher antioxidant capacity.	[153]
Functional cheese	*Spirulina platensis* and *Chlorella vulgaris* microalgae	Cheese enriched with *Spirulina platensis* (3%), *Chlorella vulgaris* (1.467%), and curcumin (1%) had a higher antioxidant activity and iron content than the control cheese.	[21]
Chocolate milk	Spray-dried microencapsulated *Spirulina* sp. LEB-18 was used in concentrations of 5 and 8.75%	The incorporation of different microencapsulated microalgae in chocolate milk presented an increase in protein content and reduced total lipids.	[154]
Vegan muffins	*Chlorella vulgaris* in concentrations of 0, 0.5, 1.0, and 1.5% (g/100 g flour)	Incorporating a mixture of spelt and wheat flour along with a 1.5% addition of microalgae made the dough more viscous, leading to a fine, porous microstructure and a crumbly texture in the muffins.	[155]
Sourdough "crostini"	Three concentrations of *Arthrospira platensis* F&M-C256 were tested: 2, 6, and 10% (*w*/*w*)	*A. platensis* F&M-C256 "crostini" showed higher protein content compared to the control.	[156]
Whole-wheat pasta	2% of *Himanthalia elongata* and 1.5% of *Spirulina*	A significant increase in fat (70.4%), protein (29.7%), ash (26.5%), and total amino acid contents in the raw algae-enriched pasta. The antioxidant activity was also higher.	[157]
Yogurt	*Spirulina platensis* in three different concentrations (0.5, 1, 1.5%, and a control without *Spirulina*)	The protein content of colored yogurt increased proportionally with added *Spirulina* powder. An increase in *Spirulina* content caused it to decrease in L^*, a^*, and b^* values.	[158]

Table 5. *Cont.*

Food Product	Amount of Added *Spirulina*	Enriched Food Product Properties	Reference
Yogurt	*Spirulina* (*Arthrospira platensis*) microencapsulated in alginate	During the storage, microencapsulated *Spirulina* yogurt had a higher pH value and showed the lowest syneresis and a constant increase in viscosity.	[159]
Feta cheese	0.5 or 1% of *Spirulina* (*Arthrospira platensis*) powder	After 60 days of storage, there were significantly higher viable counts of *Lactobacillus casei* in *Spirulina* samples in comparison with the control.	[160]
Functional sauce	0 (control), 2, 3, and 4% *Spirulina* sp. biomass (wt%)	Significantly increased protein, fiber, ash, monounsaturated fatty acid, and mineral contents and storage stability.	[161]
Bread	2, 4, 6, and 8% of *Spirulina platensis* powder	6% *Spirulina*-supplemented breads provided higher contents of total phenolic content and antioxidant activity.	[162]
Pasta	5, 10, and 20 g/100 g, of *Spirulina platensis* biomass	The incorporation of *Spirulina* resulted in an increase in protein content; however, protein digestibility was reduced as microalgae content increased.	[163]
Bread	1 and 2% of *Spirulina platensis* powder	The concentration of proteins significantly increased in the samples enriched with 1% *Spirulina* (3.17%) and 2% *Spirulina* (5.12%), while at the same time, the gluten content decreased by 5.62% and 7.41%, respectively.	[164]
Kefir	*Arthrospira platensis* at 0.05, 0.1, 0.5, 1, and 2% (*w*/*v*)	The addition of 1% *A. platensis* increased amino acid contents, palmitic and oleic acid content, and the antioxidant activities (FRAP and DPPH) of kefir.	[165]
Cookies	1, 2, and 3% of *Spirulina platensis* powder	Hardness increased with the *Spirulina* content. Microalgae had a negative impact on the overall sensory quality.	[166]
Gluten-free muffins	1, 2, and 3% of *Spirulina platensis* powder	Increasing the *Spirulina platensis* powder content made the muffins firmer and caused a decrease in the L^*, a^*, b^*, and browning index values of the muffins.	[167]

6. Conclusions and Future Perspectives

Spirulina, a nutrient-rich cyanobacterium, has garnered significant attention for its remarkable biological activities and nutritional properties, positioning it as a promising ingredient in the food industry [3]. Known for its high protein content, essential amino acids, vitamins, and minerals, *Spirulina* is increasingly being utilized in a wide array of food products, supplements, and functional foods. The future applications of *Spirulina*, particularly in the food sector, are both diverse and expansive, offering substantial benefits for health and nutrition.

One of the most promising uses of *Spirulina* in the food industry is its incorporation into bakery products. By adding *Spirulina* to items like bread, cookies, and pasta, manufacturers can significantly boost the nutritional value of these staples [3]. *Spirulina* not only enriches these products with essential nutrients but also adds a vibrant green hue, appealing to health-conscious consumers seeking natural, nutrient-dense foods. The microalga's ability to enhance the nutritional profile of everyday foods makes it an ideal ingredient for developing functional foods that support overall health and wellness. Beyond bakery products, *Spirulina* is also making its mark in the beverage industry. Its inclusion in smoothies, juices, and health drinks provides a natural source of energy, antioxidants, and immune-boosting properties. The versatility of *Spirulina* allows it to be used in various formulations, catering to the growing demand for plant-based and superfood beverages. Moreover, *Spirulina*-based supplements, including powders, capsules, and tablets, are increasingly popular in the dietary supplement market. These products are marketed for their potential to support immune function, improve digestion, and enhance overall vitality. In addition to its direct applications in food and beverages, *Spirulina's* bioactive components are being explored for innovative uses in active packaging. This type of packaging can extend the shelf life of food products by inhibiting microbial growth, leveraging the natural antimicrobial properties of *Spirulina*. Furthermore, *Spirulina's* antioxidant-rich profile is being utilized in the development of biomedical dressings, which can aid in wound healing and skin regeneration.

The commercial production of *Spirulina* is dominated by the species *Arthrospira platensis*, which accounts for an annual production of approximately 10,000 tons, with China leading as the largest producer, contributing to about 66% of the global output [168]. As interest in *Spirulina* continues to rise, the global market is projected to reach USD 968.6 million by 2028, with a Compound Annual Growth Rate (CAGR) of 13.2% from 2021 to 2028. While North America currently dominates the market, significant growth is expected in the Asia–Pacific region, driven by increasing demand for dietary supplements, efforts to combat malnutrition, favorable climatic conditions, and cost-effective production [169].

To meet the growing demand, producers are focusing on optimizing *Spirulina* cultivation to improve both economic feasibility and environmental sustainability. Innovations in production processes include the utilization of residual biomass and the recycling of waste resources, aligning with the principles of a circular economy. Even after extracting valuable compounds like phycocyanin, the remaining *Spirulina* biomass retains high levels of antioxidants, vitamins, and minerals, making it a valuable resource for skincare and cosmetic products. *Spirulina*'s extensive health benefits and versatile applications make it an invaluable asset in the food industry. As research and technological advancements continue to unlock new possibilities, *Spirulina* is poised to play a crucial role in shaping the future of nutrition, health, and sustainable food production [52].

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