UNIVERSITY OF CATANIA

Deparment of Biological, Geological and Enviromental Sciences

PhD IN EARTH AND ENVIRONMENTAL SCIENCES
XXXVIII CYCLE

Carmen Sica

SINERGY: *In SilIco-study for the evaluation of oNcoprotectivE activity by analisis by highly puRified alGal phycocYanin miRNAs regulation***"**

TUTOR

Professor M. Ferrante

CO-TUTOR

Professor G. Oliveri Conti

Professor M.V. Brundo

YY.YY 2022- 2025

Alla mia Famiglia

Sommario

Acronyms

- **ACS** = American Chemical Society
- **ADME** = Absorption, Distribution, Metabolism, and Excretion
- **ANOVA** = Analysis of Variance
- **APC** = Allophycocyanin (a type of phycobiliprotein)
- **ATP** = Adenosine Triphosphate
- **AU** = Arbitrary Units
- **BACE1** = Beta-Site APP-Cleaving Enzyme 1
- **BCL2** = B-Cell Lymphoma 2 (a gene involved in apoptosis regulation)
- **BG11** = Blue-Green 11 – a growth medium for cyanobacteria
- **BHT** = Butylated Hydroxytoluene – a synthetic antioxidant
- **BMC** = BioMed Central
- **CAA** = Cellular Antioxidant Activity
- **CALUX** = Chemically Activated LUciferase gene eXpression
- **CAN** = Canthaxanthin
- **CD59** = Cluster of Differentiation 59 – a protein that regulates complement activation
- **CHNS** = Carbon, Hydrogen, Nitrogen, Sulfur analysis
- **CLA** = Conjugated Linoleic Acid
- **CNR** = Consiglio Nazionale delle Ricerche (National Research Council, Italy)
- **CO_2** = Carbon Dioxide
- **COX** = Cyclooxygenase – enzyme involved in inflammation pathways
- **CPC** = C-Phycocyanin
- **CRC** = Colorectal Cancer
- **DAD** = Diode Array Detector
- **DESS** = Dimethyl Sulfoxide–EDTA–Salt Solution (common preservative)

- **DGCR8** = DiGeorge Syndrome Critical Region Gene 8 – involved in microRNA processing
- **DHA** = Docosahexaenoic Acid
- **DMEM** = Dulbecco's Modified Eagle Medium
- **DMSO** = Dimethyl Sulfoxide
- **DNA** = Deoxyribonucleic Acid
- **DPPH** = 2,2-Diphenyl-1-picrylhydrazyl – used in free radical scavenging assays
- **DW** = Dry Weight
- **EA** = Elemental Analyzer

Abstract

The project is taking place within the larger context of climate change as well as in an era which is changing quickly toward low-carbon industrial models. To this end it is submitted an integrated approach to industrial CO_2 emissions through the sustainable cultivation of Spirulina platensis, a filamentous cyanobacterium capable of high photosynthetic efficiency and quick biomass build-up, high content of high value bioactive compounds. This thesis is intended to prove the feasibility of biorefinery model in which Spirulina platensis will function as an environmentally friendly biotechnological platform for capturing atmospheric CO_2 and biotechnologically producing a whole spectrum of functional bio-metabolites for future use as food supply in dietary, pharmaceutical, and cosmetic applications, both for the bioprocessing process. The main hypothesis is that Spirulina can be used as a green biofactory to convert CO_2 a significant greenhouse gas [GHG] into functional metabolites in low-impact, environmentally responsive, extraction process. In fact, for validating this hypothesis, Spirulina platensis was cultured under controlled laboratory conditions (regulated CO_2 input, nutrient enrichment, and standard light and temperature parameters) to optimize metabolic output. Biomass yielded was prepared via a biorefinery-style technology to recover and value various biochemical fractions. It included the development of environmentally friendly extraction protocols using green solvents (water, limonene, p-cymene, and 2-methyltetrahydrofuran (2-MeTHF)), together with microwave-assisted extraction [MAE] in order to increase solvent economy and decrease energy consumption. Spectroscopic and chromatographic techniques were used to characterize each extract. UV–Vis spectroscopy of the aqueous fraction showed maximum absorption at 277.2 nm, 347.5 nm, and 639.8 nm, confirming the structural stability of C-phycocyanin. GC–MS analysis provided solvent-dependent selectivity: phenolic compounds were most effectively extracted by p-cymene and limonene and methyl palmitate, methyl linoleate and methyl γ-linolenate, biologically significant fatty acid methyl esters (FAMEs), by 2-MeTHF and limonene. Phycocyanin fraction with enriched protein was purified and initial in vitro biochemistry was performed. The performance of the assays showed remarkable antioxidant,

cytoprotective, and possibly anti-tumor activities, especially under condition of oxidative stress, highlighting the potential therapeutic application of C-phycocyanin for the medical field, especially in preventive medicine. Concurrently, a separate small RNA (microRNA) fraction was isolated and tested by next-generation sequencing (NGS) to identify the presence of conserved sequences, for example miR166a and miR159a. These specific microRNAs regulate growth, metabolic adaptation and stress responses in photosynthetic organisms. In the literature, miR159a has been linked to hepatoprotective and anti-inflammatory actions, implicating it in a potential biomedical application. We have not performed biological assays for the microRNA fraction. This was an intentional methodological choice to allow for the broad characterization of the phycocyanin fraction. At present, the microRNA extracts are being further purified to achieve maximal molecular integrity and also to eliminate remaining protein and polysaccharide contaminants, an important step to perform before experiments involving cell-surface exposure. This work highlights for the first time the possible application of the use of CO_2 valorization in Spirulina platensis as a sustainable bioprocessing green technology to generate bioactives with high-value for a bioprocessing process. The integrated use of eco-friendly solvents, microwave-assisted extraction and two-fold recovery of protein-RNA extracts is one of the green bioeconomies and green biotechnologies and scalable solution. In doing so, this thesis enriches and expands the work in this area by presenting an actual instance where spirulina platensis can repurposing in the form of a green carbon capture and bioactive compound bioactive chemical biophilic platform on which to use for its utilization for sustainable production. These innovations provide a foundation for further investigation into how microRNAs are functional for potential future applications and the potential to contribute to the ecosystem towards environmental and biological applications of an integrated biorefinery type model.

Chapter 1

1. Circular Economy

The term circular economy was first explicitly used by Pearce and Turner (1990). In their book entitled "Economics of Natural Resources and the Environment," they discussed the negative aspects of the traditional (or linear) economy with its open concept and proposed an alternative in the form of a closed system, called the circular economy (Ghisellini et al., 2016). In fact, the circular economy is fundamentally different from the linear economy. In a linear economy, we extract raw materials that we transform into a product that is thrown away after use. The term linear refers to the straight progression that a product can follow, with a beginning, a middle, and an end, and there is no consideration of recycling or reuse. This model is characterized by a high volume of new production. Therefore, the linear economy is a polluting system that can damage nature and the climate, causing biodiversity loss (Blomsma & Brennan, 2017).

Conversely, the circular economy aims to limit resource use, minimize waste, and maximize reuse and recycling of materials. This model adopts a systemic perspective in which materials and products are kept in circulation for as long as possible to optimize their value and reduce environmental impact. Therefore, in a circular economy, material cycles are "closed," which requires more than simple recycling (Kjaer *et al.*, 2019).

The work expands upon earlier ideas proposing that the economy should be conceived as a circular system designed to promote long-term sustainability. Although the concept of the circular economy is relatively recent, its theoretical foundations were established many years ago. The debate on circular economy has been strongly influenced by industrial stakeholders. The Ellen MacArthur Foundation, for instance, has played a significant role in promoting the concept by publishing reports and books and engaging in the World Economic Forum (Sillanpää and Ncibi, 2019). Blomsma and Brennan (2017) divided the emergence of the circular economy into several phases.

This was the time when debates on waste and resources mounted and programs like recycling, composting and waste-to-energy incineration emerged. Such ways proposed to ‘run a sustainable practice’ towards natural resources and they did so much so that circular economy became part of the discourse for authors (Blomsma and Brennan, 2017). Later, industries and governments came under pressure to embrace the developing idea and discussions in other intellectual environments, including physics and biology, deepened the conversation. Yet in this phase, waste was regarded negatively and hence as being expensive. Clear solutions for that matter were not yet available and the recycling program remained immature (Blomsma and Brennan, 2017). In the latter part of this second phase, between 1985 and 2013, people were more favorable towards waste. Product–service systems, urban mining, recycling, remanufacturing, and upgrading were devised by the development, both to prolong the resource usage and avoid landfilling. Some measures had been taken before in the earlier phase, but by this time it was more complicated and varied (Blomsma and Brennan, 2017). In addition, sustainable development attracted more attention and was perceived as a source for both economic and technological potential. Germany became the first country to implement the circular economy in 1996 through a new law establishing rules for waste management. The Chinese government, for example, embraced the circular economy as an economic model in response to environmental issues. Economic efficiency and environmentally responsible behaviour along with waste minimisation are the primary tenets of the circular economy. These concepts can often be summarized into the “3Rs”: Reduce, Reuse and Recycle (Huamao & Fengqi, 2007). According to a thorough analysis of scientific articles on circular economy, authors largely agree on the following basic principles:

- Resource sustainability: using renewable energy and renewable, recyclable, and biodegradable materials in subsequent life cycles.
- Waste elimination: waste is turned into raw materials. Circular economy advocates total elimination of waste production and designing products to become recyclable and reusable.
- Resource efficiency optimization: the flow of products, components, and materials should maximize the use of resources.
- System efficiency: the effects of the current industrial economy are not only evident but also actionable for efficiency.

- Strength lies in diversity: diversity and adaptability of products are key principles for a circular economy, as well as the long-term sustainability of products and conversion to different products in recycling cycles without waste, which transform residues into raw materials.

In addition, circular economy differs from the traditional linear model (the “take–make–dispose” paradigm) which discarded products after their lifecycle is completed. It, therefore, seeks to see waste completely wiped away with product reengineering, recyclability and reuse. (fig1)

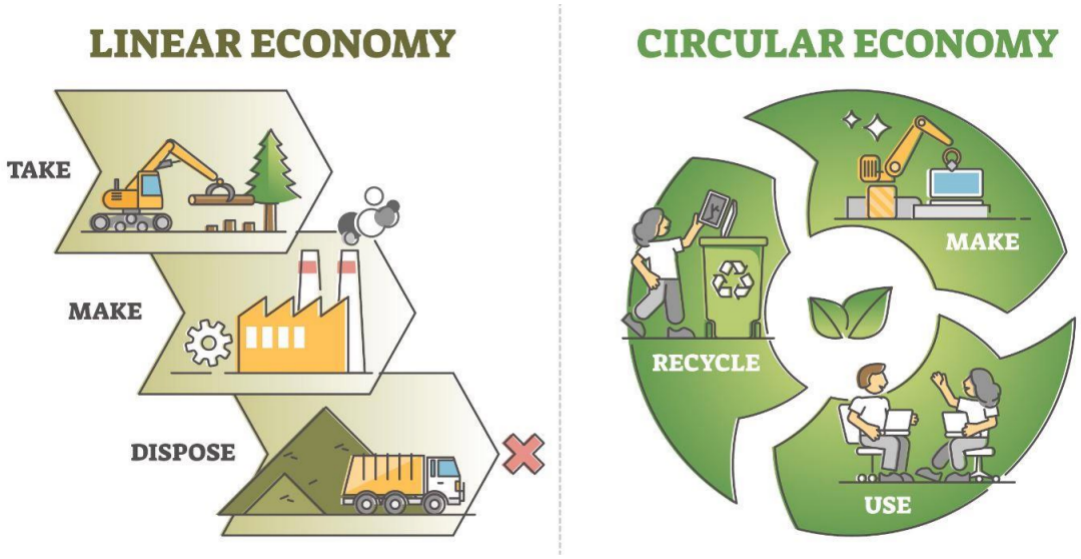


Figure 1. From the linear to the circular economy model (Adobe Stock, 2023).

To comprehend the theory of a circular economy, it is essential to examine the notion of sustainability. In 1987, the World Commission on Environment and Development characterized sustainable development as "development that meets the needs of the present without compromising the ability of future generations to meet their own needs" (WCED, 1987). Initially, sustainability primarily focused on environmental protection and social justice. Sustainability once was mostly a question of environmental protection and social justice. Subsequently, this paradigm has evolved towards economic elements and the business to reduce the environmental footprints of the business on one hand, make profits and show a social responsibility on the other (Fujii et al., 2013). A fundamental advancement was made after 2015, when sustainable development started to be framed by the United Nations Sustainable Development

Goals (SDGs) (Nikolaou et al., 2021). From an early stage of thinking, the "Triple Bottom Line" framework established an important way of sharing sustainability concepts: they include economic, social, and environmental aspects, and this approach has achieved great attraction in recent decades. So our question now is how can organizations do business with social and environmental responsibility? Nikolaou et al. (2021) explain with the Triple Bottom Line a means for companies, non-governmental organizations, and governments to estimate how much they could contribute to the sustainable future. An emphasis on environmental, social and economic (Zhao et al., 2018) have highlighted the link between circular economy practices and sustainability. Many researches have highlighted that circular strategies are very important to ensure sustainable development due to the positive environmental aspects, such as improving environmental performance variables (Nikolaou et al., 2021). Despite criticisms by some sustainability groups and many ecologists' warnings, the global acceptance of circularity dropped to 7.2% from the initial 9.1% five years earlier, suggesting less recycling and reuse effort. The European Commission has developed a new circular economy policy with the European Green Deal in March 2020 (CEAP), the EU's new strategy for sustainable growth. To the extent that one could, the EU's move to circular economy will alleviate pressures on natural resources also create economic capacity and jobs. It is also key to ensuring EU climate neutrality by 2050 and avoiding loss of biodiversity. The action plan details work on every phase of a product's life cycle and focuses on product design, production circularity, environmentally friendly consumer consumption, and waste minimisation: all to keep resources within the EU economy for a long time. It covers a range of legislative and non-legislative measures in areas where coordinated EU action can greatly benefit the economy. Within this plan, the initiatives are designed to: - Foster sustainability in EU products; - Empower consumers and public buyers; - Focus on high potential resource-intensive industries with a great potential for circularity including electronics, ICTs, batteries & vehicles, packaging materials, plastics, textiles, construction & buildings, food, water & nutrients; - Ensure waste reduction; - Make circularity benefits for individuals, regions, cities easier to achieve; - Leadership in the global progress of the circular economy. The Green Deal together with the Climate Pact approach aims at leading global efforts with 8 objectives including global climate action. Existing agricultural organic waste management practices have resulted in substantial N and P losses into atmospheric structures and local aquatic bodies with potential impacts on air quality as well as water systems

(Misselbrook et al., 2010). Agriculture is also a major contributor to greenhouse gas emissions. As global demand for food-producing meat-based products rises, the need for animal feed proteins (especially soybeans) is on the rise, which is believed to further aggravate deforestation pressures (Gasparri et al., 2013). This sector represents about a fifth of human-induced GHG emissions and constitutes almost one-fifth of total CO2 emission emissions global statistics (Gasparri et al., 2013). Moreover, the reliance of Europe on imported feed proteins is associated with food security risks mainly due to the fact that supply chains are largely under uncertainty, increasing the risks with increased volatility in feed-related risk. Agricultural GHG (GHG) emissions reduction is an essential component of the national initiatives aiming at adherence to Paris Climate Agreement (CLA) commitments (Wollenberg et al., 2016). Algae have been recognized as having potential for nutrient extraction and bioremediation related to waste, metals, carbon dioxide and organic pollutants, for many decades. Since the investigations by William Oswald in the 1950s, microalgae research has proven effective treatment of wastewater, including to be efficient for microalgal populations as described in microalgae in the collection and treatment of single culture methods in its research (Oswald et al., 1953). Programs such as the U.S National Renewable Energy Laboratory Biofuels Program coupled with the Department of Energy Aquatic Species Program recommended growth of algae through strain selection targeting lipid concentration and restoration potential through strain, particularly through strain-based lipid selection for water supply (Sheehan et al., 1998). Roadmaps based on these preliminary investigations were laid out in later research results (Barry et al., 2016). Emerging developments in the algal biotechnology field have revived as molecular genome techniques have been developed to address concerns that lead to demands associated with recycling such as those arising from waste associated requirements as defined by those concepts inherent to circular economy frameworks. This biomass derived through the cultivation of waste nutrients (digestate) is expected to be beneficial to commodity markets efficiently. Algae produces rich constituents forming protein, lipid, bioactive compounds, relevant for a wide range of industries including food, feed, fuel, pharmaceuticals, cosmetics (Singh et al., 2017). The cyanobacteria provide promising potential soil applications as slow-release fertilizers (Singh et al., 2017). Unfortunately however contributions of such protein from microalgal sources is limited related to human consumptive patterns, production limitations based mainly on identified externalities such as the challenge to regulate the use of such products,

negative climatic conditions and other prevalent issues impacting consumers in most EU countries (Vigani et al., 2015). Agricultural GHG emissions awareness may be improved by focusing on infrastructural upgrades (e.g. covered lagoon technologies) such as anaerobic digesters which harvest methane energy (Hopkins Del Prado, 2007) thereby converting agriculture food residues into biomethane through anaerobic digestion, i.e. a digestion process which generates nutrient dense digestate, thus stabilizing agricultural soil in process, reducing odor, reducing GHG, thereby enabling nutrient biostimulants input mainly with macronutrients (N, P, K, S, Mg, Ca, Fe, Na) trace elements (Co, Se, Mo, Ni) in accordance to each feedstock composition (Abinandanshanthakumar, 2015). However, limitations have been highlighted like limited growth season, limited nutrient loadings, limited availability of material, local land restricting widespread availability of digestates prompting to explore innovations in market possible uses overcoming the challenges encountered in them. Thus, through developing microalgal biorefinery systems combining digestates on-site improve the nutrient recovery that will assist to alleviate the associated impact for the given ecological issues met.

Chapter 2

2. Microalgae

Algae are a group of photosynthetic autotrophic organisms that typically thrive in various types of aquatic environments such as lakes, rivers, and seas. They are responsible for the production of atmospheric oxygen through photosynthesis, a process that converts water and carbon dioxide into carbohydrates using solar energy. The algal group is highly diverse and comprises numerous distinct phyla with unique characteristics and properties, ranging from unicellular prokaryotic cyanobacteria to more complex multicellular eukaryotic algae, as summarized in Table 1 (Tan et al., 2020).

Table 1. Main types of algae and their characteristics (Tan et al., 2020).

Type of algae	Characteristics	Examples
Cyanobacteria	Cyanobacteria, or blue-green algae, are Gram-negative bacteria that can survive in some of the most hostile habitats on Earth. Some species are known to fix both nitrogen and carbon, both essential for life on the planet.	*Prochlorococcus*, *Spirulina*, *Nostoc*, *Cyanothece*, *Anabaena*
Glaucocystophytes	Glaucocystophytes are relatively rare unicellular eukaryotic algae that contain a plastid structurally similar to cyanobacteria. They are among the descendants of the products of early endosymbiosis.	*Cyanophora*, *Glaucocystis*, *Peliaina*, *Gloeochaete*
Rhodophytes	Rhodophytes, or red algae, are mainly composed of multicellular photosynthetic eukaryotes. They are	*Batrachospermum*, *Chroodactylon*, *Bangia*, *Cyanidium*,

	characterized by their distinctive red coloration due to the presence of red pigments, such as phycobilisomes, within their chloroplasts.	*Compsopogon*
Chlorophytes	Chlorophytes, or green algae, are photosynthetic eukaryotes containing chlorophyll as their main photosynthetic pigment.	*Haematococcus*, *Chlorella*, *Dunaliella*, *Graesiella*, *Scenedesmus*
Charophytes	Charophytes are algae mainly found in terrestrial and freshwater habitats. They show features similar to land plants, suggesting that the ancestors of charophytes gave rise to terrestrial flora.	*Coleochaete*, *Micrasterias*, *Chara*, *Penium*, *Klebsormidium*
Chlorarachniophytes	Chlorarachniophytes are marine photosynthetic protists that possess secondary plastids derived from secondary endosymbiosis.	*Chlorarachnion*, *Lotharella*, *Bigelowella*
Euglenoids	Euglenoids are unicellular flagellated eukaryotes with both animal- and plant-like features. Most species are freshwater, while some inhabit marine environments.	*Euglena*, *Discoplastis*, *Phacus*, *Colacium*, *Strombomonas*
Apicomplexans	Apicomplexans are a group of obligate intracellular parasites responsible for various diseases in animals and humans.	*Plasmodium*, *Toxoplasma*, *Cryptosporidium*
Dinoflagellates	Dinoflagellates are a class of unicellular protists characterized by relatively large nuclei, golden-brown plastids, and their unique swimming mode.	*Gymnodinium*, *Karenia*, *Dinophysis*, *Alexandrium*
Heterokontophytes	Heterokontophytes are flagellated photosynthetic eukaryotes,	*Chrysophyceae*, *Parmophyceae*,

	characterized by two flagella of unequal length.	*Xanthophyceae*, *Dictyophyceae*
Haptophytes	Haptophytes are unicellular photosynthetic microalgae whose chloroplasts derive from the endosymbiosis of red algae. They typically possess two equal or unequal flagella that allow motility.	*Chrysochromulina*, *Prymnesium*, *Pavlova*, *Diacronema*
Cryptophytes	Cryptophytes are unicellular motile photosynthetic organisms, characterized by the presence of two flagella, and are typically found in freshwater and marine habitats.	*Guillardia*, *Campylomonas*, *Geminigera*, *Rhodomonas*, *Teleaulax*

Microalgae are quick to grow. Their photosynthetic efficiency is superior to most other plants and its production volume of biochemical products are large in that it allows them to be very efficient as industrial raw materials (Randrianarison and Ashraf, 2017). Their cultivation does not need fertile soils, large quantities of water sources, herbicides or pesticides; therefore, it does not compete for resources (Khan et al., 2018). Microalgae may also be grown in contaminated wastewaters (such as municipal effluents or palm oil mill effluents), which can help with bioremediation process in wastewater (Selmani et al., 2013; Posadas et al., 2017). Apart from the use for wastewater treatment, microalgae cultivation can also help to relieve atmospheric carbon dioxide via photosynthesis, which can contribute towards reducing the greenhouse effect and climate change. Despite these benefits, microalgae industrial production is still limited by limited industrial development. Low biomass productivity and small cell size in liquid cultures, for example, make harvesting of the same very expensive. A technique to overcome these problems is to enhance growth rates to compensate for poor cell density in addition to tough crop harvests. Various technologies and crop facilities have been developed over the years to increase microalgae yields. While microalgae can easily be grown under the strictest laboratory conditions necessary for its success, high productivity at large scale production becomes

even more challenging. The following characteristics should be required for a suitable microalgae culture system:

1. A suitable light source;
2. Substantial transfer of materials through the gas–liquid barrier;
3. Simplicity in practice, procedures;
4. Minimal contamination rate;
5. Low-cost in terms of construction and production;
6. Effective land-use management process (Jegathese and Farid, 2014).

Broadly, there are two types of culture systems for microalgae: open ponds and photobioreactors, each with both advantages and disadvantages. Open pond cultivation is the oldest and most basic way to grow microalgae very widely. Its low construction, maintenance, and operation costs have made this system popular in industry. Other benefits are the ease of operation and maintenance, low-energy use and ease of scaling (Costa and de Morais, 2014). Open ponds are generally composed of natural (lakes and lagoons) and artificial (circular and raceway) systems. In some cases, tanks and containers can also be employed for cultivation (Sun et al., 2016). The open pond system is generally the most economical form of cultivation. Although the cultivation area is very large, the natural cell concentration can still be relatively low in water-based cultivation, hence very high harvesting costs are needed (Tredici, 2004). Furthermore, problems like rainwater runoff (which changes salinity and pH) and bank erosion (which results in a loss of water and high turbidity) can take a toll on productivity in open pond systems (Borowitzka and Borowitzka, 1990). And protozoa and bacteria contamination can be dangerous, also making biomass hazardous and non-biomass-unused a serious issue. One way of overcoming this problem is to cultivate microalgae that can withstand such severe alkaline or saline conditions and are free from contaminants that would otherwise suppress their reproduction (Stark and O'Gara, 2012; Ugwu and Aoyagi, 2012). Because the system is an open system, the growth parameters, including the temperature and light intensity, are also more challenging to control, as it may have direct influence on growth rate (Stark and O'Gara, 2012). Collection of microalgae from organic sources is one of the earliest methods. The earliest evidence of microalgae harvesting dates back to the Aztecs who gathered Spirulina from Lake Texcoco in Mexico (Hamed, 2016). An early example of

cultivation through close association with well-preserved natural water bodies. In addition, the commercially available open-water microalgae of Dunaliella, as of the present, is in Hutt Lagoon, Australia, producing up to 6 tons of β-carotene annually from its 700-ha ponds (Borowitzka and Borowitzka, 1990). Circular ponds, the first category of artificial ponds that were used for plant-cell culture at a scale. They are named for the circular morphology of the culture basin, with a depth of 30–70 cm and diameter of around 45 m, and a rotating agitator at the center of the pond (Shen et al., 2009). The agitator promotes effective mixing and prevents biogeochemical enrichment but also inhibits the sediments of mass production (Show et al., 2017). But the size limits the design as bigger ponds would increase water resistance, leaving agitator parts under mechanical stress (Lee, 2001). Furthermore, this system has high energy used for processing and higher construction costs (Hamed, 2016). This cultivation system has already been used in Japan and Taiwan for the cultivated Chlorella that is being used for consumption (Lee, 1997). It includes several 30 cm deep, closed-loop channels, with paddlewheels that deliver algal biomass to the open loop, keeping a steady flow and avoiding the accumulation of food, salt, or nutrients throughout the system. The open topography of the raceway pond and the bottom reach the surface below the axial level, producing a broad gradient of microalgae growth in the open track (Li et al. 1). Due to their energy efficiency, raceway ponds are regarded among the best open-pond systems, as one paddlewheel is enough to flow a 5-hectare pond (Rogers et al., 2014). One such successful raceway pond cultivation in the USA is the Columbus Algal Biomass Farm that Sapphire Energy developed and successfully cultivated 520 tons of dried microalgal biomass over two years without any technical problems (White and Ryan, 2015).

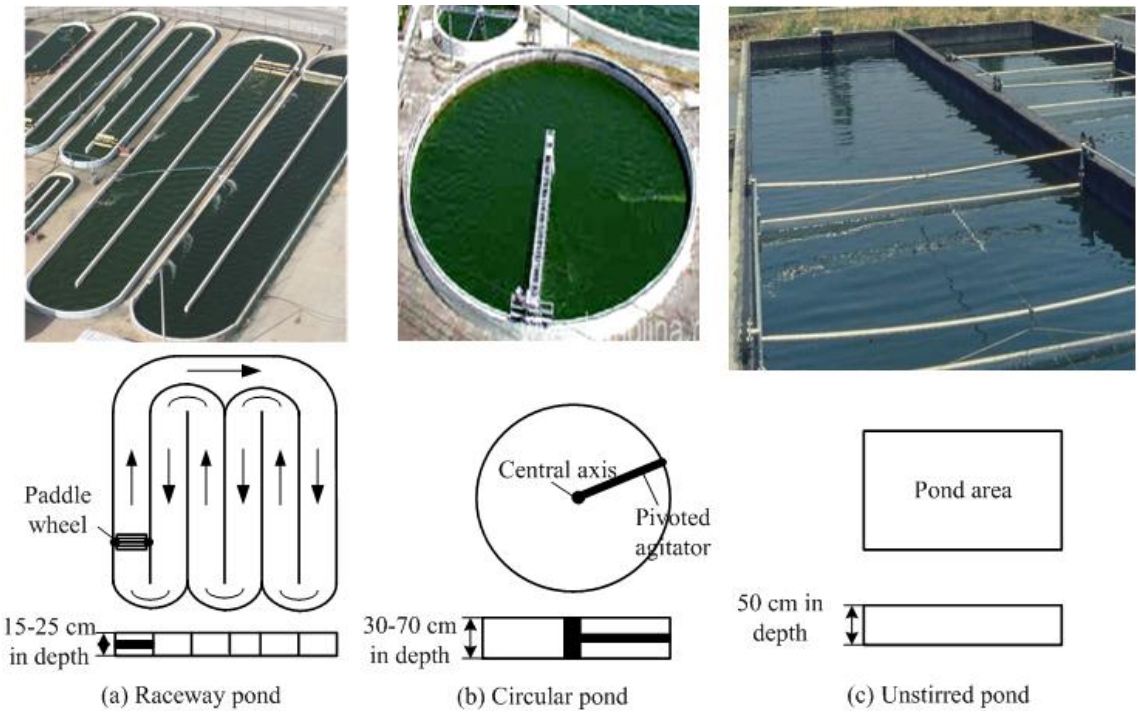


Figure 2. Three different designs of open pond systems (Shen et al., 2009).

The photobioreactor is a bioreactor system designed to cultivate phototrophic organisms such as microalgae in a closed environment that prevents direct exchange of material between the culture and the surroundings. This system overcomes several limitations typically encountered in open pond designs. Firstly, the reactor is more compact, allowing more efficient land use. Secondly, it provides highly controlled and enclosed growth conditions, making it possible to produce monoclonal algal cultures free from contamination (Posten, 2009). Moreover, highly regulated culture conditions can result in improved nutrient and metabolic efficiency, leading to higher biomass yields per unit of substrate. However, a significant drawback to the practical use of photobioreactors is their limited scalability due to design constraints, which make them uneconomical for large-scale production (Gupta ***et al.***, 2015). Additionally, the highly controlled growth environment is associated with high capital and operational costs.

The tubular photobioreactor consists of long transparent tubes (Fig.2) made of glass or clear plastic, arranged in horizontal, vertical, helical, or inclined orientations to maximize solar light capture (Hamed, 2016; Mishra ***et al.***, 2019). Microalgae cultures are circulated within the system through a mechanical pump or an airlift system (Xu, 2007). However, the main drawback of this design is poor mass transfer, as the long

tubes can cause gradients in substrate and product concentrations along the system (Torzillo and Zittelli, 2015).

The vertical column bioreactor is composed of a transparent cylindrical tube equipped with a sparger that injects air bubbles to homogenize the culture and enable CO_2 and O_2 transfer between the air and the algal biomass (Mohan ***et al.***, 2019). This system offers the highest gas–liquid mass transfer efficiency compared to other designs, as the sparger generates fine bubbles that provide a large surface area for gas exchange (Rinanti et al., 2013). In addition, the simple design results in lower energy requirements and easier operation.

Nevertheless, the cylindrical geometry does not provide sufficient light penetration for efficient photosynthesis. Furthermore, high construction costs and difficulties in cleaning the reactor limit its commercial use (Huang et al., 2017).

The flat plate photobioreactor consists of a rectangular transparent compartment with a depth of 1–5 cm (fig.3). The culture is mixed through an air recirculation system (Tamburic *et al.*, 2011). This configuration provides the largest surface area for illumination and minimizes oxygen accumulation, thereby achieving the highest photosynthetic efficiency among photobioreactor designs (Yan et al., 2016).However, the aeration design of this reactor can cause shear stress damage to algal cells (Sierra et al., 2008). Innovative designs have been implemented to improve its performance, including double-layer flat plate photobioreactors and plastic film reactors (Vo *et al.*, 2018).

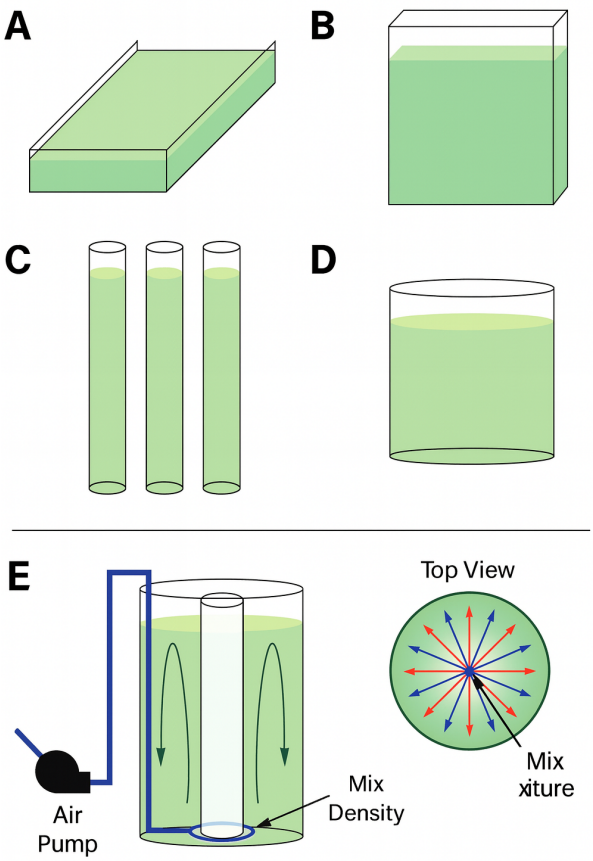


Figure 3. Different photobioreactor (Tamburic et al., 2011)

The conditions required for microalgal cultivation are critical factors influencing the metabolism of these microorganisms, thereby directing the synthesis of specific compounds of interest. Several researchers have reported that incubation temperature, medium pH, cultivation period, salinity, light intensity, and nutrient composition strongly affect the synthesis of antimicrobial agents (Noaman, 2004).

The pH regulation is a primary strategy for preventing contamination by other microorganisms such as competing algal species. Moreover, pH control is essential for efficient absorption of nutrients in the culture medium, as it directly influences the availability of various chemical elements (Lourenço and da Silva, 2006). Limiting specific nutrients in the medium can induce the production of valuable biocompounds; for example, nitrogen deprivation can shift microalgal metabolism towards the production of lipids or carbohydrates (Fogg, 1983).

Light is indispensable for photosynthesis, as it stimulates cell division and increases cell density (Nelson and Cox, 2002). Light intensity also influences the biochemical composition of the biomass (Chrismadha and Borowitzka, 1994). The fatty acid content

tends to decrease with higher light intensity, as lipids are the main components of chloroplasts, and increased light energy requires greater chloroplast activity (Guedes et al., 2010). Light has also been shown to affect antioxidant activity in microalgae. Madhyastha et al. (2009) reported that exposure to blue light during cultivation of Spirulina fusiformis altered amino acid sequences with cysteine repeats, enhancing its antioxidant capacity.

Temperature is another crucial factor for the growth of all living organisms. The specific growth rate of microalgae is directly related to gross CO_2 fixation/O_2 production (photosynthesis) and respiration rates. Both photosynthesis and respiration are temperature-dependent, with respiration increasing exponentially as temperature rises (Vonshak, 1997). Temperature strongly influences biomass yield, protein, lipid, and phenolic compound production. The optimal temperature for microalgae cultivation ranges between 35–37 °C (Fox, 1996).

Noaman (2004) had conducted a study to find out the most suitable cultivation conditions for the production of antimicrobial compounds by Synechococcus leopoliensis, and the study revealed a maximum levels of this bioactive metabolite could be obtained with a temperature of 35 °C and pH 8. Harvesting microalgae is an important step in biomass processing. Due to the large energy usage and initial investment required for the method, several studies estimate that harvesting amounts to 20–30% of the total production costs (Uduman et al., 2010; Barros et al., 2015). In general, the methods of harvesting are to eliminate as much culture medium as possible from the microalgal biomass to enable further processing, for example bioactive substances to be extracted. Different techniques of harvesting are used such as filtration, centrifugation, flocculation and flotation (Singh and Patidar, 2018). In additional cases two or more processes may be added to improve efficiency. Tan et al. (2020) summarized the different harvesting methods, the microalgal species used, and their superiority and disadvantage in Table 2. (tab 2).

Table 2. Harvesting techniques, target microalgal species, advantages, and disadvantages (Tan *et al.*, 2020).

Harvesting Technique	Microalgal Species	Advantage	Disadvantage
Cross-flow filtration	*Chlorella* sp.	High energy efficiency	Membrane fouling tendency and shear stress on fragile materials
Axial vibration membrane filtration	*Chlorella pyrenoidosa*	Reduced membrane fouling	Requires energy-consuming pumping units
Polyacrylonitrile-based membrane filtration	*Scenedesmus* and *Phaeodactylum*	Reduced membrane fouling	Requires energy-consuming pumping units
Inclined panel membrane filtration	Wild-type microalgal strains	Reduced membrane and energy costs	Membrane fouling
Ultrafiltration	*Dunaliella salina*	Lower cell shear, low energy and chemical consumption	High capital cost
Electroflocculation	*D. salina*	Cost-effective and chemical-free	High energy demand
Plant-based bioflocculation (*Moringa oleifera*)	*Chlorella* sp.	Cost-effective with limited toxicity	Contamination of microalgae-based products
Microbial bioflocculation	*Desmodesmus brasiliensis*	Cost-effective and biodegradable	Contamination of microalgae-based products
Chemical flocculation	*Chlorella* sp.	Cost-effective and easily scalable	Use of toxic chemicals

Bead-floatation	*Chlorella vulgaris*	Chemical-free and highly reusable	High cost
Magnesium coagulation–dissolved air flotation	*Chlorella zofingiensis*	No external coagulant required, high recyclability of coagulant and biomass	Use of toxic chemicals
Electrolytic flotation	*C. vulgaris*	Chemical-free, low energy consumption, suitable for continuous systems	High operating and capital costs
Foam flotation	*C. vulgaris*, *Isochrysis galbana*, *Tetraselmis suecica*	Low cost and energy requirement, highly scalable	High operating and capital costs
Ozone flotation	*Scenedesmus* sp.	Enhanced recovery of microalgal biocompounds	Requires specialized on-site ozone generation equipment

The filtration process employs a semi-permeable membrane capable of retaining microalgae on the membrane surface while allowing the liquid medium to pass through, leaving behind algal biomass to be collected (Al Hattab, Ghaly & Hammoud, 2015). This method can recover a high concentration of cells from the medium, and the various pore sizes of the filter membrane enable the system to adapt to different microalgal strains, including more delicate species that are susceptible to shear stress damage. However, this method is highly prone to fouling and clogging, thereby requiring frequent replacement of filters or fresh membranes, which may significantly increase processing costs (Milledge & Heaven, 2013). To overcome this limitation, a filter membrane made of inexpensive and readily available materials has been developed. Bejor and colleagues (Bejor, Mota, Ogarekpe et a*l.*, 2013) successfully designed a stretch-cotton membrane filter capable of achieving a harvesting efficiency of 66–93%.

Centrifugation separates microalgal cells from the culture medium based on particle size and density through centrifugal force (Soomro, Ndikubwimana, Zeng *et al.*, 2016). This technique achieves high cell recovery efficiency but is both time- and energy-intensive (Dassey & Theegala, 2013; Rawat, Ranjith Kumar et al., 2013). Furthermore, the high gravitational force applied during centrifugation may cause cellular damage, making it unsuitable for some applications where sensitive nutrients may be lost (Heasman, Diemar et al., 2000; Knuckey, Brown et al., 2006). Various centrifugal systems have been employed industrially, including disc-stack centrifuges, perforated-basket centrifuges, non-perforated basket centrifuges, decanters, and hydrocyclones.

Flocculation is a process in which free-floating unicellular microalgae aggregate to form larger particles, known as flocs, through the addition of a flocculant that neutralizes the surface charge of the cells (Muylaert, Bastiaens, Vandamme ***et al.***, 2017). Flocculating agents are generally classified into two main categories: chemical flocculants and bio flocculants. Low-cost and widely available chemical flocculants such as iron and aluminum salts have been extensively used in the industry (Bracharz, Helmdach, Aschenbrenner et al., 2018). The study conducted by Chatsungnoen and Chisti (2016) demonstrated that metallic salts such as aluminum sulfate and ferric chloride can remove up to 95% of microalgal biomass under standard conditions. However, these chemicals are not environmentally friendly due to their toxicity and require additional downstream removal processes, thereby increasing production costs (Rinanti & Purwadi, 2018).

In contrast, bioflocculants are safer and more eco-friendly than their chemical counterparts. They are also generally more cost-effective and often do not require pretreatment before downstream processing or culture medium recycling (Nguyen, Le, Show et al., 2019). Most bio flocculants used are biopolymers such as acrylic acid and chitosan, either naturally occurring or synthetically produced (Pugazhendhi, Shobana, Bakonyi et al., 2019). It has been reported that low dosages of chitosan can achieve up to 90% cell recovery, compared to chemical flocculants like aluminum sulfate which require higher concentrations to obtain similar results (Zhu, Li & Hiltunen, 2018).

Flotation employs small bubbles that attach to microalgal cells, allowing them to float to the surface of the culture medium for easy harvesting (Laamanen, Ross & Scott, 2016). The advantages associated with flotation systems include relatively high harvesting efficiency, simple operation, and high processing productivity at low cost (Ndikubwimana, Chang, Xiao *et al.*, 2016). There are three main types of flotation

systems, differentiated by their bubble generation mechanisms. Dissolved air flotation saturates the culture with compressed air before releasing it at atmospheric pressure, a method widely used in wastewater treatment but limited by high energy demand and chemical use (Niaghi, Mahdavi & Gheshlaghi, 2015). Dispersed air flotation instead uses a sprayer to generate bubbles, which requires less energy compared to dissolved air flotation (Alhattab & Brooks, 2017).

The third method, electroflotation, applies electrolysis to generate microbubbles from electrodes to trap free-floating microalgae (Baierle, John, Souza et al., 2015). In addition to harvesting, this method also enables simultaneous cell disruption when alternating current is applied (de Carvalho Neto et al., 2014). However, the system is highly energy-consuming, and electrodes require frequent replacement due to fouling, further increasing production costs. Microalgae are unicellular microorganisms that grow in freshwater or saltwater, displaying various morphologies with diameters or lengths of approximately 3–10 μm. The term "microalgae" encompasses both prokaryotic and eukaryotic organisms (Ferreira, de Souza-Soares & Costa, 2013). Cyanobacteria and bacteria share many structural similarities; however, they are classified as microalgae due to the presence of chlorophyll and other photosynthetic pigments. The so-called green algae are named for their content of chlorophyll ***a*** and ***b*** in the same proportions as higher plants (El Gamal, 2010). These photosynthetic organisms are important in aquatic ecosystems and are known to participate in ~40% of global photosynthesis (Moreno-Garrido, 2008). Microalgae metabolism responds to environmental changes with corresponding intracellular modifications. Therefore, modification of culture conditions including the availability/presence or absence of specific nutrients can enhance the biosynthesis of specific molecules. Since wide research has been conducted on the metabolic products of microalgae not only to understand their nature but also to identify substances, all of which are promising for human use as well. Extract screening or the isolation of microalgae metabolites from different types has been the typical methods to evaluate the biological activities of these components. Microalgae have been reported to be abundant sources of a plethora of biocompounds with commercial relevance (Volk & Furkert, 2006). Microalgal bioactive compounds can be derived by primary metabolism (e.g., proteins, fatty acids, vitamins, and pigments) or by secondary metabolism. Antifungal, antiviral, antialgal, antienzymatic or antibiotic effect of these compounds may be detected (Volk, 2006). Some of these compounds (cyanovirin, oleic acid, linolenic acid, palmitoleic acid,

vitamin E, vitamin B12, β-carotene, phycocyanin, lutein, and zeaxanthin) have been reported to be antimicrobial, antioxidant, and anti-inflammatory and are considered candidates for the treatment and prevention of various diseases (Smee, Bailey et al., 2008; Ibañez & Cifuentes, 2013; Markou & Nerantzis, 2013). These compounds generally bioaccumulate in the biomass, but in some species, they are excreted into the medium as extracellular metabolites (exometabolites). Microalgae constitute a naturally derived source of potent active molecules of great interest. Researchers and industries have been paying considerable attention to these compounds in the past years as they have shown promise in numerous life science applications. Applications include food and feed product production from biomass and bioactive compounds in the form of medicines developed from biomass (Harun, Singh et al., 2010). Being autotrophic organisms, microalgae rely on light energy and inorganic nutrients (carbon dioxide, nitrogen, phosphorus, etc.) for biosynthesis of high value-added biocompounds for the nutritional and therapeutic functions, for example lipids, proteins, carbohydrates, pigments, and polymers. Microalgae have various diverse biological activities and can produce a wide array of chemical compounds, including carotenoids, phycobiliproteins, polyunsaturated fatty acids, proteins, polysaccharides, vitamins, sterols, and other substances such as these are some of the different kinds of chemical compounds reported in recent research (Markou & Nerantzis, 2013; Bhagavathy, Sumathi & Jancy Sherene Bell, 2011; Palavra & Coelho, 2011). Microalgal-origin bioactive compounds with antimicrobial, antiviral, anticoagulant, antienzymatic, antioxidant, antifungal, anti-inflammatory, and antitumoral properties have been documented (Priyadarshani & Rath, 2012; Blunt & Copp, 2006; Mayer & Hamann, 2005; Rodríguez-Meizoso, Jaime, Santoyo et al., 2008; Carvalho, Costa-Neves, Conserva et al., 2013). The bioactive compounds extracted from different microalgae such as *Arthrospira (Spirulina), Botryococcus braunii*, *Chlorella vulgaris, Dunaliella salina, Haematococcus pluvialis, and Nostoc* have been researched (table 3).

Table 3. Bioactive compounds from selected microalgae

Microalgae	Bioactive Compounds
Spirulina sp.	Polysaccharides
Spirulina platensis	Phycocyanin, C-phycocyanin, phenolic acids, tocopherols (vitamin E), neophytadiene, phytol, n-3 PUFAs, fatty acids, oleic acid, linolenic acid, palmitoleic acid
Spirulina fusiformis	Diacylglycerols
Haematococcus pluvialis	Astaxanthin, lutein, zeaxanthin, canthaxanthin, β-carotene, oleic acid
Chlorella sp.	Carotenoids, sulfated polysaccharides, sterols, n-3 PUFA fatty acids
Chlorella vulgaris	Canthaxanthin, astaxanthin, peptides, oleic acid
Chlorella minutissima	Eicosapentaenoic acid (EPA)
Chlorella ellipsoidea	Zeaxanthin, violaxanthin
Dunaliella salina	trans-β-carotene, cis-β-carotene, β-carotene, oleic acid, linolenic acid, palmitic acid
Dunaliella sp.	Diacylglycerols
Botryococcus braunii	Linear alkadienes (C25, C27, C29, C31), triene (C29)
Chlorella zofingiensis	Astaxanthin
Chlorella protothecoides	Lutein, zeaxanthin, canthaxanthin
Chlorella pyrenoidosa	Lutein, sulfated polysaccharides
Nostoc linckia and *Nostoc spongiaeforme*	Borophycin
Nostoc sp.	Cryptophycin

2.1.1 Spirulina

Figure 4. Arthrospira platensis (Romano I., Bellitti M. R., Nicolaus B., et al., 2000).

Spirulina (***Arthrospira***) is a prokaryotic cyanobacterium (Fig. 4) belonging to the Cyanophyta family that originated more than 3 million years ago, shaped today's oxygen-rich atmosphere, and has been important in regulating the planetary biosphere (Romano I., Bellitti M. R., Nicolaus B., et al., 2000). In 1981, ***Spirulina*** was approved by the U.S. Food and Drug Administration (FDA) with the issuance of GRAS (Generally Recognized As Safe) status. The FDA declared that ***Spirulina*** can be legally marketed as a food or dietary supplement without risks to human health (Costa J. A. C., Morais M. G., 2013). ***Spirulina*** has a high protein value and high digestibility and contains significant amounts of essential polyunsaturated fatty acids and phenolic compounds (Ambrosi M. A., Reinehr C. O., Bertolin T. E., et al., 2008). Thanks to properties such as its high nutritional value and the presence of active biocompounds, this microorganism is one of the most studied microalgae worldwide (Borges J. A., Rosa G. M., et al., 2013). The protein content of ***Spirulina*** ranges from 50–70% (w/w) of its dry weight, carbohydrate content from 10–20% (w/w), and lipid content from 5–10% (w/w). This microalga is rich in vitamins B1, B2, B12, and E (notably vitamin B12). In addition, ***Spirulina*** has a high content of pigments, minerals, and trace elements (about 6–9% (w/w) of dry biomass), of which the most important are iron, calcium, magnesium, phosphorus, and potassium (Costa J. A. C., Morais M. G., 2013). Several studies have demonstrated the use of this microalga for pigment production owing to its antioxidant properties (Antelo F. S., Costa J. A. V., Kalil S. J., 2008; Silveira S. T., Burkert J. F. M., et al., 2007). β-Carotene accounts for about 80%

of the carotenoids present in ***Spirulina***, and other components, such as tocopherols, phycocyanin, and phycoerythrin, are part of its composition.

2.1.2 Nostoc

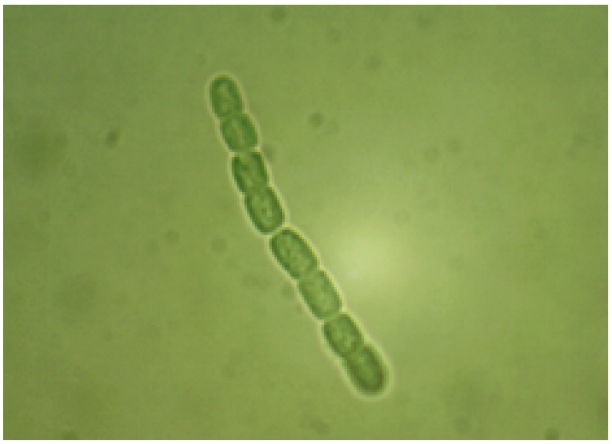


Figure 5. Nostocaceae (Cyanophyta) (Anshuman S., Deepika M., et al., 2013)

Nostoc is an edible microalga belonging to the Nostocaceae (Cyanophyta) that forms spherical colonies linked together as filaments. This microalga exhibits heterocysts with a pattern of homogeneous cells and regular spacing along the filament (Fig.5) (Anshuman S., Deepika M., et al., 2013). Heterocysts fix atmospheric nitrogen for the synthesis of amino acids in the microalgal biomass. In the absence of a nitrogen source during microalgal cultivation, heterocysts form, avoiding nitrogen limitation for cell growth (Maldener I., Muro-Paster A. M., 2010). ***Nostoc*** biomass has been used medically and as a dietary supplement because of its content of proteins, vitamins, and fatty acids. The medical value of this microalga has been demonstrated by its use in the treatment of fistula and certain forms of cancer (Temina M., Rezankova H., et al., 2007). Historically, biomass of this microorganism has been described as anti-inflammatory and is also reported to aid digestion, control blood pressure, and strengthen the immune system. Several studies suggest that ***Nostoc*** produces various compounds with antimicrobial, antiviral, and antitumor activity. These findings have encouraged large-scale cultivation, and *Nostoc* has great economic potential due to its nutritional and pharmaceutical importance. Cyanovirin, a proteinaceous molecule produced by a *Nostoc* microalga, has shown positive effects in the treatment of HIV (Boyd M. R., Gustafson K. R., McMahon J. B., et al., 1997) and influenza A (H1N1) (Smee D. F., Bailey K. W., Wong M.-H., et al., 2008). *Nostoc* contains a spectrum of polyunsaturated fatty acids (PUFAs) that includes essential fatty acids such as linoleic,

α-linolenic, γ-linolenic, octadecatetraenoic, and eicosapentaenoic acids (Wang M., Xu Y.-N., Jiang G.-Z., et al., 2000). Essential fatty acids are precursors of prostaglandins, arousing considerable interest from the pharmaceutical industry.

2.1.3 Chlorella

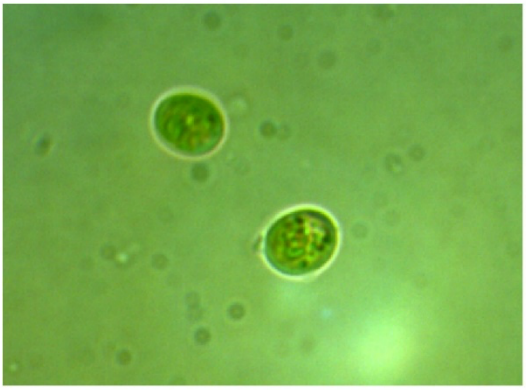


Figure6 Chlorella (Masojídek J., Prášil O., 2010)

Spirulina and Chlorella account for most of the microalgal biomass market, with annual production of about 3,000 and 4,000 tons, respectively (Masojídek J., Prášil O., 2010). ***Chlorella*** sp. (Fig. 6) is a eukaryotic genus of unicellular green microalgae belonging to the Chlorophyta. This microalga was popularized by the Japanese—traditional consumers of seaweeds—who generally value and use it as a dietary supplement. ***Chlorella*** microalgae are rich in chlorophyll, proteins, polysaccharides, vitamins, minerals, and essential amino acids. This microalga is composed of ~53% (w/w) protein, 23% (w/w) carbohydrates, 9% (w/w) lipids, and 5% (w/w) minerals and trace elements (Costa J. A. C., Morais M. G., 2013). These nutrient levels can be varied by manipulating culture conditions. The biomass of this microalga is also rich in B-complex vitamins, particularly B12, which are vital in the formation and regeneration of blood cells.

Like ***Spirulina***, ***Chlorella*** has FDA-issued GRAS status and can therefore be used as food without risks to human health if grown in a suitable environment under proper hygiene and good manufacturing practices (Costa J. A. C., Morais M. G., 2013; Costa J. A. V., Radmann E. M., et al., 2006). ***Chlorella*** contains bioactive substances with medicinal properties. Experimental studies with ***Chlorella*** have demonstrated

antitumor, anticoagulant, antibacterial, antioxidant, and antihyperlipidemic effects, as well as hepatoprotective properties and immunostimulatory activity of its enzymatic protein hydrolysate (Cha K. H., Kang S. W., et al., 2010; Kokou F., Makridis P., et al., 2012; Li L., Li W., et al., 2013). Many antioxidant compounds may be responsible for ***Chlorella***'s functional activities. Antioxidants such as lutein, α-carotene, β-carotene, ascorbic acid, and α-tocopherol have been identified and are active against free radicals. Some of these compounds are not only important as natural colorants or additives but may also help reduce cancer incidence and prevent macular degeneration (Zhao L., Sweet B. V., 2008). The most important bioactive compound in ***Chlorella*** is β-1,3-glucan, an active immunostimulant that reduces free radicals and blood cholesterol. The efficacy of this compound against gastric ulcers, sores, and constipation has been reported. It has also been shown to have a preventive effect against atherosclerosis and hypercholesterolemia, as well as antitumor activity (Spolaore P., Joannis-Cassan C., 2006).

2.1.4 Dunaliella

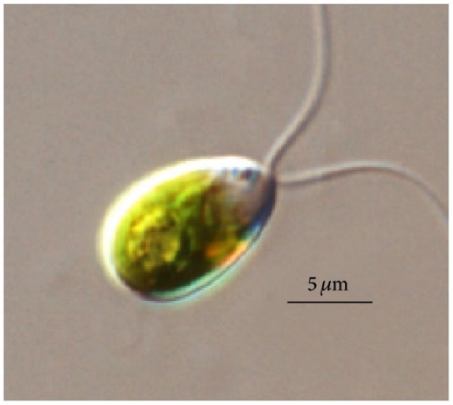


Figure7 Dunaliella (Preetha K., John L., et al., 2012)

Dunaliella is a halotolerant, unicellular green microalga belonging to the Chlorophyceae (Fig.7). This microalga is widely studied for its tolerance to extreme habitats, physiological traits, and numerous biotechnological applications. *Dunaliella* is a source of carotenoids, glycerol, lipids, and other bioactive compounds such as enzymes and vitamins (Preetha K., John L., et al., 2012; Hosseini Tafreshi A., Shariati M., 2009). This microalga is an important source of natural β-carotene, capable of producing up to 14% of its dry weight under high salinity, light, and temperature, as well as nutrient limitation (Francavilla M., Trotta P., Luque R., 2010). Beyond β-carotene, this microalga is rich in proteins and essential fatty acids and can be consumed safely, as evidenced by its GRAS recognition. Compounds present in *Dunaliella* biomass exhibit several biological activities, including antioxidant, antihypertensive, bronchodilatory, analgesic, muscle-relaxant, hepatoprotective, and anti-edema properties. Microalgal biomass can also be used directly in food and pharmaceutical formulations (Costa J. A. C., Morais M. G., 2013; Madkour F. F., Abdel-Daim M. M., 2013). Chang and colleagues (Chang T., Ohta S., Ikegami N., et al., 1993) demonstrated that *Dunaliella* cells contained antibiotic substances. According to these authors, the crude extract of this microalga strongly inhibited the growth of *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, and *Enterobacter aerogenes*.
In another study, *Dunaliella* also showed antibacterial activity against various microorganisms of importance to the food industry, including *Escherichia coli*,

Staphylococcus aureus, *Candida albicans*, and *Aspergillus niger* (Hosseini Tafreshi A., Shariati M., 2009). Under ideal growth conditions, *Dunaliella* can be stimulated to produce about 400 mg of β-carotene per square meter of cultivation area.

2.1.5 Biocompounds

Bioactive compounds are physiologically active substances with functional properties in the human body. There is great enthusiasm for the development and production of various biocompounds that can potentially be used as functional ingredients, such as carotenoids, phycocyanins, polyphenols, fatty acids, and polyunsaturated compounds (Plaza M., Santoyo S., et al., 2010). Recently, interest has grown in the production of bioactive compounds from natural sources, driven by a growing number of scientific studies demonstrating their health benefits (Herrero M., Castro-Puyana M., et al., 2013). Natural products are important in the search for new pharmacologically active compounds and generally play a role in drug discovery for human diseases (Newman D. J., Cragg G. M., 2012). Many clinically validated, commercially available drugs with antitumor and anti-infective activity originated from natural products. Microalgae are a natural source of interesting biocompounds. They are known to produce various therapeutically effective compounds that can be obtained from the biomass or secreted extracellularly into the medium (Bhagavathy S., Sumathi P., Jancy Sherene Bell I., 2011). These microorganisms contain many bioactive compounds—proteins, polysaccharides, lipids, vitamins, enzymes, sterols, and other high-value substances of pharmaceutical and nutritional importance—that can be exploited commercially (Priyadarshani I., Rath B., 2012).

Oxidative damage caused by reactive oxygen species to lipids, proteins, and nucleic acids can lead to many chronic diseases such as heart disease, atherosclerosis, cancer, and aging. Epidemiological studies have shown an inverse association between fruit and vegetable intake and mortality from diseases such as cancer, a phenomenon attributable to the antioxidant activity of these foods (Li H.-B., Cheng K.-W., Wong C.-C., et al., 2007). Microalgal biomass is considered a rich natural source of antioxidants, with potential applications in food, cosmetics, and medicine (Li H.-B., Cheng K.-W., Wong C.-C., et al., 2007). Antioxidant compounds such as dimethylsulfoniopropionate and mycosporine-like amino acids have been isolated from microalgae and are potent chemical blockers of UV radiation (Mata T. M., Martins A. A., Caetano N. S., 2010). In addition to these, pigments, lipids, and polysaccharides with antioxidant activity can also be found in microalgal biomass. Carotenoids and phycocyanins are the pigments most widely used in scientific research. C-phycocyanin (C-PC) is a blue photosynthetic pigment belonging to the phycobiliprotein group and is abundant in cyanobacteria,

Rhodophyta, and cryptophytes (Gupta A., Sainis J. K., 2010). Phycocyanin has applications as a nutrient and as a natural food and cosmetic colorant. It is commonly extracted from ***Spirulina*** biomass (Viskari P. J., Colyer C. L., 2003) and ***Porphyridium cruentum*** (Bermejo Román R., Alvárez-Pez J., 2002) and ***Synechococcus*** (Gupta A., Sainis J. K., 2010). Among carotenoids, β-carotene and astaxanthin are particularly important; they are used in the food and pharmaceutical industries for their antioxidant and pigmentation properties and, in algal metabolism, protect photosynthetic tissues from light and oxygen damage (Cazzonelli C. I., 2011). ***Dunaliella salina*** is recognized as a major biological source of β-carotene, producing more than 14% on a dry-biomass basis (Spolaore P., Joannis-Cassan C., 2006). ***H. pluvialis*** is a source of astaxanthin, producing 1–8% of dry biomass (Hejazi M. A., Wijffels R. H., 2004). Polysaccharides are a class of high-value components with applications in foods, cosmetics, textiles, stabilizers, emulsifiers, and medicine (Arad S., Levy-Ontman O., 2010). Their therapeutic action is based on macrophage stimulation and immunomodulation; the biological activity of sulfated polysaccharides depends on sugar composition and the position and degree of sulfation (Kim M., Yim J. H., Kim S. Y., et al., 2012). Microalgae capable of producing these compounds include *Chlorella vulgaris*, *Scenedesmus quadricauda* (Mohamed Z. A., 2008), and *Porphyridium* sp. (Tannin-Spitz T., Bergman M., et al., 2005).

Microorganisms such as microalgae exhibit antimicrobial capabilities due to their ability to synthesize a broad spectrum of bioactive compounds. These include fatty acids, acrylic acids, halogenated aliphatic molecules, terpenoids, sterols, sulfur-containing heterocycles, carbohydrates, acetogenins, and phenolic substances (Prakash J. W., Johnson M., Jeeva S., 2011). In particular, the antimicrobial potency of microalgal extracts has been primarily attributed to their lipid content. Compounds such as α- and β-ionone, β-cyclocitral, neophytadiene, and phytol are among the major contributors (Amaro H. M., Guedes A. C., Malcata F. X., 2011).

Microalgal extracts have demonstrated activity against various human pathogens including *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. This antimicrobial activity has been linked to polyunsaturated and saturated fatty acids like γ-linolenic, eicosapentaenoic, hexadecatrienoic, docosahexaenoic, palmitoleic, lauric, oleic, lactic, and arachidonic acids (Amaro H. M., Guedes A. C., Malcata F. X., 2011; Smith V. J., Desbois A. P., Dyrynda E. A., 2010). These fatty acids disrupt microbial membranes by increasing

permeability, leading to the leakage of intracellular components, impaired respiration, and nutrient uptake. Their effectiveness largely depends on chain length and the number of unsaturated bonds, with fatty acids longer than ten carbons (>C10) capable of lysing bacterial protoplasts (Amaro H. M., Guedes A. C., Malcata F. X., 2011).

In addition to fatty acids, microalgae also synthesize extracellular acidic sulfated polysaccharides (EPS) that exhibit both antimicrobial and antiviral effects (Raposo M. F. D. J., de Morais A. M. M. B., de Morais R. M. S. C., 2014). These sulfated polysaccharides—primarily composed of xylose, glucose, and galactose—demonstrate enhanced antiviral potential when their sulfate content is high (Arad S., Levy-Ontman O., 2010; Raposo M. F. D. J., de Morais A. M. M. B., de Morais R. M. S. C., 2014). Their inhibitory mechanism involves interactions with positively charged cellular sites or directly with viral particles, thereby blocking viral entry into host cells (Amaro H. M., Guedes A. C., Malcata F. X., 2011).

Species such as *Spirulina* (*Arthrospira*) have already demonstrated the ability to produce sulfated polysaccharides that are active as antiviral agents in *in vivo* and *in vitro* models (De Jesus Raposo M. F., De Morais R. M. S. C., De Morais A. M. M. B., 2013). Other eukaryotic genera like *Chlorella* and *Dunaliella* also secrete polysaccharides at relatively high levels (Rodríguez Meizoso I., Jaime L., Santoyo S., et al., 2008). The antibacterial capacity of *Spirulina* has also been correlated with its volatile compound profile, which is primarily composed of heptadecane (40%) and tetradecane (35%) (Plaza M., Herrero M., 2009).

Numerous studies confirm the antiviral potential of microalgal polysaccharides against pathogens such as encephalomyocarditis virus, herpes simplex virus types 1 and 2 (HSV-1, HSV-2), human immunodeficiency virus (HIV), viral hemorrhagic septicemia virus, swine fever virus, and varicella-zoster virus (Amaro H. M., Guedes A. C., Malcata F. X., 2011; Smelcerovic A., Knezevic Jugovic Z., Petronijevic Z., 2008). For instance, carrageenan has been shown to bind directly to human papillomavirus (HPV), thereby inhibiting both viral attachment and internalization (Raposo M. F. D. J., de Morais A. M. M. B., de Morais R. M. S. C., 2014).

2.2 Application Fields of Microalgae

Microalgae represent a diverse category of photosynthetic microorganisms that contribute significantly to the expanding array of valuable bioresources applicable in various industries and environmental contexts. Their biochemical composition, which includes lipids, proteins, polysaccharides, and pigments, imparts crucial nutritional, pharmacological, and functional benefits. Consequently, microalgae are appealing candidates for the formulation of functional foods and animal feed, in addition to their roles in cosmetics and agricultural biostimulants.(fig8).

Figura 8. Different types to use microalgae (Kandasamy S., Zhang B., 2022).

Unlike terrestrial crops, microalgae are not reliant on land or soil-based feed sources; they can thrive in non-arable land as well as saline or wastewater environments without being affected by food pests. This resilience, coupled with their efficient photosynthesis and ability to utilize industrial CO_2 emissions, enhances their potential within sustainable biomass production systems (Chisti & Yan, 2011). However, not all algal strains possess the capability to accumulate significant lipid content—approximately half of their dry biomass—which poses challenges for commercial algal biofuel production due to the high costs associated with lipid extraction and harvesting. As a result, the biofuel sector has shifted its focus towards generating high-value compounds

such as specialty lipids, pigments, and bioactive ingredients suitable for cosmetics and pharmaceuticals.

Furthermore, microalgae produce a range of secondary metabolites exhibiting antioxidant, antibacterial, antiviral, anti-inflammatory, and anticancer properties. Notably studied substances include carotenoids, phycobiliproteins, and sulfated polysaccharides for potential use in nutraceuticals and dermatological applications (Foley et al., 2011). Chlorella has shown considerable promise as an antitumor agent (Iwamoto, 2004), while extracts from Spirulina, Dunaliella salina, Nannochloropsis oculata, and Alaria esculenta find applications in cosmetics due to their anti-UV properties and skin regeneration capabilities (Stolz & Obermayer, 2005). From a nutritional standpoint, microalgae are rich sources of essential vitamins (B12, A, E), minerals (iodine, selenium), omega-3 fatty acids, β-carotene along with high-quality proteins and peptides. Spirulina is particularly noted for its antioxidant qualities and antitumor effects alongside being an important "super food" rich in γ-linolenic acid and B-complex vitamins. Likewise, Chlorella contains chlorophylls along with β-1,3-glucans and all essential amino acids; other species like Porphyridium and Haematococcus are notable for their EPA content as well as arachidonic acid (AA) and astaxanthin levels (Wells et al., 2017; Becker et al., 2004). Despite these advantages however; sensory attributes such as taste and odor remain barriers to consumer acceptance in food applications. In aquaculture and livestock nutrition sectors; microalgae serve as sustainable feed additives that facilitate the production of renewable proteins fats along with bioactive pigments. They can replace fish meal or oil—which often contains harmful persistent organic pollutants like PCBs—and dioxins (Hasegawa et al., 2007). Genera that are most frequently used for EPA include Nannochloropsis; Isochrysis; Chaetoceros; Chlorella; Spirulina; Dunaliella; Phaeodactylum while Thraustochytrium alongside Schizochytrium dominate DHA production efforts (Patil et al., 2005). From an energy perspective too—microalgae are explored as substrates for anaerobic digestion aimed at biogas generation—primarily methane—for electricity or heat purposes (Holm-Nielsen et al., 2009). Nonetheless—the energy yield from this approach remains limited compared to alternative feedstocks such as livestock waste or energy crops. Research into ethanol production from microalgal biomass has also been conducted but is currently not regarded as a viable option. In addition to bioproducts—microalgae play a vital role in wastewater treatment processes along with environmental remediation efforts. Specific strains including Chlorella; Chlamydomonas;

Scenedesmus; Nannochloropsis are employed within phycoremediation frameworks designed to eliminate excess nitrogen phosphorus heavy metals among other pollutants thanks largely to their remarkable nutrient uptake capabilities. Such systems offer dual benefits: biomass generation alongside water purification thus representing an eco-friendly economical method for nutrient recovery while managing waste effectively. Despite this widespread adoption remains limited due largely to the small number of commercial facilities operating worldwide—approximately only around forty at present.

Chapter 3

3. Arthrospira platensis

ArthroSpira platensis, also known as *Spirulina* (fig.9 tab. 4), is an ancient lower prokaryotic plant belonging to the category Cyanophyta, Cyanophyceae, and Oscillatoria. Morphologically, S. platensis consists of a single or multicellular filamentous body, 200–500 μm long, 5–10 μm wide, cylindrical, with a regular spiral folding, either loose or tight, like a watch spring.

As an ecological microscopic cyanobacterium, S. platensis mainly grows in alkaline salt lakes and is characterized by its rapid growth and low consumption of energy and water per kilogram (Xiaopeng et al., 2023).

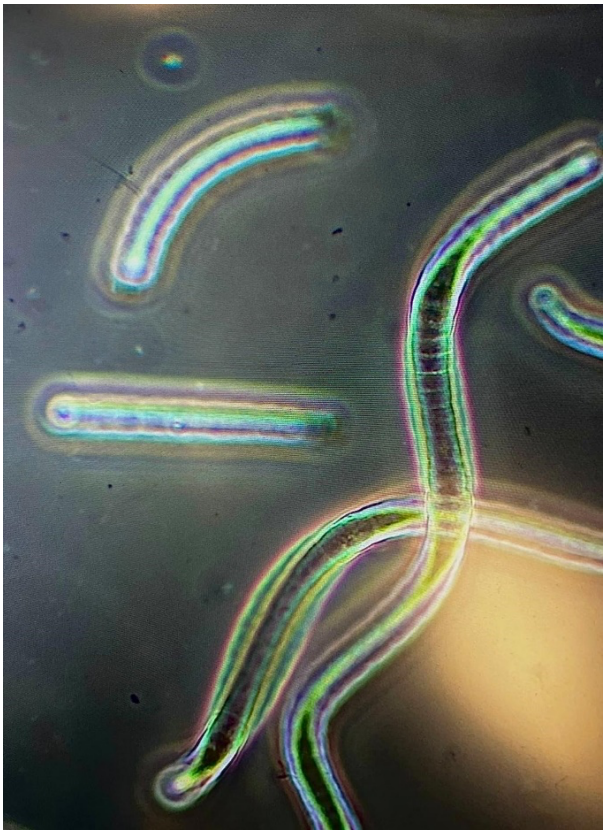


Figure 9. Spirulina in optic microscope

Table 4. Taxonomic classification

Bacteria	**Domain**
Eubacteria	Kingdom
Cyanobacteria	Phylum
Cyanophyceae	Class
Oscillatoriophycideae	Subclass
Oscillatoriales	Order
Oscillatorlaceae	Family
Arthrospira	Genus
A. platensis	Species

The life cycle of *Arthrospira platensis* is cellular in type. It begins from a single spiral that divides into cells called hormogonia, which progressively develop to form a new spiral (Sánchez et al., 2003).

The cycle includes three main phases:

1. Fragmentation of the trichome
2. Enlargement and maturation of hormogonial cells
3. Elongation of the trichome

During maturation, the trichome breaks into 2–4 cell (Fig. 10) chains through specialized cells called necridia, which dissolve (lysis), releasing discs that move and form new hormogonia (Ciferri,1983).

These hormogonia separate from the mother cell and develop into new trichomes, losing the ends of the necridial cells. The ends thus become rounded with thin walls (Ciferri, 1983).

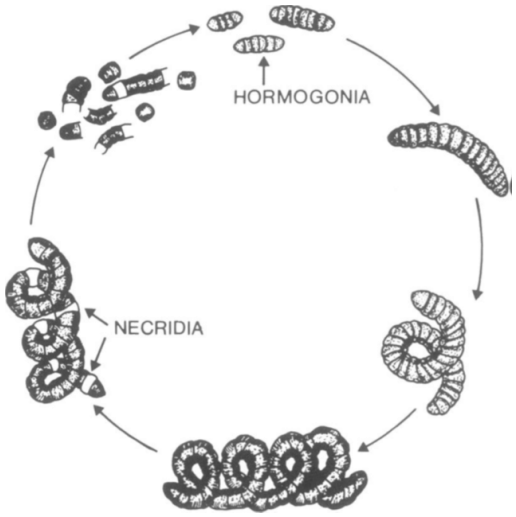


Figure 10. Life cycle of Spirulina platensis according to Ciferri (1983).

Arthrospira platensis is a cyanobacterium capable of adapting and thriving in most environments and water bodies, making it exceptionally easy to cultivate. However, different approaches must be adopted for the production of *A. platensis* in indoor or outdoor settings. Many producers have opted for indoor cultivation or tank systems, since outdoor cultivation requires large land areas, which are usually not available to the producer (Markou, 2014).

For indoor cultivation to be effective, a transparent container is required to allow *A. platensis* to absorb as much light as possible. Biomass production is directly correlated with light exposure, which makes it essential for the culture to receive a high amount of sunlight.

The use of artificial light in *A. platensis* cultures is advantageous for producers because of the reduced space requirements, better control over biomass density, and greater stability of concentration, which results from a constant day–night cycle (Markou, 2014). It is important to note that although artificial light offers advantages in terms of culture control, it also involves higher installation costs and significant energy consumption throughout the cultivation process (Markou, 2014). Regarding the light source, LED lights are preferable to fluorescent lights, since LEDs have little or no impact on the culture temperature (Naqqiuddin, Sukumaran, Almahrouqi, Omar & Ismail, 2015).

The color of light affects the growth and development of the exposed cyanobacterium. In a study conducted by the Agricultural University of Athens, it was observed that light color influenced biomass production, lipid content, chlorophyll content, and protein content (Markou, 2014). Studies have shown that microalgae seem to prefer blue or red light, greatly benefiting when the light corresponds to the pigmentation of their light-

harvesting complexes (Schulze, Barreira, Pereira, Perales & Varela, n.d.). This suggests that *Arthrospira platensis* develops best when exposed to blue or green light, as shown in Table 5, where blue light achieves the highest percentages in more than half of the categories. In Table 5, it can also be seen that higher biomass is obtained with warmer LEDs (such as red and pink), while higher levels of chlorophyll, lipids, and carbohydrates are obtained with blue LEDs. Moreover, carotenoids do not seem to be affected by light emission, showing minimal differences among different LED colors. The highest protein content, as shown in Table 5, is obtained with white light (50.1%), with results similar to green light (49.8%). This preference for blue light is related to the photosynthetic system of *A. platensis* and to its chlorophyll b content, which enables the cyanobacterium to thrive under blue light.

Table 5. Graph of the effect of light on A. platensis based on an experiment conducted at the Agricultural University of Athens (Markou, 2014).

Light color	**Biomass productivity ($mg \cdot L^{-1} \cdot d^{-1}$)**	**Phycocyanin (%)**	**Chlorophyll (%)**	**Carotenoids (%)**	**Proteins (%)**	**Lipids (%)**	**Carbohydrates (%)**
Blue	4.68	17.6%	1.42%	2.79%	42.1%	6.0%	11.3%
Green	9.73	11.7%	1.21%	2.75%	49.8%	4.8%	10.5%
Yellow	15.43	9.8%	1.19%	2.84%	47.3%	4.3%	9.8%
White	15.75	9.9%	1.16%	2.75%	50.1%	4.6%	8.8%
Red	30.69	9.3%	1.04%	2.73%	47.1%	4.4%	10.8%
Pink	30.89	8.2%	1.06%	2.78%	45.2%	3.8%	8.8%

Fluorescence spectroscopy is an electromagnetic spectroscopy that excites the electrons of a sample to analyze its fluorescence (Perkin Elmer, 1981). *Arthrospira platensis* shows a peak performance at 760 nm at 77 K mainly because it possesses a very low-energy chlorophyll a, which results in the absence of phycoerythrin in its phycobilisome (Akimoto et al., 2012). These fluorescence bands are due to the excitation energy produced in the transfer process between phycocyanin and allophycocyanin, which is believed to occur between photosystem I and photosystem II (Akimoto et al., 2012). For *A. platensis* to grow properly indoors, a proper nutrient balance is required to allow its propagation. For this specific strain, the use of a prepared medium known as Zarrouk's[1] medium is recommended, as it promotes the growth of *A. platensis* cells.

[1] Zarrouk's culture medium has been tested by scientists as a very effective medium in which A. platensis (along with many other cyanobacteria) can grow effectively. However, many scientists have modified the original culture

Zarrouk's medium is a nutrient-rich environment suitable for the growth of cyanobacteria. Composed of various chemicals, this medium provides the ideal conditions for the prosperity of *A. platensis*. The modified Zarrouk's medium includes potassium in different forms, an element that has proven fundamental in maintaining a high growth rate of *A. platensis* (Rajasekaran Chandrasekaran, 2016). In addition, A5 micronutrients have improved the growth, chlorophyll content, and biomass in *A. platensis*, making them a key element in the modified medium (Rajasekaran Chandrasekaran, 2016). It is important to use filtered water for the culture and for the preparation of the medium, to prevent the introduction of foreign algae into the culture (Jourdan, 2001). Finally, maintaining a pH equal to or greater than 10 reduces the likelihood of exopolysaccharide accumulation, which does not damage the culture but gives it an unpleasant appearance.

For indoor cultivation, a 20 L transparent tank, which can be plastic or glass, is recommended to allow adequate illumination of the culture (Naqqiuddin et al., 2015). Since this is indoor cultivation, it is necessary to include an aeration pump, a porous stone, and plastic tubes to ensure proper aeration of the tank (Naqqiuddin et al., 2015). This allows proper gas exchange, i.e., adequate inflow and outflow of accumulated gases. To obtain a good culture, the tank should be prepared before introducing the cyanobacteria, in order to avoid disturbances to the culture during setup. To avoid contamination, the use of a cover is recommended, given the possibility of absorption of toxins and heavy metals (Palaniswamy & Veluchamy, 2017).

To establish a culture of *Arthrospira platensis*, a live starter sample must be obtained from a supplier. This "starter kit" contains the mother strain suspended in a predetermined medium, which can then be transferred into the prepared tank. When selecting strains for cultivation, it is preferable to choose those with a higher proportion of spiral filaments, as these facilitate the harvesting process (Jourdan, 2001). Once transferred to the tank, the culture requires regular movement to ensure that all filaments receive light and CO_2 evenly. Agitation also prevents aggregation and sedimentation within the tank ("Growing Spirulina at Home Information," 2017). Exopolysaccharides (EPS) are sugar residues secreted by microorganisms into their surrounding environment as a protective barrier against diffusion (Nwodo, Green & Okoh, 2012). In some cases, this secretion results from an excess of sugars that the cell

---

medium to increase the growth potential of A. platensis to a maximum of approximately 5.8 µg to 6.1 µg, the average specific growth rate measured by scientists. (Rajasekaran Chandrasekaran, 2015)

expels to maintain carbohydrate reserves (Nwodo et al., 2012). In controlled indoor cultivation, EPS secretion has no particular role, yet these compounds have important medical and industrial applications. Spirulan, the exopolysaccharide produced by *A. platensis*, has been reported to inhibit lung metastasis in humans and to reduce tumor cell proliferation and adhesion (Nwodo et al., 2012).

Once the culture has been successfully established, harvesting becomes relatively straightforward. However, older biomass tends to become sticky and more difficult to handle. Harvesting is best carried out in the morning, when temperatures are lower and protein content is higher (Jourdan, 2001). Sunlight during these hours also supports the drying process of *A. platensis*.

The harvesting process involves two key steps: filtration and removal of the residual medium. Filtration is performed by passing the culture through a fine cloth, such as gauze, using gravity. Before harvesting, the culture should be stirred, since spiral filaments tend to float and form a surface layer. If only this layer is collected, the proportion of straight filaments increases, making the process more laborious (Jourdan, 2001). After filtration, it is necessary to expel the remaining medium so the product can be consumed or dried for further use. Filtration can be accelerated by moving the cloth, allowing biomass aggregation while preventing cells from adhering to the fabric. By applying pressure on the cloth bag, the remaining medium is released. When the liquid exiting the bag turns greenish, the process must be stopped, as this indicates product loss (Jourdan, 2001). Washing the product with freshwater is not recommended because osmotic shock may damage the cells and increase susceptibility to fermentation (Jourdan, 2001).

Arthrospira platensis can also be harvested at small scale through sedimentation, a process in which solids and liquids are separated by gravity. However, this method is time-consuming and energy-intensive, and therefore not recommended (Al Hattab, 2015).

Large-scale or mass production of *A. platensis* is best suited to outdoor systems, which naturally require more space and different conditions compared to indoor cultivation. Outdoor ponds used for cultivation are typically shallow (12–15 cm deep) and lined with cement, PVC, or other durable plastic materials (Habib, 2008). Ponds should also include a paddle wheel to maintain mixing of the culture, which may vary in design (see Figure 11). Proper mixing ensures that all cells are exposed to sufficient sunlight, which would otherwise be limited if the culture remained static. Experiments have shown that

unshaded outdoor cultures yield higher growth and productivity compared to indoor or shaded outdoor systems (Sukumaran et al., 2018).

The paddle wheel is the most critical component of outdoor ponds. Its shape and size may vary depending on the system, but its function remains the same: to provide aeration and mixing. This agitation improves nutrient assimilation and prevents photoinhibition (Sukumaran et al., 2018). As illustrated in Figure 11, circular ponds typically use a pivoting agitation system, whereas raceway ponds, such as those in Hainan (China), perform better with paddle wheels.

Large-scale production can be managed using different methods, ranging from industrial approaches such as vacuum or pressure filtration to simpler sieving systems. The method chosen largely depends on the available budget and the time required for harvesting. Filtration is among the most widely used methods due to its versatility and simplicity, requiring a permeable medium that retains the cells while allowing the liquid to pass through (Al Hattab, 2015). The pressure gradient needed for filtration can be provided by gravity, pressure, or vacuum, depending on the desired energy consumption and available resources.

Vacuum filtration (Figure 11) separates solids by trapping microalgal cells on a filter while drawing the liquid through it (Al Hattab, 2015). The type of vacuum filter used depends on the filtering membrane. These membranes include drum filters, suction filters, filter thickeners, belt filters, and starch-precoated filters. It has been observed that drum filters tend to clog, while thickeners yield low solid content at the expense of high energy consumption (Al Hattab, 2015).

The efficiency of the process depends on both the pore size of the membrane and the size of the cells to be harvested.

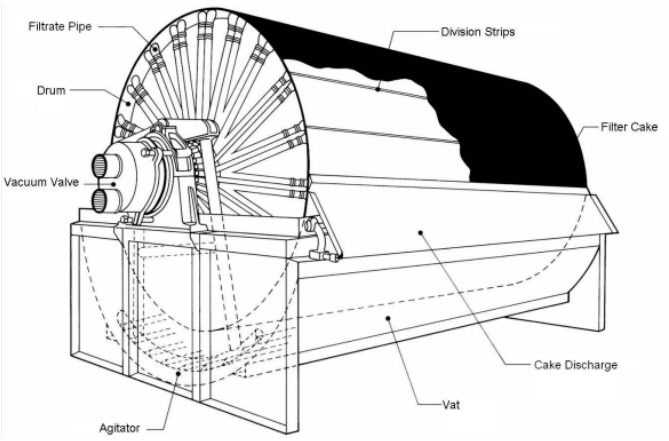


Figura 11 . Vacuum filtration system, adapted from OMMI Ltd. (2025)

Pressure filtration separates particles from their liquid suspension by pressurizing the mixture. By increasing the pressure above atmospheric levels, a liquid flow is generated that facilitates the formation of "cakes" on the filter (Al Hattab, 2015). There are two main options for pressure filtration: the plate-and-frame filter or the pressure vessel, as shown in Figure 12. The first method forces the liquid vertically through the filter using high pressure, while pressure vessels pump the fluid through filter cloths that retain the microalgae within, forming cakes (Al Hattab, 2015). This is an energy-efficient method, but its effectiveness depends on the type of microalga, making it particularly suitable for *Arthrospira platensis*.

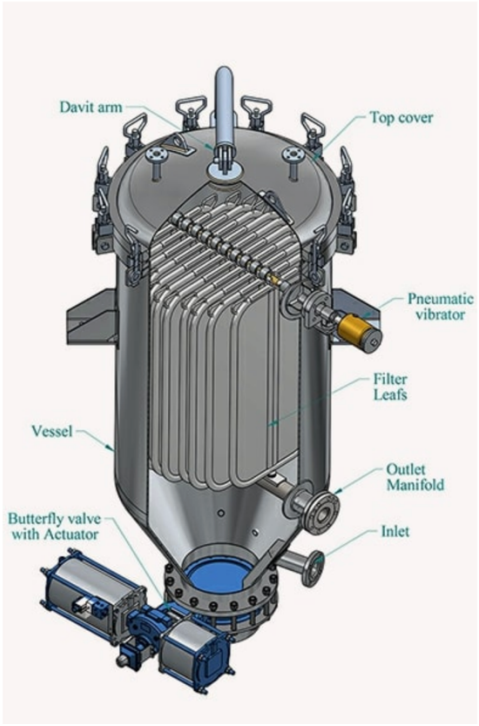
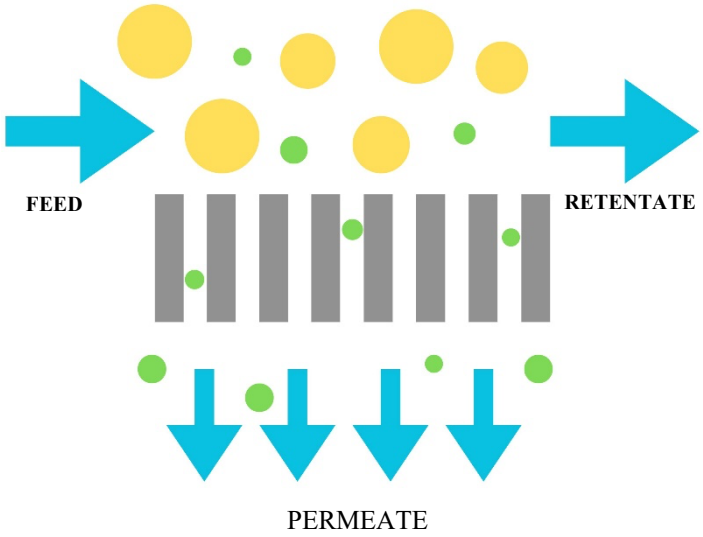


Figura 12 Vertical pressure filtration system, adapted from Sharplex Filters India Pvt. Ltd. (2025).

Tangential flow filtration is one of the most effective methods for large-scale harvesting. In this process, the culture flows tangentially over a membrane, which retains larger particles while allowing smaller ones to pass through. This separation is illustrated in figure 13, where the retained particles (retentate) are separated from those that permeate. This is a highly efficient method because it allows for the complete separation of debris and microalgal cells in a cost-effective manner (Al Hattab, 2015).



Figur 13. Schematic representation of tangential filtration, adapted from Al Hattab (2015)

With a large range of bioactive compounds and potential health benefits, Arthrospira platensis has received considerable attention in recent decades (Fathi, 2018). Spirulina is rich in certain nutrients, such as proteins, vitamins, minerals, and antioxidants, which have also been related to different therapeutic outcomes including immune modulation, anti-inflammatory and antitumor activity (Farag et al., 2015; Lupatini et al., 2017; Zahir et al., 2019; Park, Lee & Kim, 2018). It is widely recognized as a protein source of choice in many cases and a promising way to tackle protein deficiency in developing countries, providing an alternative to soy and eggs (Chaves et al., 2021). Furthermore, these new pigments (ex: phycocyanin) became a focus mainly for the pharmaceutical, nutraceutical and food applications of the novel pigments (Eilam & Khattib, 2023). Spirulina contains protein, which constitutes 50–70% of total dry weight (Valente et al., 2021; Evans, Smith & Moritz, 2015). This is notable since Spirulina contains all necessary amino acids in one source creating a protein source and the same proteins can be found in animal products like meat and eggs (Wild, Steingaß & Rodehutscord, 2018). Other important amino acids are leucine (7.67%), lysine (4.37%), methionine (2.39%), phenylalanine (4.42%), threonine (4.88%), tryptophan (1.93%), valine (6.37%) (AlFadhly, 2022). Moreover, major non-essential amino acids (61.19%) comprise alanine (7.52%), arginine (7.65%), glycine (5.24%), and serine (4.16%) also, which influence Spirulina protein composition (Morsy & Sharoba, 2014; Bortolini et al., 2022). Because of this, an excellent food protein candidate for food consumers such as

vegetarians and vegans (AlFadhly, 2022). Lipids have lower intake of Spirulina than protein but are still significant in biochemical contents (Bortolini et al., 2022). 5–10% of the dry biomass contain lipids containing polyunsaturated fatty acids (PUFAs) which are the principal polyunsaturated fatty acids generated by the biomass (Bonos, Kasapidou et al., 2016). A well-known omega-6 fatty acid with anti-inflammatory properties is γ-linolenic acid (GLA; 18:3n–6)—the major fatty acid (Martins et al., 2021). Relatedly, linoleic (18:2n–6), oleic (18:1c9), and palmitic acid (16:0) (Spinosa, Costa & Prates, 2022; Wild, Steingaß & Rodehutscord, 2018) make up other related fatty acids. Carbohydrates make up approximately 15–20% of the dry weight of Spirulina (Chaiklahan, Chirasuwan et al., 2022). These are primarily polysaccharides with structural and storage properties. Sulfated polysaccharides (such as calcium spirulan, or Ca-SP) have been known to have antiviral, anti-inflammatory and immunomodulatory applications (Pugh, Ross et al., 2001; Hayashi et al., 1996). Spirulina contains carbohydrates such as glucose, rhamnose, xylose, mannose and galactose (Pugh, Ross et al., 2001) that serve in complex structures that are important for cell wall strength as well as bioactivity (Chen, Xu, Yu et al., 2020). Spirulina pigments produce a blue green coloration (Christaki et al., 2011). The main pigment, phycocyanin, can account for 47% of dry weight (Bortolini et al., 2022; Sousa et al., 2008). Apart from its photosynthetic role, phycocyanin is also a highly powerful antioxidant (Chaiklahan, Chirasuwan et al., 2022). Spirulina also contains chlorophyll and carotenoids, vitamins B and E (de Marco et al., 2019). Spirulina also contains vitamins rich in B1, B2, B3 and B9 (Bortolini et al., 2022; Janda-Milczarek et al., 2013), so as Spirulina has high levels of vitamin B, then vitamin B12 too (Liestianty et al., 2019). It also has its own vitamin E and provitamin A (β-carotene). There are also high iron, calcium, magnesium, zinc, and potassium contents, with iron being in the range of 28–50 mg/100 g dry weight, indicating to Spirulina high utility for individuals with iron-deficiency anemia (Liestianty et al., 2019; Moradi et al., 2023). Ferulic acid and caffeic acid are examples of phenolic compounds with antioxidant, antimicrobial and anti-inflammatory activities (Ilieva et al., 2024; Rojas et al., 2020). Spirulina has antioxidant, anti-inflammatory, immunomodulatory, antiviral, antitumor, antidiabetic, hypolipidemic and cardioprotective properties (Konícková et al., 2014; Carbone et al., 2021; Anvar et al., 2021; Calella et al., 2022). These bioactivities tend to be associated with compounds including phycocyanin, sulfated polysaccharides as well as essential fatty acids (Chaiklahan, Chirasuwan et al., 2022; Ferdous & Yusof, 2021). Spirulina is

widely used as a natural colorant and dietary supplement due to its nutritional value and phycocyanin content (Christaki et al., 2011; Su et al., 2023). In the food, the incorporation of this vitamin adds many more nutrients into the diet and has a beneficial role to play in supplementing human nutritional deficiency (Wild et al., 2018; Evans et al., 2015). It has also been added as an alternative nutrient to the vegetable consumed in livestock, poultry, aquaculture and pet feed (Valente et al., 2021; Martins et al., 2021; Spinosa, Costa & Prates, 2022). It improves growth efficiency, immune function, pigmentation and disease resistance (Pestana et al., 2020; Watanuki et al., 2006; Teimouri, Amirkolaie & Yeganeh, 2013). It is well established as therapeutic in immune system support, cancer prevention, and chronic disease control (Bortolini et al., 2022; Anvar et al., 2021; Konícková et al., 2014). Its components (fig. 14) may serve as antioxidants, antivirals, and immunomodulators (Hayashi et al., 1996; Carbone et al., 2021; Gromek et al., 2024). It has been backed by clinical studies in allergies, diabetes, cardiovascular diseases and obesity (Yang, Lee & Kim, 1997; DiNicolantonio et al., 2020).

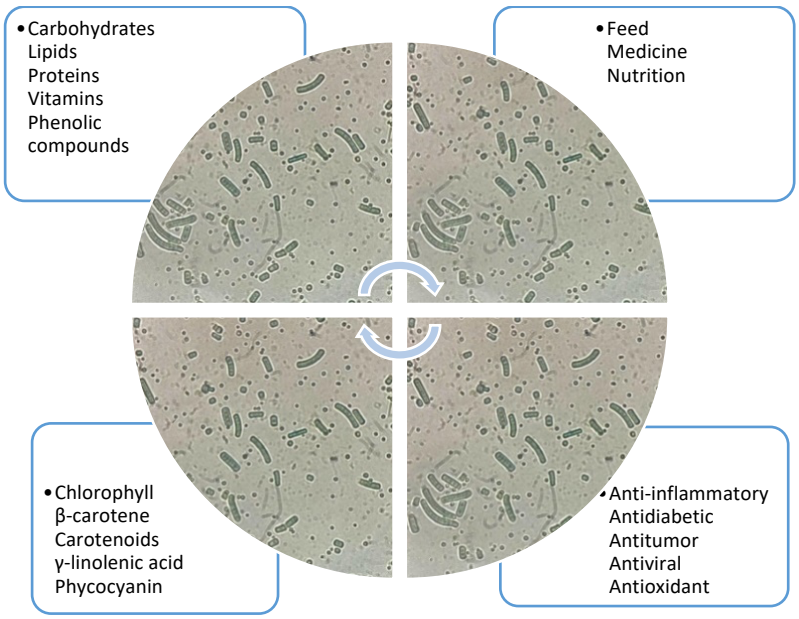


Figura 14. Main nutritional components, pigments, and biological activities of Spirulina platensis, adapted from Bortolini et al. (2022).

Chapter 4

4. Phycocyanin

Phycobiliproteins are the main fluorescent proteins present in Spirulina responsible for light absorption (fig15). They are divided into phycoerythrin (PE), phycocyanin (PC), and allophycocyanin (APC), according to the color of their pigment (Hsieh-Lo, Castillo et al., 2019). Phycocyanin is localized in the phycobilisomes, a supramolecular protein complex that functions as an antenna of the photosynthetic apparatus at the thylakoid level, attached to the thylakoid membrane (Wu, Liu, Miron et al., 2016).

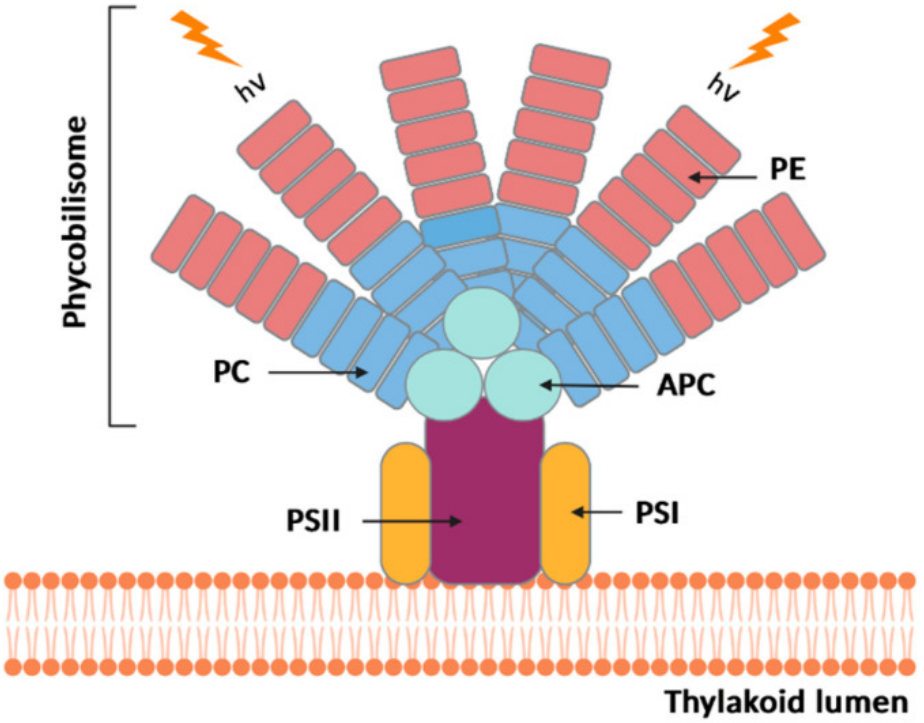


Figure 15.Structural organization of phycobilisomes. This complex is composed of phycoerythrin (PE), phycocyanin (PC), and allophycocyanin (APC), arranged to transfer energy (hv) unidirectionally to the reaction center with high efficiency.

Phycocyanin, a high-value protein, is composed of an alpha (α) polypeptide ranging from 10 to 19 kDa and a beta (β) polypeptide ranging from 14 to 21 kDa, which together form a monomer. The α subunit is covalently bound to one phycocyanobilin,

while the β subunit carries two phycocyanobilins (Yuan, Li, Shan et al., 2022). Phycocyanobilin is a bilinic chromophore of phytochromes, characterized by an open-chain tetrapyrrole group, which binds proteins through a thioether linkage and imparts the distinctive blue color to phycocyanin (Yuan, Li, Shan et al., 2022) (Figure 16 A, B, C). The monomers assemble into ring-shaped trimers, which further organize into hexameric structures, providing stability to the phycocyanin molecule (Yuan, Li, Shan et al., 2022).

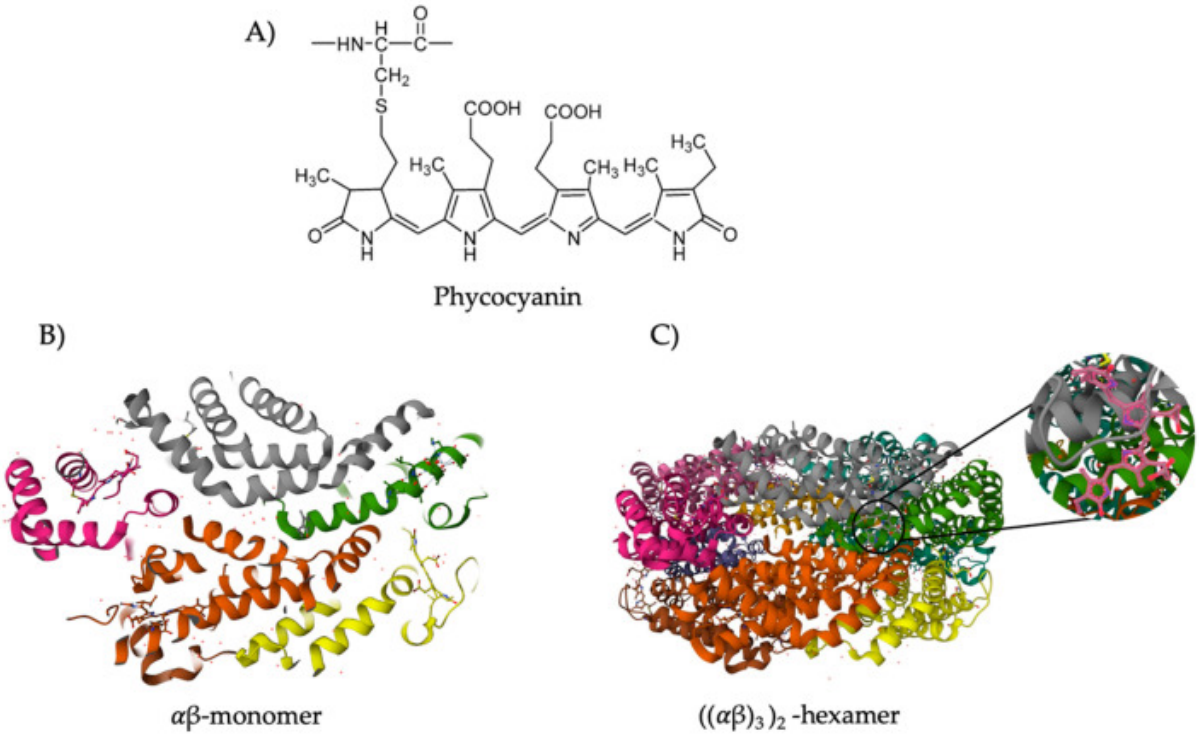


Figure 16. (A) Chemical structure and protein (α, β) of phycocyanin from Spirulina platensis. (B) Representation of the αβ monomer and (C) the trimeric three-dimensional structure (α3β3) of phycocyanin. The α subunit is shown in green, orange, and pink, while the β subunit is shown in gray, yellow, and brown. (C) also highlights a phycocyanobilin chromophore of phycocyanin. The α and β subunits carry one and two phycocyanobilins, respectively. α: alpha; β: beta. (Yuan, Li, Shan et al., 2022; Pagels, Guedes et al., 2019).

The extraction of C-PC is mainly influenced by the following physical and chemical parameters: temperature, pH, solvent type, biomass-to-solvent ratio, and biomass form (dried or fresh); these parameters are described below.

Temperature affects the extraction of intracellular compounds by altering the structure of the cell membrane, increasing the mass transfer rate of intracellular compounds into the extraction medium. In the case of C-PC, yield increases with temperature up to a

certain point; beyond this, degradation and denaturation occur. It has been reported that extraction yields increase at 30–50 °C but further increases lead to reduced C-PC concentration (Su, Liu et al., 2014).

pH directly affects the solubility of C-PC due to the influence of solvent ionic strength on protein structure. Optimal pH conditions are reported between 6 and 7 (Su et al., 2014; Vali Aftari et al., 2015), since C-PC is unstable at pH values below 5 and above 8 (Patil & Raghavarao, 2007; Su et al., 2014). The isoelectric point of C-PC varies between 4.1 and 6.4 depending on the cyanobacterial species (Fernández-Rojas et al., 2014). Near the isoelectric point, protein-water interactions are reduced, making extraction more difficult (Silveira et al., 2007). Extract purity is also lower at acidic pH (<5) (Patil & Raghavarao, 2007).

To control extraction pH, aqueous buffer solutions are typically used within the pigment's stability range. The most common buffer is sodium phosphate (pH 6–8). In addition to buffers, distilled water and aqueous solutions such as $CaCl_2$ (1.5%, w/v) and NaCl (0.15 M) have also been used (Jaeschke, Teixeira et al., 2021).

In general, higher biomass-to-solvent ratios increase extraction yield, though they reduce extract purity. Common ratios reported are 1:25 (Silveira et al., 2007), 1:20 (Ferreira-Santos et al., 2020; Su et al., 2014), 1:15 (Li et al., 2020), and 2:25 (Silveira et al., 2007; Vali Aftari et al., 2015). Besides yield and purity, processing costs must also be considered, since higher solvent volumes increase expenses.

C-PC is usually extracted from dried biomass, with fewer studies testing fresh biomass (Jaeschke et al., 2019; Martínez et al., 2017; Patil & Raghavarao, 2007; Sarada et al., 1999). It remains unclear whether freeze-dried or fresh biomass influences yield. However, rehydration of Spirulina powder (2 h in buffer) improved yields compared to dried biomass (Tavanandi & Raghavarao, 2020). Conventional drying is not recommended, since prolonged exposure to high temperatures (up to 60 °C) reduces C-PC content (İlter et al., 2018).

Cell disruption methods are among the most critical factors affecting extraction yield and purity. An ideal method allows selective release of the target compound with minimal energy input. Both conventional and emerging methods for C-PC extraction are outlined below.

Freeze–thaw cycles are frequently used in laboratories to disrupt cyanobacterial cells (Moraes et al., 2011; Tavanandi et al., 2018). Intracellular freezing increases cell volume due to ice crystal formation, followed by shrinkage during thawing. Freezing

also alters cell membrane pressure conditions and generates osmotic stress from localized changes in electrolyte concentration, contributing to membrane rupture (Acker & McGann, 2003; Roquebert & Bury, 1993).

Mixing and homogenization are simple extraction methods that involve agitating biomass in the extraction solvent.

- *Mixing* uses low agitation intensity (typically a magnetic or rotary stirrer). Osmotic pressure variations can induce cell lysis, while shear forces from agitation also damage cells. Extraction, however, requires long processing times (4–24 h) (Chaiklahan et al., 2011; Silveira et al., 2007).
- *Homogenization* (commonly with an Ultra-Turrax) disrupts cells through strong shear forces, releasing intracellular compounds within minutes. The main drawback is non-selectivity, as cellular debris are also released (İlter et al., 2018). Higher temperatures during mixing or homogenization improve extraction yield but reduce purity (Silveira et al., 2007).

Bead milling is a mechanical disruption method in which high-speed beads collide with microbial cells. Disruption occurs through shear forces generated by collisions, bead friction, and turbulence (Suarez Garcia, Lo, Eppink, Wijffels & van den Berg, 2019). Due to its efficiency, bead milling is often used as a positive control in microalgal extraction studies and to determine total C-PC content (Jaeschke et al., 2019; Käferböck et al., 2020; Pott, 2019).

Conditions are optimized according to cell function, medium viscosity, and flow rate, resulting in intricate parameter interaction (Montalescot et al., 2015). Beads are usually glass, zirconium, ceramic, and steel; smaller sized beads (0.2–0.6 mm) exhibit higher disruption as a consequence of high collision frequency (Suarez Garcia et al., 2019; Zinkoné et al., 2018). The density of beads impacts performance: low-density glass beads exhibit better functionality in low-viscosity media, whereas these high-density zirconia beads work best in viscous suspensions (Günerken et al., 2015). Other significant parameters are volume/geometry of chamber, the agitation speed, and biomass concentration. Cellular disruption is increased by additional impact forces and frequency of collisions (Postma et al., 2015) at higher speeds of agitation. High-pressure homogenization (HPH) is a mechanical cell disruption method in which biological suspensions are pumped through a narrow space subjected to intense shear forces. A pressure drop (up to 2000 atm) produces high fluid velocities in shallow passages. In

addition to the shear forces, cell damage is the consequence of the pressure drop in the nozzle and the environment (de Carvalho et al., 2020; Koubaa et al., 2020). Temperature is likewise an important parameter as it is thought that the temperature increases by 19.4°C due to a pressure drop from 800 to 1 atm in an aqueous suspension. As a result samples are subjected to in-cycle cooling (de Carvalho et al., 2020). HPP is a process of high pressure application to the liquid that is applied and delivers pressure to the matrix in a closed system. It is a non-thermal method and is highly applied to food processing and has been newly applied as an extraction protocol. In high pressure conditions air escapes from cell vacuoles and membrane construction is lost and increases cell wall permeability. As a result, HPP typically reduces extraction time and yields concentrated extracts (Huang, Hsu, Yang & Wang, 2013). Ultrasound is based on ultrasound transmission of ultrasonic waves (20 kHz to 10 MHz) across biological matrices. The wave propagation leads to several physical and chemical phenomena, such as agitation, cavitation, shear, shock waves, and free radical formation. Ultrasound (US) applied for intracellular compound extraction results in acoustic cavitation as the principal driving force. Ultrasonic waves generate alternating compression and rarefaction zones causing the bubbles in liquids. Bubble expansion and contraction with the wave cycles lead to a violent collapse. This phenomenon is called acoustic cavitation and produces localized temperatures of about 5000 K as well as pressures of up to 50 MPa. In an experimental setting, turbulence, collision and agitation result from collapsing bubbles, which thin cell membranes and cause rupture of the cells, which enhances extraction production (Tiwari, 2015). Non-ionising microwave waves (MW) are generally considered to provide energy as fast as 300 MHz up to 300 GHz. Through biological matrices, heat is generated as MW pass ionic conduction and also dipole rotation. Ionic conduction is the migration of an ion due to an alternating electric field resulting in frictional heating. Dipole rotation aligns dipolar particles with the alternating field; molecular oscillation is capable of creating collisions, yielding heat (Vinatoru, Mason & Calinescu, 2017). Microwave-assisted extraction can be carried out with solvents (for non-volatile) or without solvents (for volatile). For this kind of compound, or a non-volatile one, the solvent needs to be chosen based on its dielectric properties and the solubility of C-PC. If the dielectric loss tangent of the biomass is higher than that of the solvent, heating is more rapid, promoting cell rupture. Conversely, if the solvent has a higher dielectric loss tangent, MW enhances diffusion through the solvent (Vinatoru et al., 2017). MW power and extraction time are the main

parameters determining extraction efficiency. Results of C-PC extraction have found optimal conditions at 133 W for 166 seconds (İlter et al., 2018). Li et al. According to (1999), C-PC is stable at temperatures below 40 °C, however, in higher temperatures, pigment degenerates, while optical density decreases sequentially. Optical density declines considerably at >50 °C, and drops by 75% at >70 °C. It was also reported that sugar solutions enhances thermal stability of C-PC. Light has little impact on C-PC: there were no optical density changes at 5000 lx under pH 5 after 60 h.

Thangam et al. (2013) showed that C-PC has general apoptotic characteristics including DNA fragmentation, nuclear condensation, membrane blebbing, and cell shrinkage through fluorescence and phase contrast microscopy. Inhibited proliferation, based on the C-PC treatment of human tumor cells, was indicated by arresting the cell cycle at the G0/G1 level and by blocking DNA synthesis. The findings of Basha et al. (2008) are similar. Zong et al. (2001) studied the antitumor activity of C-PC against HeLa cells. Their study demonstrated that inhibition of tumor cells reached 31% at a concentration of 80 mg/L, and the inhibition mechanism was thought to shift cells from the S or M phase into the G1 phase, preventing cell division into the tumor-cell and eliminating DNA synthesis.

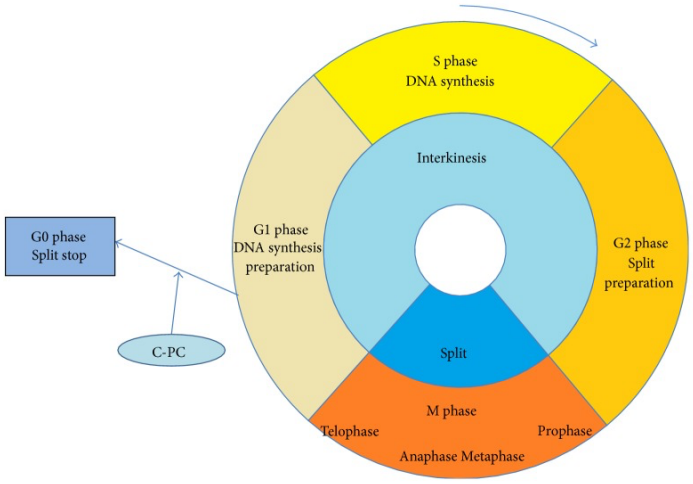


Figure 17 COX-2 is an inducible enzyme highly expressed in inflammatory and tumor cells (Liu & Zhang, 2006; DuBois, Shao et al., 1996).

COX-2 is an inducible enzyme highly expressed in inflammatory and tumor cells (Liu & Zhang, 2006; DuBois, Shao et al., 1996). Recent studies have shown that COX-2 is closely linked to tumor formation (Huang, Zhang, Xie & Lin, 2015) and progression (Sheehan, Sheahan, O'Donoghue et al., 1999; Chen, Markosyan, Connolly et al., 2014), as well as tumor angiogenesis (Tsujii, Kawano et al., 1998) and metastasis (Sheehan et al., 1999).

Therefore, COX-2 inhibitors are considered promising candidates for cancer therapy. Their antitumor effect is thought to follow two main mechanisms (Fig 17).

The first relies on blocking the COX-2 pathway and reducing the formation of COX-2-derived products, such as prostaglandin E2 (PGE2) (Williams, Smalley & DuBois, 1997). Studies have also reported inhibitory effects of C-PC on TPA-induced alterations of ODX, COX-2, and IL-6, highlighting its role in tumor initiation, promotion, and progression (Gupta & Gupta, 2012). Acting as a COX-2 inhibitor, C-PC can bind VEGF1 and suppress colon cancer through the angiogenic pathway (Saini & Sanyal, 2014). Ravi et al. (2015) further showed that C-PC reduces PGE2 levels.

Reduction of intracellular cAMP concentrations by lower levels of PGE2 has been reported (Sales, Katz, Davis et al., 2001) and is associated with increased expression of E-cadherin, so that malignant tumor response was reduced. Furthermore, depleted PGE2 drives the expansion of T and B immune cells, which reinforces immunogenesis (Liu & Jiang, 2014). Despite the high antitumor activity of C-PC, however, high molecular weight and complex secondary structure prevent accurate detection of small antitumor molecules and mechanisms. To circumvent this problem, a great number of investigators have designed enzymatic hydrolysates and subunits of C-PC. Wang et al. (2001) isolated C-PC peptides by enzymatic hydrolysis and column chromatography, obtaining four peptide groups. Their activity and that of normal C-PC and enzymatic hydrolysates were evaluated on HeLa and 293T tumor cells. There were the highest inhibitory effects of groups 1 and 4 on HeLa cells, with group 4 demonstrating the greatest inhibition on 293T cells. Hydrolysis was still limited, resulting in peptide fractions with unknown composition that need further structural and mechanistic analysis. Zhang and colleagues (Sun, Liang & Xu, 2010; Zhang, Li & Gong, 2010) isolated C-PC subunits and examined the effect on SPC-A-1 lung cancer cells. More importantly, as compared to the α subunit, this β subunit displayed higher inhibitory effects. Subhashini et al. (2004) indicated that the β subunit inhibits the growth of many apoptosis cells in most tumor cells through interaction with membrane-bound tubulin

and GAPDH and binds to the caspase-3 and caspase-9. GAPDH down-regulation resulted in pre-S-phase escape, arrest the cycle at G0/G1, and inhibited tumor cell proliferation. C-PC has antioxidant and antiinflammatory properties (Romay, Armesto et al., 1998) including scavenging of hydroxyl and oxygen free radicals, which were noted by Romay for the first time. These observations were subsequently corroborated by Bermejo, Piñero & Villar (2008). ROS (for instance, superoxide anions (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (•OH)) are generated naturally by the cell itself during normal metabolism, mainly in mitochondria. At the physiological level, these molecules are key players in cell signaling and in immune responses. However, when produced too much, these reactive oxygen species (ROS) can oxidize lipids, proteins, as well as DNA for the development of chronic diseases such as inflammation, cardiovascular and neurodegenerative disease, diabetes, and cancer to name a few (Gupta, Dwivedi & Khandelwal, 2011). In the last few years, the compounds of Spirulina platensis such as C-phycocyanin (C-PC) have been studied due to their very high antioxidant and anti-inflammatory activity. C-PC is a biliprotein pigment, which is involved in photosynthetically mediated processes and in this process scavenges ROS and reactive nitrogen species (RNS) to assist in reestablishment of redox homeostasis. Several researchers have characterized the cytoprotective activity of C-PC. For example, Fernández-Rojas, Medina-Campos et al. (2014) found that C-PC alleviates oxidative stress induced by cisplatin and keeps endogenous antioxidant enzymes active in a dose-dependent manner. These components not only inhibit the accumulation of oxidative damage markers but also aid cellular antioxidants defense. Noteworthy, studies by Lim, Jeong and Chang (2012) showed that C-PC inhibits lipid peroxidation and disrupts intracellular apoptosis signaling pathways, such as phosphorylated ERK, Bax, caspase-9 and caspase-3. Similarly, Farooq et al. (2009) found that C-PC protects renal cells exposed to oxalate by decreasing ROS generation, stabilizing mitochondrial membrane potential, inhibiting lipid peroxidation, and enhancing ATP generation. In diabetics with nephropathy, C-PC has also been presented to block NADPH oxidase-mediated superoxide production in renal mesangial cells (Zheng, Inoguchi, Sasaki et al., 2013) and proved its capability to attenuate oxidative insult down to the cellular level. Lipid imbalances, mitochondrial injury and oxidative stress are some of the central features associated with CVDs. C-PC exerts various protection actions on the cardiovascular system, modulating oxidative and inflammatory pathways. Riss et al. (2007) showed that C-PC ameliorates oxidative-

inflammatory injury in atherosclerotic animal models via radical scavenging and/or COX-2 (cyclooxygenase-2) activity suppression, antioxidant enzyme activity enhancement, and regulation of plasma lipid profile. Sheu et al. (2013) demonstrated that C-PC was found to improve serum cholesterol, triglycerides, and low-density lipoprotein (LDL) and to increase catalase, superoxide dismutase, and glutathione peroxidase [GPx] activity as previously demonstrated. Such results emphasize its hypolipidemic and antioxidant functions, implying that C-PC supplementation can be beneficial to the prevention and advancement of CVDs and atherosclerosis. Li et al. (2013) demonstrated that C-PC inhibited atherosclerotic plaque development mediated by up-regulating CD59 expression, suppressing smooth muscle cell proliferation, endothelial apoptosis and lipid accumulation in the vascular wall, which resulted in a decline in atherosclerotic plaques. In addition to antioxidant properties, C-PC demonstrates pronounced anti-inflammatory effects. Remirez et al. (2002) demonstrated for the first time that it acts as a natural and selective COX-2 inhibitor, which demonstrated a large decrease in inflammation after S. platensis administration. Subsequent studies (see e.g. Romay, González et al. (2003) and Romay, Ledon & Gonzalez (2000)) also confirmed these findings, which characterised (a) the radical-scavenging and anti-inflammatory activity of C-PC in vitro and in vivo. C-PC also showed dose-dependent inhibition of inflammatory processes, decreasing tissue edema in a wide range of experimental models. In a comparative study on C-PC nutritional formulations, similar to the effects of carprofen used for the treatment of osteoarthritis is evidenced. Martinez, Chen et al. (2015) reported that C-PC supplementation reduced levels of pro-inflammatory cytokines (for that we are considering TNF-α, IL-6, MMP-3, NO and sulfated glycosaminoglycans) and pointed out its use as therapy for anti-inflammatory as a natural anti-inflammatory agent. Collectively, these investigations verify that C-PC works by two mechanisms: the scavenging of free radicals itself, and by regulating molecular cascades for the inflammation, apoptosis, and lipid metabolism. The ability of C-PC to increase endogenous anti-oxidant defenses but decrease inflammation mediators makes it an antioxidant molecule that might be a viable bioactive candidate for the prevention and control of oxidative stress-induced diseases. Within the wider framework of this thesis, these results provide reinforcement for the rationalization investigating Spirulina platensis extracts as multifunctional biocompounds capable of buffering against cellular damage and improving biological resiliency overall.

Chapter 5

5. MicroRNAs

MicroRNAs (miRNAs) are a highly conserved class of endogenous, small non-coding RNAs approximately 22 nucleotides long that directly regulate gene expression at the post-transcriptional level. They are derived through a series of steps that consist of transcription of primary miRNAs (pri-miRNAs), nuclear cleavage into precursor miRNAs (pre-miRNAs), and subsequent cytoplasmic transformation to mature miRNAs with the RNAse III enzyme Dicer (Ha & Kim, 2014). These mature miRNAs are then capable of binding to the 3' untranslated regions (3' UTRs) of specialized messenger RNAs (mRNAs) to repress or degrade the target mRNA. However, increasing evidence shows that their regulation is not limited to canonical 3' UTR interaction, such as binding to coding structures, 5' UTRs, and potentially transcribed-based on gene promoters; they thereby modulate the expression of the gene through various pathways (Broughton et al., 2016). Remarkably, some miRNAs also serve as enhancers of gene expression under the specific physiological or pathologic conditions, depending on the cellular situation (Vasudevan, 2012). Moreover, their localization to separate cellular cell compartments such as nucleus and mitochondria (Makarova et al., 2016) indicates their broader participation in both transcriptional and post-transcriptional regulation. MiRNAs are essential during embryonic development and in subsequent postnatal development. According to Fu, Hayder & Peng (2013), they participate in the regulation of many steps of development, including pluripotency in embryonic stem cells, setting the cell fate, formation of the lineage (or the gene), tissue morphogenesis, and apoptosis/programmed cell death. This functional flexibility is also often tissue- and temporally-dependent. For instance, miR-1 is essential for cardiac and skeletal muscle maturation, regulating the expression of muscle-specific genes and aiding in terminal differentiation (see Chen et al., 2006). MiR-124 acts as a master regulator in neurodevelopment by repressing non-neuronal (RNA) transcripts, stabilising neuronal identity, and supporting neurogenesis and normal brain function (Makeyev et al., 2007). Likewise, let-7 family of miRNAs which were first described regulates timing of development and favors stem cell differentiation through downregulation of oncogenes including RAS and HMGA2, preventing aberrant formation (Roush & Slack, 2008).

Transformation in miRNA expression have been firmly established as a signature of a variety of diseases. According to Paul et al. (2018), dysregulation miRNA is a key mechanism that is now one of the critical mechanisms underlies the pathophysiology of different human disorders such as neoplasia, cardiovascular dysfunction, neurodegeneration, autoimmune diseases and metabolic syndromes. In cancer, miR-21 is often overexpressed promoting the malignant advancement by targeting important tumor suppressors, PTEN and PDCD4 (Chan et al., 2005). Conversely, miR-15a/16-1 are frequently deleted in chronic lymphocytic leukemia that leads to an accumulation of anti-apoptotic proteins such as BCL2 to enhance cell viability (Calin et al., 2002). Decreased miR-29a/b levels in neurodegenerative disease have been associated with Alzheimer's, owing to the upregulation of BACE1, an important enzyme implicated in amyloid plaque formation (Hebert et al., 2008). Likewise, miR-375 plays a key role in pancreatic β-cell function, and its perturbation has been associated with reduced insulin secretion in type 2 diabetes (Poy et al., 2004), while miR-146a is involved in innate immunity and is modified in inflammatory-related diseases like rheumatoid arthritis (Taganov et al., 2006). In addition to their intracellular functions, miRNAs are present in extracellular biofluids including blood plasma, saliva, and urine, where they show outstanding stability as they either contain extracellular vesicles (e.g., exosomes) and/or bind RNA sites (as with Argonaute; Hayes et al., 2014; Huang, 2017). This unprecedented stability has created novel opportunities to use miRNAs as an ultra-disease neutral predictor of diagnostic status, prognosis and therapeutic monitoring in the clinic. Moreover, extracellular miRNAs also contribute to cell–cell communication, affecting gene expression in recipient cells and thus spreading their functional reach across tissues and organs. One promising but more recently under-studied area is for studies on plant−derived miRNAs and their mammalian effects. Originally thought species-specific, new evidence hints on dietary miRNA activities in cross kingdom applications and biological functions in animals. Importantly, soy-derived miR-159a has been shown to exert anti-inflammatory effects in murine models of liver disease, specifically through its action on the GSK-3β pathway and inhibition on hepatic fibrosis (Yu et al., 2021). Concurrently, miR-159a has also been shown to inhibit the colorectal tumor growth by downregulating oncogenic targets including TCF7 and MYC (Liu et al., 2021). In a similar manner, miR-166a from Lycium barbarum, has exhibited antiproliferative properties against renal carcinoma cells, which is suggestive of a therapeutic approach against kidney cancers (i.e., Zhang et al., 2023). Such miRNAs

have been encapsulated into plant-derived nanocarriers and utilized in vivo models, such as treatment of atherosclerosis, indicating their therapeutic potential when administered systemically (Yang et al., 2024). Collectively, these discoveries emphasize miRNAs as master regulators of gene expression in normal development and disease, and emerging personalized medicine-mediated tools. Their high specificity can modulate entire regulatory networks effectively, which has made them one of the dominant agents for modern molecular therapeutics today (Table 6).

Table 6: Overview of selected microRNAs (miRNAs) with known biological functions, associated pathological conditions, and their emerging or established roles in therapeutic applications.

miRNA	Biological Function	Associated Condition	Therapeutic/Pharmaceutical Potential	Source
miR-1	Muscle differentiation	Cardiac arrhythmia	Regulates ion channels and cell cycle genes	Chen et al., 2006
miR-124	Neuronal differentiation	Glioblastoma, neurodevelopmental disorders	Suppresses non-neuronal gene expression	Makeyev et al., 2007
let-7	Stemness inhibition, developmental timing	Various cancers	Represses oncogenes (*RAS*, *HMGA2*)	Roush & Slack, 2008
miR-21	Cell proliferation, anti-apoptotic	Breast, colon, lung cancers	Targets *PTEN*, *PDCD4*	Chan et al., 2005
miR-15a/16-1	Apoptosis induction	Chronic lymphocytic leukemia	Suppresses *BCL2*	Calin et al., 2002
miR-29a/b	Amyloid regulation	Alzheimer's disease	Targets *BACE1*	Hebert et al., 2008
miR-375	Insulin secretion	Type 2 diabetes	Modulates insulin granule exocytosis	Poy et al., 2004
miR-146a	Immune regulation	Rheumatoid arthritis, lupus	Suppresses *IRAK1* and *TRAF6*	Taganov et al., 2006
miR-159a	Flower development (plant); tumor suppression	Colon cancer, liver fibrosis	Inhibits *TCF7*, *MYC*, *GSK-3β* – potential cross-kingdom therapeutic agent	Liu et al., 2021; Yu et al., 2021
miR-166a	Leaf polarity (plant); anti-proliferative	Kidney cancer, vascular inflammation	Inhibits cell growth pathways – used in plant-derived nanovesicles	Zhang et al., 2023; Yang et al., 2024

Chapter 6

Purpose of the Study

The research work presented in this thesis is a part of a more general scientific landscape aimed at the sustainable valorization of microalgal biomass, specifically Spirulina platensis. Within an existing approach based on the frameworks of circular bioeconomy and industrial decarbonization, this activity is foundational to both the recent transition to ecological standards and modern biotechnological innovation. The objective was in this context to develop eco-friendly extraction strategies for bioactive compounds recovery with high potential (phycocyanin and microRNAs), and to evaluate the biological activities of these compounds in vitro (cell-based assay). The experimental procedures of Spirulina platensis were performed alongside Plastica Alfa S.r.l. in horizontal tubular photobioreactors with a total working volume of about 4,000 liters. This semi-industrial set-up allowed photocirculation through industrially derived carbon dioxide as the carbon source of the photosynthetic growth pathway, encouraging the reuse of waste gases and contributing to emission mitigation strategies. This is also an example of the practice that the industry has taken advantage of in circular economy, in this case, they converted an industrial by-product into a valuable biological resource. The harvested biomass was frozen in a portion and freeze dried to preserve the biochemical profile and structural integrity, in order to follow the extraction procedures properly. The specific objective of the study was to improve green and low-impact extraction through use of eco-sustainable solvents, such as water, limonene, p-cymene, and 2-methyltetrahydrofuran (2-MeTHF). They were chosen for their ability to biodegrade, low toxicity, and compatibility with principles of green chemistry. Simultaneously, process intensification techniques were implemented, in particular by means of microwave-assisted extraction (MAE) which facilitate faster extraction times, lower energy consumption, and higher extraction yield. These approaches made it possible to recover a number of bioactive fractions rich in phycocyanin, fatty acids, phenolic compounds and carotenoids. Spectrophotometric and chromatographic techniques, namely ultraviolet–visible spectroscopy, high-performance liquid chromatography (HPLC), and fatty acid methyl ester (FAME) profiling, were then performed for respective extraction steps in order to characterize the extracted fractions

from the microbial studies and determine the compositional properties and potential bioactive composition. Specific focus was put on the aqueous fraction of the extracts collected with a high level of phycocyanin. This pigment-protein complex (members of the phycobiliprotein family) is among the biologically most important units of Spirulina platensis known for its antioxidant, anti-inflammatory, cytoprotective roles. It has been reported many times in science that phycocyanin scavenges the reactive oxygen species (ROS), prevents lipid peroxidation, modulates inflammation, and protects cellular materials from oxidative damage. It has numerous properties that render it a compound important to pharmaceutical, nutraceutical and cosmetic industries with a focus on anti-aging and protective agents. Alongside pigment extraction, a small-RNA fraction was isolated and characterized along with this. microRNAs were detected as an important constituent of the analysis. Mainly they are miR166a and miR159a. MicroRNAs, as small non-coding RNA molecules, are of particular importance for regulating gene expression at the post-transcriptional level which in turn impacts fundamental cellular processes such as proliferation, differentiation, apoptosis and stress responses. Based on available information it has been reported that miR166a regulates oxidative stress and also exerts an adaptive mechanism on cell metabolism and miR159a may be a potential modulator of hepatic disease pathways. Some of these microRNAs have not been assayed in biological studies in the current project, but the identification of these microRNAs, in the Spirulina platensis biomass, would be a useful starting point for further studies investigating their functional roles and therapeutic strategies. These experiments have been conducted in the National Research Council (CNR) facility in Catania, where the cell line was maintained in a controlled environment. Oxidative stress was applied via hydrogen peroxide (H_2O_2) at a concentration of 30 μM at a well established toxic level that produces reactive oxygen species. Three experimental groups were set: untreated controls, oxidatively stressed cells, and two pre-treated groups treated with either liquid or lyophilized phycocyanin. In colorimetric assays cell viability was determined to assess the capacity by which phycocyanin maintained cellular viability in response to a stress situation. First steps showed that phycocyanin was protective towards oxidative stress, the result being that at least partial viability of cells was kept where it is in untreated oxidatively damaged controls. While the confirmed data is being validated, these findings confirm our hypothesis that phycocyanin has strong antioxidant and cytoprotective activities, which implies that it might be a bioactive component of pharmacotherapy and drug preparation. Additional

investigations are ongoing and will be implemented across additional cellular models to clarify the protein-level, molecular interactions leading to its biological actions, including redox regulation and cell conservation pathways. In conclusion, the experimental work presented herein demonstrates the potential relevance of Spirulina platensis and its applications in a dynamic and sustainable form of biological resource, with the potential to foster environmental and industrial development. The combination of green extraction methods and process intensification demonstrates that high extraction capacity has been achieved with a great reduction in production environmental impacts. This direction is in line with the bioeconomy's guiding principles that value be gained from renewable bio-materials and with green chemistry with sustainable techniques which propose to replace the use of toxic processes and solvents with environmentally-friendly alternatives. Finally, this doctoral study aims to support the development of environmentally responsible technologies in order to achieve value for microalgal biomass by linking the responsibility of eco-protection with the process innovation. Spirulina platensis is known by the industry as a promising source of multifunctional bioactive compounds for cosmetic, nutraceutical and pharmaceutical applications. Using renewable inputs, circular carbon management and environmentally friendly extraction, this work represents a concrete illustration of how microalgal biotechnology could serve towards industrial decarbonization and sustainable industries. The proposed method allows the establishment to further the development of algal biorefinery and the inclusion of Spirulina products into the green market towards the development of more resilient and greener models of scientific and industrial progress.

Chapter 7

Materials and Methods

7.0 Cultivation

For the controlled cultivation of Spirulina platensis, a tubular photobioreactor system (model ALGAE SPEED_V6_540) was installed at Plastica Alfa S.p.A. (Caltagirone, Italy) (Fig. 18 A, B). To begin the cultivation, Spirulina platensis was precultivated in sterile laboratory-scale Erlenmeyer flasks on a modified BG11 medium as the nutrient base in a pre-cultured environment. Up to a certain point the culture was kept under continuous light and the temperature was regulated, and only then did the exponential growth phase start. The algal suspension then was placed in 2 L glass bottles where the culture volume was slowly increased. Biomass accumulation was monitored by gravimetric analysis, with dry weight per liter (g/L) determined for growth rates and physiological health. After obtaining sufficient biomass concentration, the culture was expanded to two photobioreactor configurations: the ALGAE SPEED_V6 system (nominal working volume 500 L) and a second outdoor vertical tubular arrangement (capacity 3000 L). The decision to test both photobioreactor scales was motivated by biomass yield, reproducibility and system scalability issues. Both systems were checked weekly during the growth to measure algal growth behavior. Finally, the photobioreactor was fully automated by using a PLC (Programmable Logic Controller) enabling for real-time and fine control of cultivation parameters (pH, temperature, light exposure and nutrient concentration) while allowing close monitoring to maintain optimal growth by the respective growth models and monitoring the dry biomass regularly in order to control this data. Interestingly, the nutrient medium was developed based on an adjusted BG11 formulation enriched to accommodate high density culture with a longer growth cycle. The formulation performed well for extended production of biomass as well as for a long-term culture maintenance. Weekly-measured data showed similar growth kinetics between the two systems, with respect to the specific growth rate during the exponential phase. Nonetheless, the final volumetric biomass concentration of 3000 L system also showed a high final volume of biomass, demonstrating its potential applicability for utilization at industrial scale, and to the downstream bioprocessing. This finding was in line with the literature reference that has

claimed to state big photobioreactor facilities to have a greater light distribution, better CO_2 utilization efficiency and more stable temperature regulation, a factor that result in better algal productivity. These results served as the infrastructure for later production and biochemical characterization of bioactive substances obtained from the harvested biomass.

A) 

B)

Fig 18 A) Initial cultivation of Spirulina platensis in Erlenmeyer flasks under controlled laboratory conditions using modified BG11 medium. (B) Scale-up of the algal culture in a tubular photobioreactor system (model ALGAE SPEED_V6_540) for large-scale biomass production.

7.1 Dry weight

DW is essential for determining biomass concentration (Cx, g L^{-1}) for both batch and continuous cultivation, where the DW measurement can be used to monitor the growth curve or verify the attainment of a steady state. In the latter case, a minimum of three consecutive measurements with similar values, taken on different days, are needed to confirm that the culture has reached steady conditions. Nitrocellulose filters with a 0.22 µm pore size are used for the analysis. These filters are first dried in an oven at 110 °C for 1 hour, and then weighed to determine the tare mass (g). Afterwards, a known volume of culture sample (usually 10 mL) is filtered using a vacuum flask and a manometer. After filtration, the filters are dried again in the oven for approximately two hours to remove residual moisture and then weighed again to obtain the gross mass (g). The biomass concentration is then calculated by the following relationship:

$$DW\ (g\ L^{-1}) = \frac{(W_{gross} - W_{tare})}{V_{sample}}$$

where *Wgross* and *Wtare* represent the filter's weight after and before filtration, respectively, and *Vsample* is the volume of the culture used for the measurement. This method provides an accurate and reproducible estimation of the microalgal biomass concentration over time (Fig19).

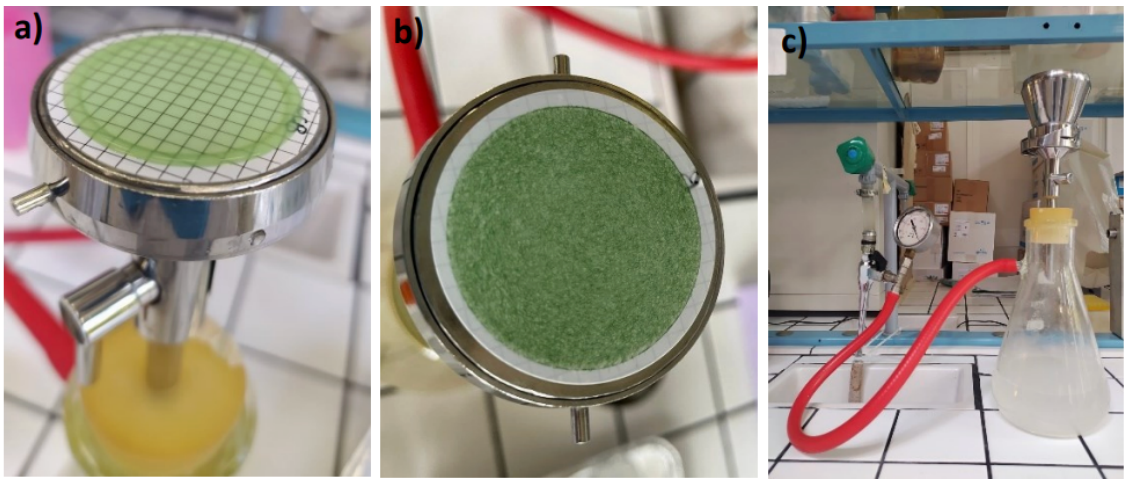


Figure 19 a) Dry weight of an Arthrospira platensis sample grown under heterotrophic conditions; b) Filter containing the algal biomass; c) Vacuum pump with filtration apparatus.

7.2 Composition

The elemental composition of carbon (C), hydrogen (H), and nitrogen (N) was analyzed using a Thermo Scientific Flash Smart Elemental Analyzer (model EA 1112, Germany). The instrument was configured with helium as carrier gas at a flow rate of 130 mL/min, alongside an oxygen flow of 250 mL/min and a reference gas stream at 100 mL/min. The combustion furnace was maintained at 900 °C, while the reduction furnace operated at 680 °C, in accordance with manufacturer guidelines and prior literature (Baldé et al., 2019; Kalinichenko et al., 2017). Separation was performed using a 6 × 5 mm packed column (Cromlab, Spain) held at 50 °C. Chromatographic acquisition lasted 420 seconds. Calibration was performed using aspartic acid (Sigma, USA) as the elemental standard. All samples were analyzed in triplicate.

The protein content was estimated using nitrogen values and a nitrogen-to-protein conversion factor of 5.13, recommended for green algae biomass (Lourenço et al., 2002).

To assess the energetic value of the samples, the Higher Heating Value (HHV) was calculated based on the empirical formula reported by Trigueros et al. (2021):

$$HHV\ (kJ/kg) = 3.55C^2 - 232C - 2230H + 51.2CH + 131N + 20{,}600$$

where C, H, and N represent the experimentally determined percentages of carbon, hydrogen, and nitrogen.

Furthermore, the quantitative determination of mineral and metal elements (including trace elements) was carried out using Inductively Coupled Plasma Mass **Spectrometry** (ICP-MS). This analysis was performed at a specialized analytical facility in Spain, following standard protocols for high-sensitivity elemental quantification in biological matrices.

7.3 Lyophilization

Spirulina platensis biomass and spirulina extract were stored with freeze-drying, a dehydration method that utilizes sublimation under vacuum of frozen water that has

been extensively used to preserve the chemical and structural integrity of thermosensitive bioactive compounds. This started with freezing of newly grown algal biomass at −40°C with an ultra-freezer in laboratory setting. This frozen step was carried out for at least 24 hours in order to retain the full solidification of both intracellular and extracellular water. After being frozen, the biomass was transferred to a bench-top freeze-dryer (e.g., Christ Alpha 1-2 LDplus or equivalent), with a vacuum pump to ensure that the dryer-to-wet point was at a low pressure for the entire drying process. The lyophilization was carried out at an average chamber pressure of 0.1 mbar. The shelf temperature was increased from −40°C up to 0°C during primary drying duration of 48h. This controlled process of increasing the temperature so slowly permitted water to sublimate from solid ice without entering the liquid phase, to eliminate hydrolysis, and retain thermal and oxidative degradation sensitive compounds (polyunsaturated lipids, pigments, or phenolic). In another drying step, residual moisture of the biomass was removed by maintaining the vacuum and then slightly increasing the shelf temperature to about 20°C, and the final moisture level was less than 5%, enough to survive for the long term and to extract quality (Fig 20). The choice of this method was due to its stability to thermolabile compounds, inhibition of microbial growth, and solvent penetration into subsequent extraction protocols (Tang & Pikal, 2004; Abdelaziz et al., 2020).

Fig 20 a) and b) Lyophilization Spirulina and extract

7.4 Extraction methods

Preliminary review of scientific literature was performed to identify the best extraction method for various classes of bioactive compounds. Critical parameters including extraction temperature, time and solvent-to-biomass ratio were identified. According to Martí-Quijal et al. (2021) the ideal extraction temperature of thermolabile compounds for microalgae and cyanobacteria is typically between 40°C and 60°C, while Zakaria et al. (2022) investigated solvent-to-biomass ratios ranging from 20 to 90 mL/g using similar matrices. However, for a complete evaluation of the compound stability in various extraction conditions and to assess the effects of particular green solvents, we also stretched the temperature to go beyond commonly accepted limits and limited to two fixed temperatures: 80°C for 5 minutes and 180°C for 5 minutes. The 80°C temperature was selected based on several reports that it is an effective and safe temperature for extraction of phenolic compounds and fatty acids from algal biomass without the degradation of these target molecules (Saini et al., 2023; Arora et al., 2020). However, 180°C was chosen as a high-temperature strain condition for the thermal degradation levels testing. The above high temperature (>140–160°C) is conducive to degradation of thermolabile compounds, including some antioxidants, polyphenols, and unsaturated fatty acids (Domingues et al., 2019); this justifies the above limit and further reinforces the conservative efficiency of 80°C. Microwave-Assisted Extraction (MAE) at both temperatures was used with a controlled power setting and fixed exposure time. The use of such technique has been well validated for both polar and non-polar compounds recovery from microalgae (Günerken et al., 2015). Also a freeze-thaw cycle extraction was performed with water as the solvent, with two temperature changes between −20°C and room temperature. This approach allowed the separation of water soluble materials such as phenolic compounds, sugars, but not fatty acids which are not soluble as they are unreuspended, and because it is a poor fluid and due to water's limited ability to extract lipids. In parallel, 4 green solvents were tested by polarity and selectivity for bioactive compounds:

(A) D-limonene (97% purity, Sigma-Aldrich)

(B) 2-Methyltetrahydrofuran (≥99.0%, stabilized with 250 ppm BHT)

(C) Para-cymene (≥97%, Sigma-Aldrich)

(D) Milli-Q ultrapure water

These solvents were chosen based on previous applications in microalgal extraction, along with their green chemistry profiles and compatibility with microwave-assisted processes.

7.5 Microwave-Assisted Extraction (MAE)

The microwave-assisted extraction process was conducted in an Anton Paar Microwave reactor Monowave 450 (Austria). In this experiment, approximately 1 gram of *Spirulina* was mixed with 20 mL of water or green solvent maintaining a 1:20 (w/V) ratio (Zakaria et al., 2022). The extraction process with water, involved subjecting the suspension to microwave assisted heating up to temperatures of 80°C and 180°C and then maintaining this temperature for a duration of 5 minutes each.
With the other green solvents we chose only two temperature 80°C and 180°, for 5 min. After all technique we filtered and took the liquid extract, leaving the solid (fig21).

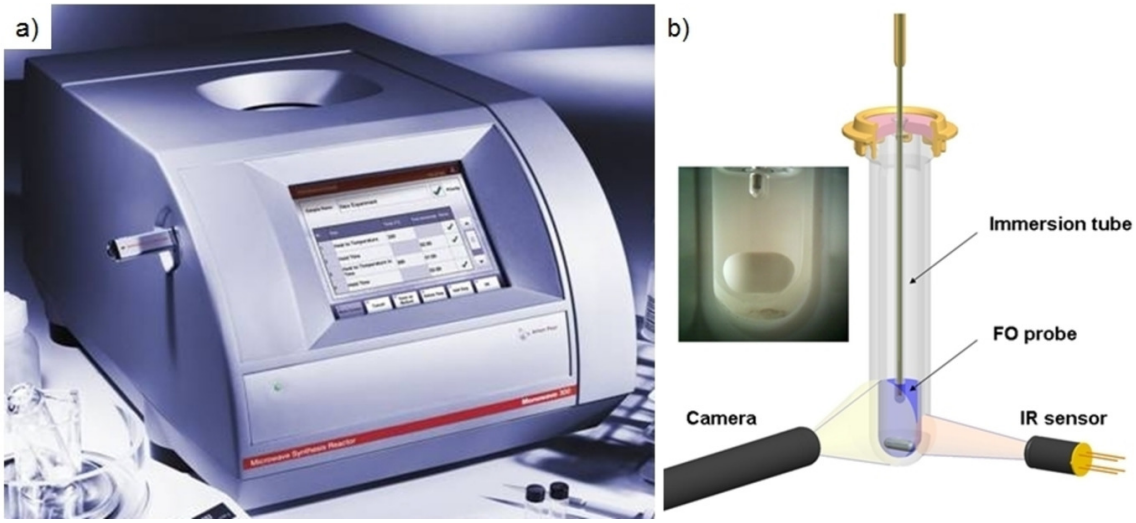


Figure 21 a) Microwave b) imagine inside of microwave

7.6 Freezing and Thawing extraction

The Spirulina-water mixture was centrifuged at 150 rpm for 30 minutes. Post centrifugation, the sample was frozen at -20°C for 2 hours. This step was designed to preserve and stabilize the bioactive compounds by slowing down metabolic activities and preventing enzymatic degradation.
After freezing, the sample was thawed at room temperature for 2 hours, allowing the frozen constituents to return to a workable state.

The thawed sample was then centrifuged at 847 rpm for 15 minutes to further purify the extract by removing any remaining solid residues (Vernès et al., 2015).
The entire cycle (freezing, thawing, and centrifugation) was repeated four times to ensure maximum extraction efficiency and consistency. Each repetition was critical to breaking down the cell walls thoroughly, for to the release of a different biocompounds (Fig 22).

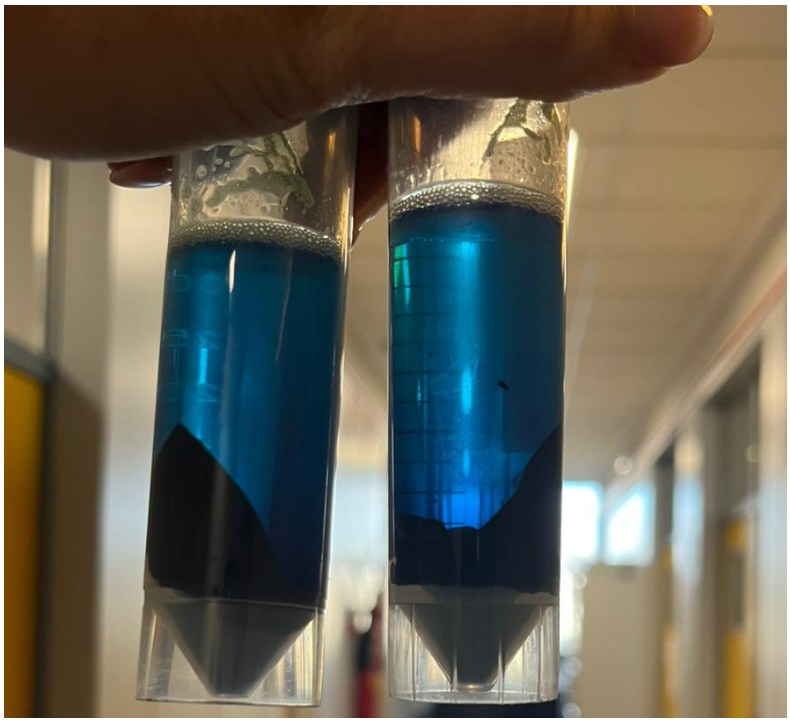


Figure 22 Phycocyanin after the extraction

7.7 Extraction of Phycocyanin from *Spirulina platensis*

Phycocyanin extraction was performed using ultrapure water as the sole solvent, following a sustainable and buffer-free approach to preserve the native structure of the protein and ensure its compatibility with food and cosmetic applications. Specifically, 1.00 g of dried Spirulina platensis biomass was suspended in 20 mL of ultrapure water in a 50 mL Falcon tube. The mixture was subjected to three consecutive freeze–thaw cycles (−20 °C and room temperature) to promote cell lysis through osmotic and mechanical stress, avoiding harsh treatments. After the final cycle, the suspension was centrifuged at 10,000 rpm for 10 min at 4 °C. The blue supernatant, containing the solubilized phycocyanin, was carefully collected and stored in the dark at 4 °C until further analysis. This methodology, relying exclusively on water as the extracting

medium, allows for a gentle extraction process that maintains the biochemical activity of phycocyanin, facilitating its direct application in sensitive matrices such as functional foods and dermocosmetic formulations.

7.8 Total phenolic content determination

The total phenolic content **(TPC)** of *Spirulina platensis* extracts was quantified using the Folin–Ciocalteu colorimetric assay, a well-established analytical method based on the oxidation–reduction reaction between the Folin–Ciocalteu reagent and phenolic hydroxyl groups acting as reducing agents (Santos et al., 2019). Briefly, 5 µL of each extract were combined with 60 µL of sodium carbonate solution (Na_2CO_3, 75 g/L) and 15 µL of Folin–Ciocalteu reagent, followed by the addition of 200 µL of ultrapure water to achieve the final reaction volume. The mixtures were thoroughly homogenized and incubated at room temperature to allow full color development, after which the absorbance was measured using a UV–Vis spectrophotometer (Synergy HT, BioTek Instruments Inc., USA) at the appropriate wavelength. The quantification of phenolic compounds was performed by comparison with a calibration curve constructed using gallic acid as the reference standard, in the concentration range of 50–1500 mg/L. Results were expressed as gallic acid equivalents (GAE)**,** and the total phenolic content of each extract was calculated as milligrams of GAE per gram of dry weight (mg GAE/g dw) of *Spirulina***.** This value reflects the efficiency of the extraction process and provides an indication of the antioxidant potential associated with the phenolic fraction of the biomass.

7.8.1 Extraction of Phenolic Compounds for HPLC Analysis

After following a method modified from Robards (2003) and Luthria (2006), phenolic compounds extraction was performed from Spirulina platensis biomass in the specific physicochemical characteristics of investigated matrix. It was adopted in order to obtain as much free and conjugated phenolic substances (phenolic constituents in microalgal biomass are available in variable proportions depending on cultivation and environmental conditions) as well as to optimize the recovery of these. The lyophilized biomass was finely ground to a homogenous powder with a mortar and pestle prior to extraction, thus increasing the specific surface area and facilitating solvent permeation

into the cell matrix. To prepare powdered sample, this was further treated with solid–liquid extraction (SLE) via a hydroalcoholic solvent system of 70% methanol (v/v) acidified with 0.1% hydrochloric acid (HCl). The extraction with a solid-to-liquid ratio of 1:20 (w/v) is studied because it has previously been reported to be efficient in providing diffusion and solubilization of phenolic molecules from complex biological matrices. The methanol-water mixture with mild acidification has been widely used in the literature to extract polyphenols (Luthria, 2006; Khoddami et al., 2013) and is characterised by its capability to disrupt the presence of hydrogen bonds, and to increase the solubility of both polar and moderately non-polar phenolic molecules including flavonoids, phenolic acids, and their glycosylated analogues. Acid also prevents oxidative degradation and promotes the hydrolysis of esterified or bound phenolic structures, making total extractable phenolics higher. The extraction procedure was performed at a temperature of ~40 °C and an ultrasonic bath level of 30 min, in controlled atmospheres, through ultrasonic amplification. Ultrasonic-assisted extractions (UAE) allow the microalgal cell wall matrix, mainly containing polysaccharides and peptidoglycan, to be degraded by acoustic cavitation and mechanical shear forces. This physical effect improves the release of intracellular compounds into the solvent phase, which also reduces extraction time and improves yield (Yang et al., 2017). After extraction, suspensions were centrifuged at 4500 × g for 10 min, allowing for the deposit of insoluble residues and debris. As the supernatant had a phenolic-rich extract in it, it was taken from the pellet without resuspension, and filtered through a 0.45 μm PTFE membrane. The filtered sample provided an opportunity to filter out residual particulate matter, avoiding possible interferences during downstream spectrophotometric and chromatographic analysis. Clarified extracts were kept in amber glass vials at 4 °C to minimize phenolic compounds' susceptibility to oxidation and degradation due to light influence before quantification. Selecting extraction conditions and solvent system allowed to achieve a tradeoff among selective extraction efficiency and stability of the compounds for reproducibility and reliability between the replicates.

7.8.2 HPLC Analysis

Phenolic compound detection was achieved through high performance liquid chromatography (HPLC) and diode array detector (DAD). A reversed-phase C18 column (250 × 4.6 mm; 5 μm) was utilized and fixed at constant temperature. The

mobile phase was made up of water that was acidified with 0.1% phosphoric acid (phase A) and acetonitrile (phase B). Separation was accomplished by applying a linear gradient from 5% to 35% of phase B in 30 min and performing the washing and re-equilibration step. The flow rate was kept at 1.0 mL/min and the injection volume was 20 µL; according to López-Lázaro (2011), detection was performed by selecting the wavelengths of the compounds: 280 nm for the simple phenolic acids, 320 nm for hydroxycinnamic acids, and 360 nm for flavonoids. Peak identification was achieved through comparison of retention time and UV emission to pure analytical standards. Quantification was done via external calibration by making a linear calibration curve for each compound ($y = m \cdot x + b$) and calculating concentrations in mg/L depending on peak area (Miller & Miller, 2018).

7.9 Antioxidant potential

The TEAC (Trolox Equivalent Antioxidant Capacity) assay evaluates the ability of antioxidants to neutralize the ABTS radical cation (ABTS*+), which is produced through a reaction between ABTS and potassium persulfate. To perform the assay, plasma and erythrocyte samples were mixed with ABTS*+ and incubated for one minute at 30°C. Absorbance was measured at 734 nm using a spectrophotometer, with 5 mM phosphate-buffered saline (pH 7.4) serving as a blank (Antolovich et al., 2002).

7.9.1 ABTS radical cation

ABTS radical cation (ABTS·+) scavenging ([2,2-azinobis(3-ethyl-benzothiazoline-6-sulfonate)]) was estimated following the protocol proposed by Re et al. [38]. Antioxidant capacity was measured using Trolox (6-hydroxy 2,5,7,8-tetramethylchroman-2- carboxylic acid) as standard and values were expressed as mg/mLTro- lox equivalent. PBS buffer (NaCl, KH2PO4, Na2HPO4, KCl, sodium acidic, distilled water) and TEAC reagent (ABTS+ 7 mM, potassium persulfate 2.45 mM) were first prepared. After that, the TEAC reagent was diluted with PBS buffer to obtain a diluted ABTS+ solution, with an absorbance of 0.7 ± 0.1 at 734 nm. Then, a volume of 1 mL of the prepared solution was added to 10 µL of liquid sample. After 6 min of incubation at 30 ◦C, the absorbance was evaluated spectrophotometrically at 734 nm. Mea- surements were run at least in triplicate.

7.9.2 Ferric reducing antioxidant power

Ferric-reducing antioxidant power (FRAP) was carried out by preparing a mixture of three solutions (10:1:1 v/v/v) as follows, acetate buffer (pH 3.6; 300 mmol/L), 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mmol/L HCl and FeCl3 6H2O in distilled water. Liquid samples (100 µL) were added to the prepared FRAP reagent (3 mL). The absorbance was read spectrophotometrically at 593 nm After 6 min of incubation at room temperature. Results were expressed by referring to a standard curve using aqueous solutions of ascorbic acid. Tests were performed in triplicate.

7.9.3 Radical scavenging assay

Radical scavenging capacity was assessed as follows, methanolic solution of DPPH (2,2-diphenyl-1-pic- rylhydrazyl) (2 mL, 6 × 10− 5 M) was mixed with the liquid extracts (50 µL). The absorbance was measured at 515 nm after 16 min of incubation. Results were compared according to the control at a time of 0 min. All assays were conducted at least in triplicate. The antiradical activity was expressed as IC50 (µg/mL), which is the extract dosage needed to achieve 50 % inhibition. Lower IC50 values indicate stronger antioxidant activity of the algal extract.

7.10 Fatty acids determination

The extraction of lipids was carried out using microwave-assisted as was described above and with tested green solvents with variations in thermal conditions; D-limonene, 2-methyltetrahydrofuran, p-cymene, and water. The samples were then processed for fatty acid profiling as per EN ISO 12966-3:2015 standards, Fig 22 A,B. This procedure involves the derivatization of fatty acids to fatty acid methyl esters (FAMEs) before chromatographic analysis. In short, the extracted lipids were first saponified, and then methylated by base-catalyzed transesterification (methanolic sodium hydroxide followed by esterification with boron trifluoride (BF_3) in methanol). FAMEs were subsequently extracted with n-heptane and washed with saturated sodium chloride solution. Chromatographic study was conducted by gas chromatography with flame ionization detection (GC-FID). Separation using a polar capillary column adapted for FAMEs was achieved under temperature-controlled conditions using Supelco SP-2560 or equivalent. Quantification was made by means of comparison with certified FAME

standards, to identify and quantitatively compare saturated, monounsaturated, and polyunsaturated fatty acids. This standardized method offers a very high level of replicability and accuracy in identifying the fatty acid composition of lipid samples, especially for the processes used in food, nutraceuticals, and bio-based materials.

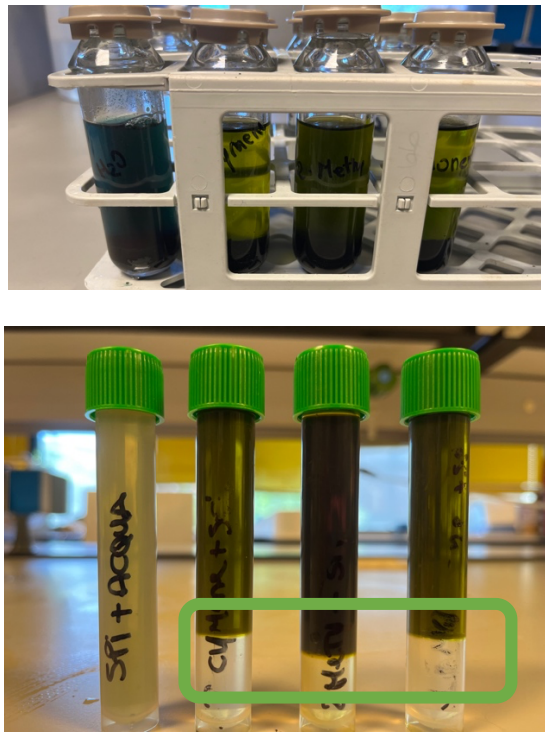


Fig 22 (A) *Spirulina platensis* biomass prepared for extraction using various green solvents.
(B) Result of the extraction process: the separation of lipid components is visible, with extracted fatty acids highlighted within the green box.

7.11 OEC Cultures

OECs were isolated from olfactory bulbs of 2-day-old mice pups (P2) as described by Pellitteri et al. (2007). After pup decapitation, the bulbs were removed, dissected out in cold (+4°C) Leibowitz L-15 medium (Sigma), and digested with collagenase and trypsin added to medium essential medium-H (MEM-H, Sigma). To stop trypsinization, Dulbecco's Modified Eagle's Medium (DMEM, Sigma) supplemented with 10% Fetal Bovine Serum (FBS, Sigma) was used. Cells were resuspended and then plated in flasks with fresh complete medium DMEM/FBS, and antibiotics (Sigma).

After 24 h from the first plating, cytosine arabinoside (10–5 M, antimitotic agent; Sigma) was added for 48 h to reduce the number of dividing fibroblasts. The purification procedure of Chuah and Au (1993) was used to reduce the number of contaminating cells. The percentage of p75/S-100-positive cells in our cultures was about 90–95% (data not shown). In the last passage, OECs were plated on 25-cm2 flasks and cultured in DMEM/FBS supplemented with bovine pituitary extract (Sigma).

6.9.1 Treatment of OECs

Purified OECs were replated on 14-mm-diameter poly-L-lysine (PLL, 10 μg/ml, Sigma)–coated glass coverslips at a final density of 0.3 × 104 cells/coverslip and grown in DMEM/FBS (Sigma). In some cultures, 24 h post-seeding, Ghre (Abcam), reconstitute by adding deionized water, was added to the culture medium. We tested different concentrations of Ghre (data not shown), but we found that the optimal concentration was 2 μM, in accordance with previous report (Stoyanova et al. 2013). Control cultures (CTR) were fed with DMEM/FBS. Both of the experimental cell cultures, with and without treatment, were grown at different times (1, 3, 7 days) and incubated at 37 °C in a humidified 5% CO2–95% air mixture. All cultures were fed with DMEM/FBS; in particular, in those treated with Ghre at 7 days, the peptide was replenished for the second time after 3 days; successively, immunocytochemical procedures were assessed.

To evaluate cellular viability by MTT, OECs were plated in 96 multiwells, incubated at 37 °C and grown both with and without Ghre treatment, following the same experimental protocol of the cells grown on 14-mm-diameter poly-L-lysine–coated glass coverslips.

7.12 MTT Bioassay

Cellular viability survival at the Ghre treatment time was evaluated by the 3-[4,5-dimethylthiazol- 2-yl)-2,5-diphenyl] tetrazolium bromide (MTT, Sigma) reduction assay using a quantitative colorimetric method. MTT was added, to each multiwell at final concentration of 1.0 mg/ml, and incubated for 2 h in a CO2 incubator. Media were removed gently and MTT solvent (acid-isopropanol/SDS) was added; cells were then placed on an orbital shaker for 15 min. Absorbance was observed using a multiskan reader at 570 nm. This colorimetric reaction is resulting from the conversion of the tetrazolium ring of MTT (yellow compound) to formazan (blue salt). Quantitative measurements were done at 570 nm spectrophotometrically. For this analysis U937

mononuclear cells were harvested from the American Type Culture Collection (Manassas, VA, USA). Cells were cultured in a 5% CO_2 environment in RPMI 1640 medium (GIBCO, Invitrogen) with 10% fetal bovine serum, 100 ng/mL streptomycin, 100 U/mL penicillin, and 2 mM L-glutamine. Cells were thawed, grown, and seeded (2×10^5 cells/well) onto 6-well tissue culture plates. Cultivation was conducted using two types of medium — one with and one without incorporation of 10 µM astaxanthin (ASX, Sigma-Aldrich, St. Louis, MO, USA), bacterial lipopolysaccharides (LPS, 10 µg/mL), and 10 µM zinc protoporphyrin IX (ZnPP IX, Sigma-Aldrich, St. Louis, MO, USA). ASX was dissolved in dimethyl sulfoxide (DMSO) and diluted in the media, resulting in a total DMSO concentration of 0.5% maximum. Control cells did not contain ASX and/or LPS. After growing, cells were plated in 96-well plates at a density of 8×10^3 cells/well. A concentration of 0.5 mg/mL and 200 µL of medium was added to all wells (20 µL of MTT). Plates were incubated for 4 h at 37 °C for the dissolution of the formed formazan. Next, 220 µL of the (MTT + medium) solution was removed and 150 µL of DMSO added to each well. The reduced MTT was measured using an ELISA reader (Bio-Rad, Hercules, CA, USA) at 570 nm. Cells were then pretreated with phycocyanin (both lyophilized and liquid/Spirulina extract) for 24 h before being exposed to hydrogen peroxide for 60 min. Control points were collected every 10 min to examine the protective effect of phycocyanin.

7.13 miRNA Extraction

Total RNA was extracted using Trizol® (Invitrogen, Waltham, MA, USA) followed by clean-up using the modified RNeasy®Mini Kit (Qiagen, Hilden, Germany) (Eldh et al., 2012). Briefly, 100 mg of frozen tissue was ground using a prechilled mortar and pestle into a fine powder. The ground tissue sample was mixed with 1 mL of TRIzol reagent and homogenized until no visible debris remained. The homogenized samples were then incubated at room temperature for 5 min. Chloroform (0.2 mL) was added to the homogenate, vortexed vigorously for about 15 s, and incubated at room temperature for 2 to 3 min. After centrifugation, the aqueous layer of the sample was transferred to an RNeasy column from a mini RNA isolation kit (Qiagen, Hilden, Germany) for purification.

Residual DNA was eliminated by performing on-column DNase I (Qiagen, Hilden, Germany) digestion at 37 °C for 30 min. The integrity of the extracted RNA was

determined by gel electrophoresis, and its concentration was measured using a biospectrometer (Eppendorf BioSpectrometer, Hamburg, Germany).
A Qubit RNA HS (High-Sensitivity) assay kit (Life Technologies, Darmstadt, Germany) was used to determine the concentration of RNA in samples. Briefly, the RNA samples were diluted to a final concentration between 250 pg/µL and 100 ng/µL using the buffer provided before loading onto the Qubit 2.0 Fluorometer (Life Technologies, Darmstadt, Germany).
The extracted RNA was analyzed using the Agilent High-Sensitivity RNA assay kit on the 2100 bioanalyzer (Agilent Technologies, Waldbronn, Germany) in complement with the RNA 6000 Nano LabChip kit. Data analysis was performed in accordance with the Agilent protocol.

7.13.1 miRNA Analysis

According to literature, miRNA levels increase under stress conditions and reach a certain threshold (Mendell & Olson, 2012; Olejniczak et al., 2018). Based on this knowledge, it was hypothesized that direct actions on the algal genome could modulate miRNA production. Many researchers have attempted to enhance the biosynthesis of these bioactive molecules primarily through two strategies: (i) nutrient limitation, particularly nitrogen deprivation, which significantly inhibits algal growth and can lead to cell death; or (ii) applying thermal shocks or altering photoperiods, which also result in negative effects on culture stability. Despite awareness of this crucial mechanism, no mapping of miRNAs in *Spirulina* has been reported to date. This represents another important aspect addressed in the present research project.

7.14 Protein Extraction Protocol

Building upon the detergent combinations that have been previously established for the purpose of efficient protein extraction, as detailed by Deb-Choudhury et al. in their 2016 study, a comprehensive protocol was developed to effectively harness the synergistic actions of various chemical reagents. This protocol specifically highlights the collaborative effects of TCEP, which is classified as a

reducing agent, in conjunction with IAA, an alkylating agent. The combination of these two reagents has been shown to facilitate the successful breaking of disulfide bonds within proteins, thereby enabling a controlled denaturation process. This controlled denaturation is crucial, as it not only aids in the extraction of proteins but also effectively inhibits the formation of aggregates, which can complicate subsequent analyses.

Moreover, the inclusion of the detergent SDC (sodium deoxycholate) plays a pivotal role in this protocol by significantly enhancing the destabilization of cell membranes. This destabilization is essential for the efficient release and solubilization of even highly hydrophobic proteins, including those that are intimately associated with the plasma membrane. The synergistic effects of these reagents collectively promote the separation of a broader range of proteins from both the cytosolic and membrane compartments, ultimately leading to an increased yield of the extraction process. This increase in yield is not merely quantitative; it also offers distinct advantages for subsequent analytical steps, as outlined in the works of Deb-Choudhury et al. (2016) and Danko et al. (2022).

To prepare the OPB buffer, which comprises a total volume of 50 mL, specific reagents were meticulously dissolved. This included TCEP at a concentration of 10 mM, CAA (chloroacetamide) at 40 mM, and ABC (ammonium bicarbonate) at 100 mM, with careful attention to maintaining a pH of approximately 8.5. In instances where pH adjustment was deemed necessary, approximately 60 µL of 25% NH_4OH (ammonium hydroxide) was incorporated into the mixture. Following this step, 1% of the detergent SDC was introduced into the buffer, with thorough mixing employed to avoid the formation of foam that could interfere with the extraction process.

In the event that any aggregation or incomplete dissolution was observed, the solution was subjected to gentle heating at 56 °C until it was completely dissolved, ensuring the homogeneity of the buffer. Subsequently, a 1 mL volume of this diluted buffer was added to approximately 3–4 mg of microalgal biomass. The resulting suspension was then homogenized through careful pipetting, conducted gently to avoid any mechanical damage to the target molecules, which is critical for preserving their structural integrity.

To facilitate effective protein extraction, the samples underwent probe sonication for a total of five cycles, with each cycle consisting of 20 seconds of sonication

at 50% power followed by a 20-second pause. This methodical approach allows for the efficient extraction of proteins while minimizing potential damage. At the conclusion of the extraction process, each sample was divided into two equal volumes for further analysis. One portion was centrifuged for 10 minutes at a speed of 9000 rpm, resulting in the separation of solid fractions from liquid fractions. Consequently, three distinct analytical fractions were extracted from each sample: the whole suspension, the pellet, and the supernatant. All of these fractions were then incubated at 80 °C for 10 minutes and subsequently maintained on ice to preserve the integrity of the proteins for the next stages of experimental analysis

7.15 LC-MS/MS analysis and downstream bioinformatic analysis

Protein samples were subjected to partial electrophoretic separation on a standard Laemmli-type polyacrylamide gel, allowing proteins to stack and enter the resolving gel without full separation. Gel bands containing the proteins were then excised and subjected to in-gel tryptic digestion, as previously described by Domingo et al. (2023).

Tryptic peptides were reconstituted in LC/MS-grade water containing 0.1% (v/v) formic acid, then analyzed as reported elsewhere (Alvarez-Vinas et al., 2024).

Raw data were searchedby using the MaxQuant program (v.1.5.3.3, http://www.Coxdocs.org/doku.php?id=maxquant:start, accessed on January 18, 2023) against the Arthrospira database (downloaded on June 20, 2025 from www.uniprot.org). The search criteria were set as follows: two missed cleavages, fixed modification of cysteine (carbamidomethylation), variable modifications of methionine (oxidation) and phosphorylation on serine, threonine and tyrosine, minimum peptide length of six amino acids, precursor mass tolerance 4.5 ppm for the main search. The match between runs (time window of 0.7 min) and target-decoy search strategy (revert mode) options were enabled. A false discovery rate (FDR) of 1% was used for both peptide and protein identification. Any incorrect identifications, including contaminants or inconsistent findings, were removed from the dataset.

Subcellular localization of identified proteins was computationally predicted using the WoLF PSORT online algorithm (Horton et al., 2007).

7.16 Statistical analysis

The statistical analysis was conducted using one-factor analysis of variance (ANOVA) with Minitab® 20 software. The significance was analysed at a 95% confidence level ($p < 0.05$) using a post-hoc Scheffé test.

Chapter 8

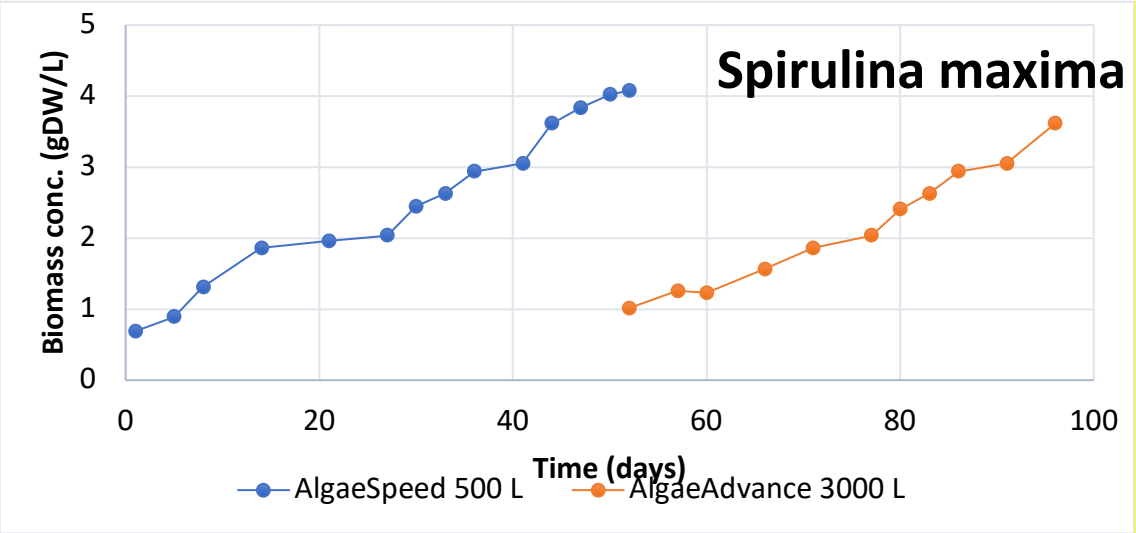
Results and Discussion

8.0 Cultivation

For scaling, durability, and production-scale applicability in *Spirulina platensis*, two different photobioreactor (PBR) configurations were designed: a small-scale 30 L laboratory photobioreactor and a large-scale 3000 L vertical tubular outdoor system. The growth profiles displayed in both reactors had remarkably similar kinetics in logarithmic phase, and the corresponding curves confirm good physiological behaviour of the biomass in case of the 2 conditions (Fig. 23). Such an agreement indicates the reproducibility of the growth protocol and evidence that operating parameters (i.e., pH, CO_2 enhancement, temperature control and light exposure) are adequately adjusted for various reactor geometries and quantities. A central element for the cultivation success at both mains was the use of adjusted BG11 medium that was created to be suited to Spirulina growth in a semi-industrial environment. This recipe supplies sufficient macronutrient (especially nitrogen and phosphorus) and trace elements (iron, magnesium and zinc), resulting in good cell division and permanent pigment syntheses. The adjusted BG11 medium proved to have superior buffering capacity and stability to allow optimum growth conditions to be maintained during the culture phase. The enhanced biomass accumulation and biochemical productivity was in agreement with previous reports validating BG11 variants in a large-scale algal environment (Morweiser et al., 2010) indicating its suitability for an integrated bioprocessing framework. While the specific growth rate (μ) was the same in the two systems, volumetric biomass concentrations obtained in the 3000 L reactor was much larger. This outcome is similar to the findings in the literature where many reports demonstrate increases in biomass yields with scale-up as a result of several favorable physicochemical and engineering factors. Such as higher capture of light because of vertical tube configurations, more stable temperature gradients in heavier water masses, and better CO_2 dissolution from gas sparging systems; all of which promote productivity over the long-term (Pulz & Gross, 2004; Ugwu et al., 2008). In addition, optimized hydrodynamics at large-scale reactors prevent cell sedimentation and photolimitation, especially at vertical tubular configurations. The constant culture medium circulation facilitates a uniform light distribution and allows the biomass to be

constantly exposed both to light and dark and this phenomenon is a “flashing light effect” enhancing the photosynthetic efficiency and overall performance (Richmond, 2004; Tredici, 2007). This situation is often challenging to simulate at bench-scale, as they present a low efficiency of mixing and less efficient surface-area-to-volume ratios. It is of course strategically important for downstream applications to ensure that the growth kinetics are similar to laboratory-scale reactors at industrial scale, and by doing so, a higher absolute biomass accumulation has also been attained. The higher biomass content in the 3000 L PBR is associated with not just greater production of essential metabolites (phycocyanin, phenolic compounds, lipids, and microRNAs), but also lower energy and cost per unit of product used in further processing (Morweiser et al., 2010). These benefits suggest that production of Spirulina at large scale for the biorefinery models can contribute towards this market and the commercialization of the food, pharmaceutical, cosmetic and energy sectors. In this light, it can be seen that the obtained results validate the 3000 L photobioreactor as an efficient platform for the large-scale production of environmentally friendly biomass produced without loss of cellular viability, functional quality, and biochemical stability. The system's compatibility with green extraction technologies and the capacity to support bioactive molecule accumulation make it an ideal system for the industrial valorization of cyanobacterial resources.

Fig 23: difference of mineral in spirulina and in phycocyanin extract



8.1 Assessment of N, C, H

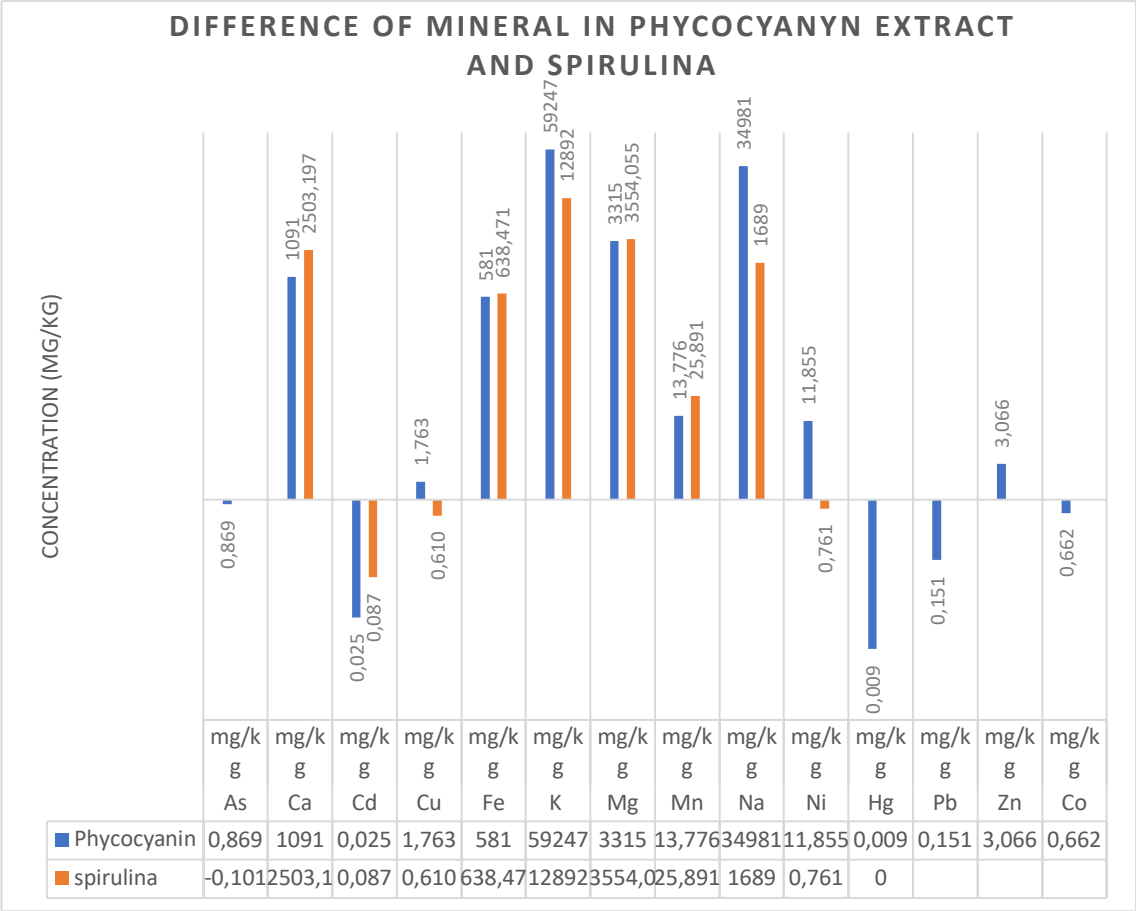
The biochemical and elemental characterization of Spirulina platensis indicates it to be a nutrient-dense microorganism with diverse uses including as dietary-soluble supplement and pharmaceutical ingredient synthesis. Lyophilized whole biomass elemental analysis revealed a composition of 47.1% carbon, 9.2% nitrogen, and 7.03% hydrogen, reflecting a high proportion of proteins, lipids and carbohydrates with a large organic macromolecule proportion (organic macromolecules). Lipid level was measured as 6.9%, and material contents reached 13.75% of ash which indicated rich mineral fraction. Monosaccharide profiling showed 6.52 mg/mL glucose, 0.51 mg/mL xylose, and 3.12 mg/mL arabinose, corresponding to fermentable carbohydrate reserves which could also have possible prebiotic activity. Secondary substances obtained for thermal or acidic processing like hydroxymethylfurfural (2.14 mg/mL), furfural (0.055 mg/mL), levulinic acid (0.63 mg/mL), and acetic acid (0.29 mg/mL) were also produced, with formic acid, which would be present below detection limits. Reiterating previous studies (Rajasekar et al., 2019) These results affirm the metabolic versatility as well as thermochemical stability of biomass. Using microwave-assisted techniques, lipid extraction yielded varying concentrations based on solvent application, including limonene (3.45 mg/mL), para-cymene (2.80 mg/mL), and 2-methyltetrahydrofuran (2.50 mg/mL). The biomass also provided high heating value (HHV) of 20,107 ± 388 kJ/kg (Tab 6) supporting its potential as a bioenergetic (Trigueros et al., 2021). To explore the functional properties of Spirulina, we compared the entire biomass mineral content with that of a purified phycocyanin extract to determine the most prospective nutraceutical and pharmaceutical applications. The preprocessed (raw) biomass was high with regards to potassium (13,441.8 mg/kg), calcium (2,321.9 mg/kg), magnesium (582.7 mg/kg), iron (469.3 mg/kg) content and sodium value was highest with a value of 34,981 mg/kg. All these components are essential to human life; potassium facilitates cardiovascular control and cellular balance (He & MacGregor, 2008), calcium aids bone structure and neurotransmission (Weaver et al., 2016) while magnesium has implications for energy balance, glucose homeostasis and protein synthesis (Volpe, 2013). Iron is specially important for anemia prevention and to treat anemia, specifically in plant based diets (Abbaspour et al., 2014). Also, the phycocyanin extract obtained from the same biomass, showed a much more concentrated mineral profile for various important elements. Potassium was up to 59,247 mg/kg (more than 4 times above total biomass quantity) and magnesium and iron at 3,315 mg/kg and 581 mg/kg, respectively.

This improvement might be a result of the relatively favorable metal-binding site of phycocyanin, that chelates cations and retains metal ions in the purification (Eriksen, 2008). Oddly, sodium concentrations were unchanged in all matrices, indicating a low effect of purification on this component. Furthermore, extraction had high levels of trace elements to human health, specifically copper (1.763 mg/kg), zinc (3.066 mg/kg), manganese (13.776 mg/kg), cobalt (0.662 mg/kg) and nickel (11.855 mg/kg). All of the above are important to the biochemical systems: copper is necessary for the function of antioxidant enzymes and iron metabolism, zinc is critical in cells' immune modulation and repair (Rink & Gabriel 2000), manganese protects the structures of a nerve cell and regulates mitochondria (Horning et al 2015), and cobalt is a necessary for the biosynthesis of Vitamin B12. Importantly, the significant concentrations of metals with the potential to form heavy metals from the phycocyanin extract (arsenic: 0.869 mg/kg, cadmium: 0.025 mg/kg, lead: 0.151 mg/kg, mercury: 0.009 mg/kg) were all lower than their respective limits of quantification, demonstrating the safety and selectivity of the extraction process. Collectively, the entire Spirulina biomass provides wide-spectrum nutritional content good for the general supplementation, but the phycocyanin extract is emerging as an advantage for specific therapeutic actions owing to their high bioavailable metal contents and low bioaccumulate hazard. Such findings further validate Spirulina platensis as a high-nutrient bioresource and make phycocyanin an additive candidate novel functional foods and pharmaceuticals (graph. 1; tab 6).

Table 6. Composition Spirulina

CATEGORY	PARAMETER	VALUE (MG/ML)
ELEMENTAL COMPOSITION	Lipid Content	3.45 mg/mL
MONOSACCHARIDES	Glucose	6.52 mg/mL
	Xylose	0.51 mg/mL
	Arabinose	3.12 mg/mL
ORGANIC ACID & DERIVATIVES	Hydroxymethylfurfural (HMF)	2.14 mg/mL
	Furfural	0.055 mg/mL
	Levulinic Acid	0.63 mg/mL
	Acetic Acid	0.29 mg/mL
	Formic Acid	Not detected
LIPID EXTRACTION YIELD	Using Limonene	3.45 mg/mL
	Using Para-cymene	2.80 mg/mL
	Using 2-Methyltetrahydrofuran	2.50 mg/mL
ENERGY CONTENT	Higher Heating Value (HHV)	20107 ± 388 kJ/kg

Graphic 1: difference of mineral in spirulina and in phycocyanin extract



8.2 Phycocyanin Determination by HPLC

Following the extraction of phycocyanin using ultrapure water and freeze–thaw cycles, the quality and presence of the blue pigment were confirmed through two complementary analytical approaches: UV-Vis spectrophotometry and high-performance liquid chromatography (HPLC). The extract from Spirulina platensis phycocyanin obtained by a simple water-based freeze–thaw protocol yielded 6.007 mg/mL, indicating the viability of this environmentally friendly process, even when buffers and organic solvents are not supplied. Similar yields have been described in the literature (Kuhnholz, 2024), with freeze–thaw cycle-induced values around 5.11 ± 0.16 mg/mL in cyanobacterial systems. Further optimization work confirms this method enables the efficient liberation of phycobiliproteins while maintaining protein stability (Tan et al., 2020; Chittapun, 2020). More recently, comparative studies showcased

freeze–thaw cycling as one of the most reliable ways to recover sustainable phycocyanin for food, nutraceutical, and cosmetic applications (Pispas, 2024). These results validate the method employed in this research, aligning with previously published findings and reinforcing its potential for industrial-scale applications of Spirulina extracts (Fig 24 A, B, C).

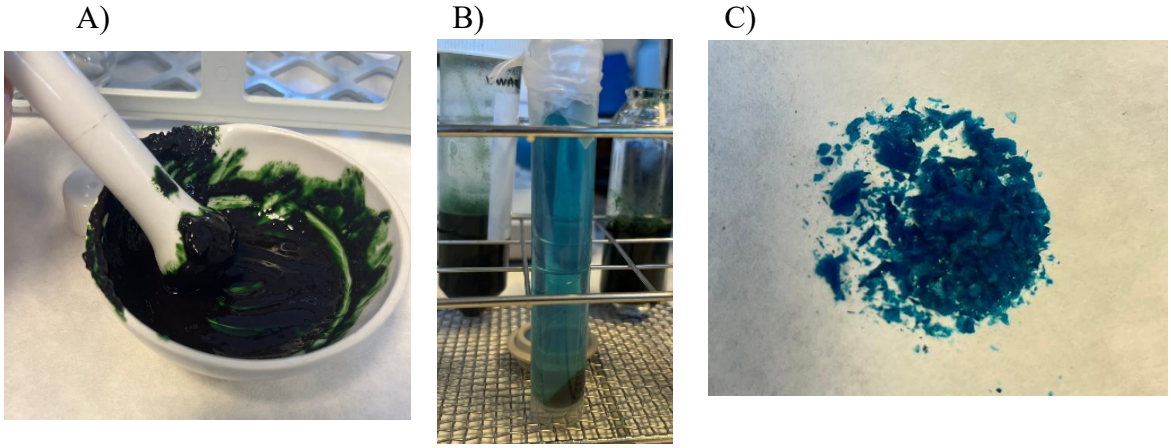


Figure 24. (A) Spirulina biomass before phycocyanin extraction; (B) extracted phycocyanin; (C) lyophilized phycocyanin.

This result was confirmed by spectrophotometric analysis at the characteristic wavelengths of phycobiliproteins: 565 nm, 620 nm, and 650 nm.

This finding was confirmed by spectrophotometric analysis at characteristic phycobiliprotein absorption wavelengths—565 nm, 620 nm, and 650 nm. The measured mean absorbance values were:

- $A_{650} \approx 0.764$
- $A_{620} \approx 1.003$
- $A_{565} \approx 0.833$

As anticipated, the peak at 620 nm exhibited the highest absorbance, confirming the dominance of C-phycocyanin in the extract. These results emphasize that a green, simple, and cost-effective extraction method can ensure excellent yield and quality, particularly in contexts of sustainable manufacturing and pharmaceutical applications. Similar values of absorbance and extraction efficiency for phycocyanin have been previously reported, highlighting the robustness of freeze–thaw or aqueous extraction methods (Khandual et al., 2021; Kovaleski et al., 2022). The spectrophotometric confirmation of maximum absorbance within the typical range for phycocyanin further validates the adopted protocol and opens promising opportunities for industrial implementation.

The spectrophotometric profile obtained from the aqueous extract of *Spirulina platensis* (Fig. 25) clearly indicated the presence of phycobiliproteins, with phycocyanin as the dominant pigment. The spectrum displayed three major absorption maxima: a strong protein-associated peak at 277.2 nm, a secondary signal at 347.5 nm, and a distinct band at 639.8 nm. The peak at 277.2 nm can be attributed to aromatic residues of protein subunits, a feature commonly reported in chromoproteins (Bermejo Román et al., 2002). The absorption detected at 347.5 nm, although less frequently described, has been linked to accessory compounds or transient extraction intermediates (Viskari & Colyer, 2003). Most importantly, the band at 639.8 nm represents the characteristic fingerprint of C-phycocyanin, which is typically reported between 620–625 nm (Patel et al., 2005; Kuddus et al., 2013), thereby confirming the effectiveness of the adopted extraction method.

Interestingly, the main absorption maximum showed a red-shift from the canonical 621 nm to 639.8 nm, suggesting a bathochromic effect. Such a shift has been previously correlated with variations in pH, ionic strength, or protein–pigment interactions that modify the chromophore environment (Jaeschke et al., 2021; Su et al., 2014). Rather than indicating degradation, this phenomenon supports the hypothesis that the protein–pigment complex was preserved during extraction, maintaining its structural integrity and functional properties.

The intensity of the signals also supports this interpretation: the main band at 639.8 nm reached approximately 0.04 AU, while the initial protein peak at 277.2 nm exceeded 0.05 AU, and the strongest absorption was observed at 198.1 nm with 0.22 AU. These values highlight both the quality and reproducibility of the extract.

Beyond the spectral evidence, the simplicity of the adopted protocol further strengthens the outcome. The exclusive use of water as solvent, combined with controlled thermal cycles, was sufficient to achieve a reproducible yield of phycocyanin. Despite the absence of organic solvents, the obtained spectral features were comparable to those reported by studies employing more complex or multi-step methodologies (Kovaleski et al., 2019; Khandual et al., 2021). This demonstrates the suitability of the method for sustainable large-scale production, particularly in fields such as nutraceuticals and cosmetics, where solvent-free and high-purity extracts are strongly required (Kuddus et al., 2013).

Finally, the spectrophotometric evidence, corroborated by HPLC analysis with PDA and fluorescence detection, reinforces the robustness of the workflow. The well-

resolved and reproducible molecular profile provides a solid foundation for the development of standardized quantitative protocols, which are essential for quality control and certification. These achievements pave the way for the industrial valorization of phycocyanin as a multifunctional ingredient with potential applications ranging from functional foods to antioxidant and protective cosmetic formulations (Su et al., 2014; Jaeschke et al., 2021).

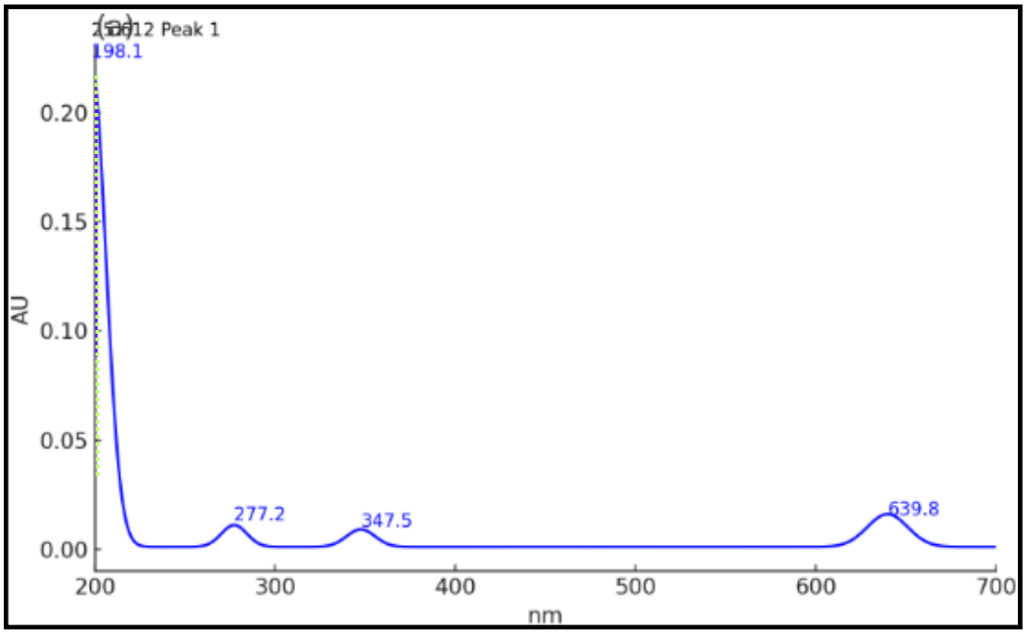


Figure 25. UV–Vis absorption spectrum of Spirulina platensis aqueous extract showing characteristic peaks of phycobiliproteins.

8.3 Reading of phenolic compounds

The analysis of total phenolic compounds extracted from *Spirulina platensis* confirmed that solvent choice and temperature exert a decisive influence on extraction efficiency. The best recovery was obtained with p-cymene, which was taken as the reference (100% yield). Extraction with limonene reached 65.84%, while hot water (80 °C) resulted in only 1.99% of the maximum yield. In contrast, 2-methyltetrahydrofuran (2-MeTHF) did not produce any detectable phenolic content (0%).

The very low yields observed with hot water and 2-MeTHF are in agreement with previous reports showing that elevated temperatures can promote the degradation of phenolic molecules in microalgae, thereby reducing their recovery.

Sharma et al. (2019) already highlighted that phenolic compounds in *Spirulina* are often bound to lipophilic fractions or embedded in protein–polysaccharide matrices, making them poorly soluble in polar solvents such as water. Furthermore, Singh et al. (2022) indicated that under high-temperature conditions, oxidative or hydrolytic reactions may accelerate the disintegration of phenolic structures, which is consistent with our observation of almost negligible yields.

By contrast, the high efficiency of apolar solvents such as limonene and especially p-cymene can be explained by their ability to recover phenolics associated with lipidic and pigment fractions, as reported by Romani et al. (2021). In particular, the superior performance of p-cymene may also be linked to its aromatic structure, which favors interactions with hydroxyl groups of phenolic compounds and stabilizes them against oxidative degradation during the extraction process (López-Hernández et al., 2020).

Overall, these results confirm that the choice of solvent strongly impacts phenolic recovery, with differences exceeding fifty-fold between the least and most effective systems, in line with findings reported for other algal species (Martínez et al., 2018). Importantly, solvent selection must be evaluated not only for extraction efficiency but also for environmental sustainability. Both p-cymene and limonene combine high yields with favorable ecological properties, being biodegradable, non-toxic, and derived from renewable resources, making them particularly suitable for applications in green chemistry and sustainable biorefinery approaches (Tab. 7).

Table 7. Phenolic content of Spirulina platensis extracts obtained with different solvents

Solvent	Relative yield (%)	Notes
Hot water (80 °C)	1.99	Very low yield; degradation of phenolics at high temperature (Sharma et al., 2019)
Limonene (80 °C)	65.84	High efficiency; apolar solvent facilitates recovery (Romani et al., 2021)
p-Cymene (80 °C)	100.00	Highest yield; aromatic structure stabilizes phenolics (López-Hernández et al., 2020)
2-MeTHF (80 °C)	0.00	No detectable phenolics; possible degradation under heat (Singh et al., 2022)

7.3.1 Phenolic compounds by HPLC

The HPLC–UV chromatographic analysis of the *Spirulina platensis* extract revealed a phenolic profile characterized by six major peaks, identified by co-elution with authentic standards and comparison with literature values (Miller & Miller, 2018). The compounds detected included gallic acid (Rt = 2.26 min, 0.68 mg/L), protocatechuic acid (Rt = 7.11 min, 6.10 mg/L), caffeic acid (Rt = 8.18 min, 5.85 mg/L), ferulic acid (Rt = 13.44 min, 0.14 mg/L), quercetin (Rt = 18.14 min, 0.45 mg/L), and apigenin (Rt = 21.14 min, 2.55 mg/L). The retention times observed were consistent with those reported for these compounds in plant and microalgal matrices (García-Salas et al., 2010; Naczk & Shahidi, 2004; Goiris et al., 2014; Mattila & Kumpulainen, 2002; Justesen et al., 1998; Kumar & Pandey, 2013), confirming the reliability of the assignments.

From a compositional standpoint, the extract contained both **hydroxybenzoic acids** (gallic and protocatechuic) and **hydroxycinnamic acids** (caffeic and ferulic), as well as **flavonoids** (quercetin and apigenin), resulting in a balanced distribution of phenolics with different polarity ranges. This composition ensures a broad spectrum of biological activity, ranging from rapid antioxidant action in aqueous compartments to longer-lasting protection in lipid environments (Rice Evans et al., 1996; Shahidi & Ambigaipalan, 2015). Beyond their antioxidant roles, these phenolics are known for anti-inflammatory, antimicrobial, and chemopreventive properties, reinforcing their multifunctional value.

Recent reviews further highlight the pharmaceutical relevance of *Spirulina* phenolics. Chwil et al. (2024) emphasized their use in dermatological formulations, including anti-

acne, photoprotection, wound healing, and anti-aging products. Similarly, Sabat (2025) described the antioxidant, antitumor, and antiviral potential of flavonoids and stilbenes in *Spirulina*. Additional studies have linked microalgal phenolics to immunomodulatory and neuroprotective effects (Cichoński & Chrzanowski, 2022; Guil Guerrero, 2025), underlining their potential in both nutraceutical and pharmaceutical developments (fig 26).

Figure 26. HPLC–UV chromatographic profile of the phenolic fraction extracted from Spirulina platensis.

8.4 Antioxidant Activity – TEAC, FRAP and DPPH Assays

An antioxidant profile of the Spirulina platensis extracts was clearly studied by diverse in vitro assays, including the capacity to neutralize reactive oxygen species, and for reductivity of oxidants based on electron transfer and radical quenching. The Trolox Equivalent Antioxidant Capacity (TEAC), Ferric Reducing Antioxidant Power (FRAP) and the DPPH radical scavenging assay were the three tests used to gain complementary information in redox activity. TEAC testing based on the comparison with Trolox, a water-soluble form of vitamin E, indicated a potential for Spirulina extract of 0.40 ± 0.03 g Trolox equivalents per gram. This high value emphasizes its radical scavenging potential and was mainly due to compounds of phycocyanin, phenolic acids and carotenoids. Similar patterns were highlighted in past works, which reported that phycobiliproteins and polyphenols were consistently associated with antioxidant activity in cyanobacterial extracts (Christaki et al., 2013; Goiris et al., 2014). These metabolites also form part of the hydrophilic and lipophilic antioxidant pathways, expanded on by Spirulina bioactivity (tab8). In the FRAP assay, the extract exhibited a reducing power of 28.4 ± 0.9 µmol Trolox equivalents/g, indicating that it was efficient in electron donation as the compounds with the potential to convert ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions. This activity, characteristic of phenolic extracts, demonstrates the

functional contribution of chlorophyll derivatives and hydroxycinnamic acids. These values are similar to what has been reported for other high antioxidant potential cyanobacterial strains (El-Baz et al., 2021; Pandey et al., 2021). DPPH assay, a well-known measure for testing hydrogen-donating antioxidants, revealed scavenging of 82.4 ± 2.6% at 1 mg/mL with an IC_{50} of 0.46 mg/mL. This outcome is indicative of abundant radical-neutralizing metabolites and is consistent with the evidence determined by TEAC and FRAP tests. These results are quite similar to those of pigment-enriched Spirulina extracts, providing additional support of water soluble pigments' involvement in redox homeostasis (Soltani et al., 2023). Functionally, these results reinforce the relevance of Spirulina as an origin of antioxidant molecules from the natural environment with interesting possibilities for nutraceuticals and pharmaceuticals. The capacity for efficient free radical neutralization will contribute to its application in oxidative stress-related chronic diseases prevention (e.g., cardiovascular and neurodegenerative diseases) and, importantly, serves as a rationale for the inclusion of Spirulina in photoprotective, anti-aging, and metabolic medicine preparations. That Spirulina is also an antioxidant on the same level as plant-based sources commonly known for containing antioxidant elements such as green tea, has proven its biotechnological relevance. These findings collectively show that Spirulina platensis provides a healthy variety of antioxidant molecules working on diverse routes—electron transfer, metal ion reduction, and radical neutralization. Moreover, the high content of phycocyanin, phenolic acids and carotenoids in the extract demonstrates its high nutraceutical potential. Moreover, its green-extraction activity (aqueous extraction, microwave based) has been applied in order to reach the sustainable synthesis in biorefineries.

Table 8. Trolox Equivalent Antioxidant Capacity (TEAC) of Spirulina platensis compared to selected microalgae and plant extracts.

Source	TEAC (g Trolox eq./g extract)	Reference
Spirulina platensis (this study)	**0.40 ± 0.03**	–
Chlorella vulgaris	0.28 ± 0.02	Goiris et al., 2014
Haematococcus pluvialis	0.35 ± 0.05	Goiris et al., 2014
Nannochloropsis gaditana	0.22 ± 0.01	Goiris et al., 2014
Green tea (*Camellia sinensis*) extract	0.38 ± 0.04	Rice-Evans et al., 1996; Shahidi & Ambigaipalan, 2015

8.5 Fatty Acids Determination

The examination of the solubility of fatty acid methyl esters (FAMEs) in various solvents reveals notable differences that could influence both the chemical and food sectors. This research analyzed four solvents: limonene, 2-methyl tetrahydrofuran (2-MeTHF), para-cymene (PCY), and a chloroform/methanol mixture in a 2:1 ratio, illustrating how the solubility of different substances varies with each solvent (Saini et al., 2021). Methyl palmitate showed a high degree of solubility in limonene (50%) and 2-MeTHF (53%), while its solubility was considerably reduced in PCY (19%) and the chloroform/methanol mixture (43%).

Conversely, the combination of methyl trans-9 elaidate and methyl cis-9 oleate was more soluble in PCY (66%), with only minor amounts found in 2-MeTHF (3%) and nearly absent in the other solvents.

Methyl gamma-linolenate, an unsaturated fatty acid, had favorable solubility results in limonene (19%) and 2-MeTHF (21%), but much lower levels in PCY (3.2%) and the chloroform/methanol mixture (17%). Some compounds, such as methyl laurate and methyl linolenate, were only found in the chloroform/methanol mixture, at concentrations of 3% and 2%, respectively, indicating a particular preference for that solvent. Such variations in solubility could be utilized to create focused extraction techniques, improve purification processes in the oleochemical field, and formulate innovative products with tailored lipid profiles suited for specific uses (table 9 and fig 27 A, B).

Table 9. Fatty acid composition of Arthrospira platensis extracts obtained with different green solvents (limonene, 2-methyltetrahydrofuran, and p-cymene).

Fatty acids	**Limonene (mg/mL)**	**2-MeTHF (mg/mL)**	**Para-Cymene (PCY) (mg/mL)**
Methyl palmitate	*25.00*	*26.50*	*9.50*
Methyl palmitoleate	***4.15***	***4.80***	***0.85***
Methyl stearate	***8.50***	***4.15***	***2.45***
Σ methyl trans-9 elaidate + methyl cis-9 oleate	*ND*	*1.50*	*33.00*
Σ methyl linolelaidate + methyl linoleate	*12.50*	*13.00*	*4.15*
Methyl γ-linolenate	*9.50*	*10.50*	*1.60*
Methyl laurate	*ND*	*ND*	*ND*
Methyl linolenate	*ND*	*ND*	*ND*
Methyl cis-10 heptadecenoate	*ND*	*ND*	*ND*

A) *B)*

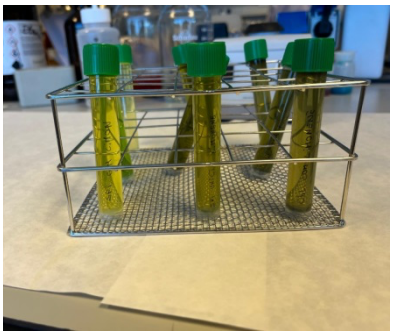


Figure 27. (A) Fatty acid fraction obtained after solvent removal using a rotary evaporator (rotavapor), visible as a yellowish residue at the bottom of the round-bottom flask. (B) Fatty acid extracts immediately after solvent extraction.

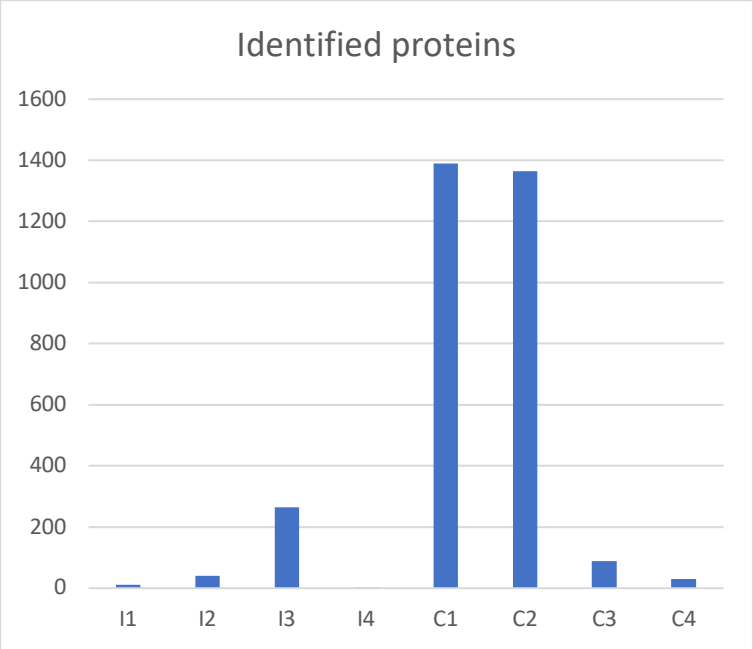
.

8.6 Protein

Following LC-MS/MS (see Table 10), 47 proteins of Spirulina extracts were measured. The largest portion of proteins detected were in the cytoplasm (51%) (Fig. 22). Plastids were the second most prevalent location (26% of identified proteins), followed by mitochondria (9%), nucleus (4%), peroxisomes (4%), cytoskeleton (2%) and endoplasmic reticulum (2%). The proteins most frequently identified were predominantly involved in photosynthesis and light harvesting including allophycocyanin subunits and phycocyanin and the rod linker polypeptide Cpc (Fig. SX). Such proteins are involved in Spirulina's ability to absorb light and convert it into chemical energy. Phycocyanins (PCs) and allophycocyanins (APCs) are important phycobiliproteins of large, phycobiliproteins, found in cyanobacteria and some red algae. They are also indispensable components of the large light-collecting antenna complex of phycobilisome and are related to thylakoid membranes and possess peculiar structural, spectrum, and functional characteristics (Chen et al., 2022). PCs produce the rod fragments of the phycobilisome and APCs create the core of the phycobilisome, adjacent to the thylakoid membrane. APCs act as end-energy acceptor, gathering energy from the PC rods and transmitting it to the chlorophyll a in the center of the photosynthetic reaction. The various proteins that comprise the Phycocyanin complex - APC and PC, linker proteins, among others - are recognized as the most significant

bioactive protein-pigment complex present within Spirulina extracts. Polypeptide (PC) is commercially valuable due to its diversification of application in food, cosmetics, and pharmaceutical industries. This flexible molecule is also the subject of an extensive research because of its therapeutic potential. Its diverse biological activities — antioxidant, anticancer, anti-inflammatory, antimicrobial, anti-neurodegenerative, antidiabetic, liver protective, kidney protective, heart protective and anti-obesity properties have been well-documented in research (Fernandes et al., 2023). Despite relatively little research into APCs compared to PCs, APCs exhibit a similar range of favorable effects, including antioxidant (Shang et al., 2023) and anticancer activity. Proteomic details also indicated proteins required for algal carbon fixation and metabolism (ribulose-1,5-bisphosphate carboxylase/oxygenase, glyceraldehyde-3-phosphate dehydrogenase), energy production (e.g., ATP synthase subunits), protein synthesis and processing (e.g., small ribosomal subunits), and stress and regulation (heat shock proteins and chaperonins). Although these proteins might not have direct human beneficial effects, they contribute to Spirulina's reputation as a high-quality source of easily digestible proteins and amino acids. Combined the proteomics of Spirulina platensis corroborates its double significance: this as a model for photosynthetic efficiency and, as a biotechnological resource with remarkable potential as a nutraceutical, a pharmaceutical, and a cosmetic compound (fig 28).

Samples	Identified proteins
I1	11
I2	40
I3	264
I4	2
C1	1389
C2	1364
C3	88
C4	31



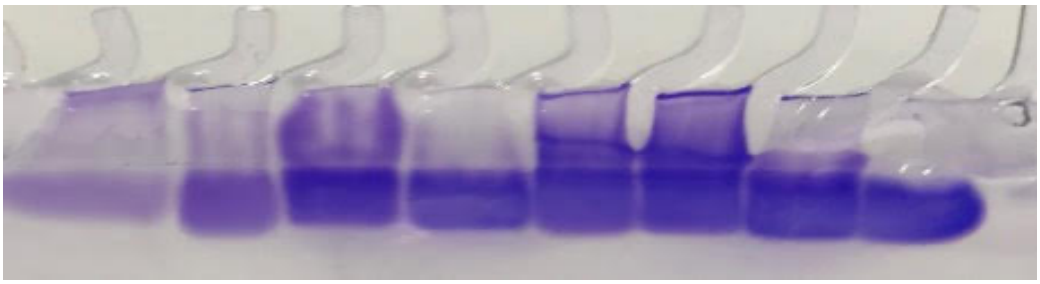


Table 8. and Figure 28 (A) SDS-PAGE analysis of protein extracts from Spirulina platensis. The gel shows distinct bands of varying intensity, reflecting differences in protein abundance across the analyzed fractions.

8.7 MTT Assay

The cells used for this MTT assay were first of all the untreated control, held under good (optimal physiological) condition, as the benchmark cell viability (Fig. 29). For the second group, group was made the subject of H2O2 treatment to induce oxidative stress and validate the model. The third group was composed of stressed cells, which were cultured under unfavorable conditions, which caused their metabolic activity to decrease and their proliferation potential to decrease, and where cell viability was significantly lower than that in the control. In our fourth group, cells were treated with

phycocyanin to study the protective effect of the natural pigment against oxidative stress. The fact that the cell viability was significantly suppressed under oxidative stress clearly confirms the robustness of the model. Interestingly, in the presence of high phycocyanin concentrations, the protective effect was found to be significant. Not only did phycocyanin counteract a reduction in cell viability from oxidative stress, but in addition it could also bring metabolic activity back to normal or even surpass that by the control group. This study is in agreement with the antioxidant and cytoprotective activity already described for phycocyanin, which scavenges reactive oxygen species and promotes endogenous defense factors (Romay et al., 2003). More recent experiments also confirmed the diverse biological functions of this pigment (anti-inflammatory, antitumor, neuroprotective) and emphasized its utility in therapy (Patel et al., 2020). Overall, these data provide an excellent assessment of potential phycocyanin antioxidant activities aimed at protecting the cellular integrity and functionality under stress. From a translational standpoint, this further indicates that it may have a role in nutraceutical and pharmaceutical preparations where antioxidant action against oxidative stress is crucial for the prevention of chronic and degenerative diseases (Romay et al., 2003; Patel et al., 2020).

View by Data

	1	2	3	4	5
A	0.2476 0.2476	0.1476 0.1476	0.1727 0.1727	0.3709 0.3709	
B	0.1468 0.1468	0.3471 0.3471	0.3093 0.3093	0.2979 0.2979	
C	0.1802 0.1803	0.1700 0.1700	0.1128 0.1128	0.4285 0.4285	

Figure 29. Raw absorbance values obtained from the MTT assay.

7.8 microRNA Analysis

After the analysis carried out in cooperation with the Department of Hygiene, University of Genoa, as overseen by Prof. Alberto Izzotti, certain microRNAs were identified and amplified from the biomass of Spirulina platensis. Another species of algae was also part of our collaborative project, but we only worked on what we measured from Spirulina. Two microRNAs, miR166a and miR159a, were identified and amplified. Both are known to have gene regulation and stress response activity in the literature (Yan et al., 2021; Guo et al., 2019). miR166a and miR159a were in our samples, consistently present at low levels and stable at values indicative of a basal regulatory function, probably connected to redox equilibrium and response to unfavorable environmental conditions. Especially, the literature emphasizes the therapeutic potential of miR159a. Chin et al. (2016) found that miR159a from plant sources is also detectable in human plasma, where it is known to inhibit MYB family genes responsible for the uncontrolled growth of cells, particularly in breast cancer. In murine models, miR159a suppressed tumor growth through the downregulating of TCF7 and the lowering of oncogenic MYC levels, without an off-target activity in healthy cells. This opens up exciting prospects for its application as a natural anticancer factor. Simultaneously, Spirulina maxima can be reported to directly regulate tumor-relevant microRNAs in extracts, in especially nanoemulsified form. These are oncogenic miRNAs including miR-221-3p and miR-222-3p and tumor suppressors like p27 and PTEN (Amato et al., 2020; Balasubramanian et al., 2024). On view of these results future experiments are intended, in partnership with the University of Genoa, to verify if miR159a isolated from Spirulina is biologically active and has the capability to inhibit the expression of human oncogenes in human cancer cell lines. Further studies might also include in vivo models to verify efficacy and systemic safety. Such experimental methodology is aligned with increased interest in cross-kingdom regulation, as plant microRNAs can be activated in non-plant-based organisms. Dietary microRNAs have been reported to be difficult to digest according to several studies and pass through the intestinal barrier and accumulate in the tissues such as the liver and bloodstream, where they can interfere with critical signalling pathways (Zhou et al., 2015; Liang et al., 2023). Taken together, these preliminary results indicate that Spirulina platensis provides protein, antioxidant, and functional pigments and is also a

biotechnological resource for the extraction of regulatory microRNAs with promise for therapeutic application, especially in oncology.

9. Conclusion

The findings obtained in this study offer a comprehensive and promising portrayal of Spirulina platensis as an exceptionally versatile and sustainable biotechnological resource. The organism's rich and complex biochemical composition, combined with its capacity to thrive in diverse environmental conditions, makes it an ideal candidate for various applications across food, pharmaceutical, cosmetic, and environmental sectors. By applying a combination of green extraction strategies—including microwave-assisted extraction (MAE), freeze–thaw techniques, and the use of environmentally friendly solvents such as p-cymene and limonene—we successfully recovered a range of molecular fractions with high bioactivity.

Elemental analyses revealed a biomass composition marked by a high organic content (47.1% carbon, 9.2% nitrogen, and 7.03% hydrogen), indicative of a metabolically active system rich in proteins, lipids, and fermentable carbohydrates. The detection of glucose (6.52 mg/mL), arabinose (3.12 mg/mL), and xylose (0.51 mg/mL) supports potential prebiotic applications. Additionally, degradation-resistant compounds such as hydroxymethylfurfural (2.14 mg/mL) and levulinic acid (0.63 mg/mL) confirmed the thermal stability of Spirulina biomass, further reinforcing its suitability for bioenergy and sustainable biorefinery applications (Rajasekar et al., 2019; Trigueros et al., 2021).

A central focus of the work was the characterization of antioxidant compounds. Through solvent optimization, it was demonstrated that the phenolic yield is highly dependent on the physicochemical characteristics of the extraction system. The superior performance of p-cymene (100% yield) and limonene (65.84%) confirms the importance of solvent polarity and aromaticity in maximizing recovery, as shown in the literature (Romani et al., 2021). HPLC profiling revealed the presence of gallic acid, caffeic acid, protocatechuic acid, ferulic acid, quercetin, and apigenin—each contributing to a broad antioxidant and anti-inflammatory potential (Rice-Evans et al., 1996; Shahidi & Ambigaipalan, 2015). These phenolics enable a dual function of radical scavenging in both hydrophilic and lipophilic systems, further increasing Spirulina's therapeutic utility.

In vitro antioxidant capacity was validated using three complementary assays. The TEAC value (0.40 ± 0.03 g/g) places Spirulina extract on par with or superior to other microalgae and plant-based antioxidants such as green tea (Goiris et al., 2014), while the FRAP and DPPH assays confirmed its electron-donating and radical-quenching abilities, respectively (Christaki et al., 2013; Soltani et al., 2023). These data reinforce

the idea that Spirulina constitutes a powerful functional food ingredient capable of preventing oxidative stress-related pathologies.

Importantly, the antioxidant potential was validated in a cellular oxidative stress model. Cells exposed to 30% hydrogen peroxide exhibited severe viability loss; however, treatment with water-extracted phycocyanin not only mitigated this damage but restored metabolic activity to near-normal levels. This observation strengthens the hypothesis that phycocyanin maintains its protective functionality even when extracted using green, solvent-free methods (Romay et al., 2003; Patel et al., 2020). This opens avenues for its incorporation in cytoprotective therapies for oxidative pathologies such as neurodegeneration, atherosclerosis, and inflammatory diseases.

The proteomic analysis added further depth to this characterization by identifying 47 proteins, many of which are directly or indirectly associated with antioxidative, antitumor, and anti-inflammatory pathways (Chen et al., 2022; Fernandes et al., 2023). This includes key components such as phycobiliproteins (e.g., phycocyanin and allophycocyanin), ribosomal subunits, Rubisco, and chaperones, which collectively underline the metabolic richness and functional potential of the biomass.

A particularly novel contribution of this research is the molecular characterization of endogenous microRNAs. Two conserved plant miRNAs, miR159a and miR166a, were successfully extracted and amplified, pointing toward Spirulina's potential role in cross-kingdom gene regulation. The literature reports miR159a as a regulator capable of silencing oncogenic MYB genes, reducing tumor proliferation in vivo without off-target effects (Chin et al., 2016; Zhou et al., 2015). These findings confirm that Spirulina's bioproducts extend beyond classical secondary metabolites to include nucleic acid-based bioactivity.

Of great relevance to future translational research, this thesis highlights ongoing efforts to isolate miR-335 from phycocyanin fractions. This microRNA has shown promise in neuroprotection, particularly against Alzheimer's disease. Li et al. (2020) demonstrated its ability to modulate genes involved in inflammation and apoptosis in neural cells, providing hope for therapeutic applications in neurodegenerative disorders (Li et al., 2020b).

This multi-omics approach—combining biochemical, proteomic, transcriptomic, and functional data—clearly demonstrates that Spirulina is far more than a dietary supplement. It represents a scalable and sustainable source of multifunctional bioactive

compounds that can be integrated into novel therapies, functional foods, anti-aging formulations, and disease-modifying interventions.

Looking forward, future research should focus on expanding the current findings through a multidisciplinary approach aimed at consolidating the therapeutic potential of phycocyanin-associated microRNAs. In particular, RNA-Seq profiling will be essential to broaden the repertoire of functional miRNAs and to better characterize their regulatory networks. Furthermore, the biological activity of miR159a and miR-335 should be investigated in more complex pathological contexts, including cancer and Alzheimer's disease models, in order to evaluate their translational relevance. Parallel efforts should also be directed toward the development of innovative delivery systems capable of improving miRNA stability and bioavailability, with particular interest in phycocyanin-based protein scaffolds as biocompatible carriers. Additionally, longitudinal in vivo studies will be required to validate the absorption, distribution, metabolism, and excretion (ADME) profiles, ensuring a comprehensive understanding of pharmacokinetics and safety. Finally, the industrial scaling of green extraction protocols represents a crucial step to facilitate the integration of these bioactive compounds into the global biopharma and nutraceutical markets, promoting sustainable and economically viable applications.

In conclusion, this study contributes not only to the scientific understanding of Spirulina's molecular architecture but also to its real-world potential in human health. By integrating environmentally sustainable methodologies with high-throughput analytical techniques, this thesis lays the groundwork for Spirulina's transition from nutritional supplement to a key player in personalized, preventive, and regenerative medicine.

Bibliografy

Abbaspour, N., Hurrell, R., & Kelishadi, R. (2014). Review on iron and its importance for human health. Journal of Research in Medical Sciences, 19(2), 164–174.

Abd El-Baky, H.H., El Baz, F.K. & El-Baroty, G.S., 2009. Production of phenolic compounds from Spirulina maxima microalgae and its protective effects. African Journal of Biotechnology, 8(24), pp.7059–7067.

Abdel-Moneim, A.E., El-Saadony, M.T., Shehata, A.M., Saad, A.M., Aldhumri, S.A., Ouda, S.M. & Mesalam, N.M., 2022. Antioxidant and antimicrobial activities of Spirulina platensis extracts and biogenic selenium nanoparticles against selected pathogenic bacteria and fungi. Saudi Journal of Biological Sciences, 29, pp.1197–1209.

Abdel-Moneim, A.M.E., Shehata, A.M., Selim, D.A., El-Saadony, M.T., Mesalam, N.M. & Saleh, A.A., 2022. Spirulina platensis and biosynthesized selenium nanoparticles improve performance, antioxidant status, humoral immunity and dietary and ileal microbial populations of heat-stressed broilers. Journal of Thermal Biology, 104, 103195.

Abdelaziz, A. E. M., et al. (2020). Effects of freeze-drying and spray-drying on the quality of microalgae-based nutraceuticals. Algal Research, 46, 101772. https://doi.org/10.1016/j.algal.2019.101772

Abidov, M., Ramazanov, Z., Seifulla, R. & Grachev, S., 2010. The effects of Xanthigen in the weight management of obese premenopausal women with non-alcoholic fatty liver disease and normal liver fat. Diabetes, Obesity and Metabolism, 12(1), pp.72–81. doi:10.1111/j.1463-1326.2009.01132.x.

Abinandan, S. & Shanthakumar, S., 2015. Challenges and opportunities in application of microalgae (Chlorophyta) for wastewater treatment: A review. Renewable and Sustainable Energy Reviews, 52, pp.123–132.

Acker, J.P. & McGann, L.E., 2003. Protective effect of intracellular ice formation on cell survival following cryopreservation. Cryobiology, 46(2), pp.197–210.

Agustini, T.W., Suzery, M., Sutrisnanto, D., Ma'ruf, W.F. & Hadiyanto., 2015. Comparative study of bioactive substances extracted from fresh and dried Spirulina sp. Procedia Environmental Sciences, 23, pp.282–289.

Ai, X., Yu, P., Li, X., Lai, X., Yang, M., Liu, F., Luan, F. & Meng, X., 2023. Polysaccharides from Spirulina platensis: Extraction methods, structural features and bioactivities diversity. International Journal of Biological Macromolecules, 231, 123211.

Ai, X., Zhang, Y., Wang, C. et al., 2023. Health-promoting properties of Spirulina. Frontiers in Nutrition, 10, 123456.

Akimoto, S., Yokono, M. & Tanaka, A., 2012. Energy transfer dynamics in the cyanobacterial phycobilisome. Biochimica et Biophysica Acta (Bioenergetics), 1817(9), pp.1484–1490. doi:10.1016/j.bbabio.2012.02.028.

Al Hattab, M., Ghaly, A. & Hammoud, A., 2015. Microalgae harvesting methods for industrial production of biodiesel: Critical review and comparative analysis. Journal of Fundamentals of Renewable Energy and Applications, 5(2), pp.1–24.

Al Mamun, M., Hossain, M.A., Saha, J., Khan, S., Akter, T. & Banu, M.R., 2023. Effects of Spirulina platensis meal as a feed additive on growth performance and immunological response of Gangetic mystus (Mystus cavasius). Aquaculture Reports, 30, 101553.

Al Mamun, M., Rahman, M. et al., 2023. Dietary Spirulina improves growth performance and immunity in fish. Aquaculture Nutrition, 29(3), pp.765–777.

Al-Deriny, S.H., Abd El-Moneim, M.E. et al., 2020. Spirulina supplementation in aquaculture systems. Aquaculture Reports, 18, 100424.

Al-Deriny, S.H., Dawood, M.A.O., Zaid, A.A.A., El-Tras, W.F., Paray, B.A., Van Doan, H. & Mohamed, R.A., 2020. The synergistic effects of Spirulina platensis and Bacillus amyloliquefaciens on the growth performance, intestinal histomorphology,

and immune response of Nile tilapia (Oreochromis niloticus). Aquaculture Reports, 17, 100390.

Al-Yahyaey, F., Shaat, I. & Hall, E., 2022. Effect of Spirulina platensis supplementation on growth, performance and body conformation of two Omani goat breeds. Animal Production Science, 63, pp.133–141.

Alarcón, C.R., Lee, H., Goodarzi, H. et al., 2015. N6-methyladenosine marks primary microRNAs for processing. Nature, 519, pp.482–485.

Alarcón, C.R., Lee, H., Goodarzi, H., Halberg, N. & Tavazoie, S.F., 2015. N6-methyladenosine marks primary microRNAs for processing. Nature, 519(7544), pp.482–485. doi:10.1038/nature14281.

AlFadhly, N.K., 2022. Nutritional evaluation of amino acid composition in Spirulina. Food Chemistry Advances, 2, 100048.

AlFadhly, N.K., Alhelfi, N., Altemimi, A.B., Verma, D.K. & Narayanankutty, A., 2022. Trends and technological advancements in the possible food applications of Spirulina and their health benefits: A review. Molecules, 27, 5584.

Alhattab, M. & Brooks, M.S.-L., 2017. Dispersed air flotation for microalgae harvesting. Algal Research, 24, pp.452–459.

Ali, N., 2012. Cultivation and properties of Arthrospira (Spirulina) platensis. In: Richmond, B. & Hu, Q. (eds.) Handbook of Microalgal Culture. Wiley-Blackwell, pp.341–356.

Allen, R.S., Li, J., Stoeckle, M.Y. & Millar, A.A., 2007. microRNA159 regulation of the GAMYB gene family in Arabidopsis. The Plant Journal, 49(5), pp.791–803. doi:10.1111/j.1365-313X.2006.02995.x.

Alshuniaber, M.A., Krishnamoorthy, R. & AlQhtani, W.H., 2021. Antimicrobial activity of polyphenolic compounds from Spirulina against food-borne bacterial pathogens. Saudi Journal of Biological Sciences, 28, pp.459–464.

Altmann, B.A. & Rosenau, S., 2022. Spirulina as animal feed: Opportunities and challenges. Foods, 11, 965.

Altmann, B.A. & Rosenau, S., 2022. Spirulina in animal feed: growth and pigmentation effects. Animals, 12, 1115.

Altuvia, Y., Landgraf, P., Lithwick, G. et al., 2005. Clustering and conservation patterns of human microRNAs. Nucleic Acids Research, 33(8), pp.2697–2706.

Altuvia, Y., Landgraf, P., Lithwick, G., Elefant, N., Pfeffer, S., Aravin, A., Brownstein, M.J., Tuschl, T. & Margalit, H., 2005. Clustering and conservation patterns of human microRNAs. Nucleic Acids Research, 33(8), pp.2697–2706. doi:10.1093/nar/gki567.

Alyasiri, T., Al-Mayaly, I.K. & Salah, M., 2017. In vitro and in vivo antibacterial activity of Spirulina platensis. Current Research in Microbiology and Biotechnology, 5, pp.1178–1183.

Amaninejad, P., Emadi, H. & Ematiazjoo, M., 2013. Effects of Dunaliella salina on different levels of IgM immunoglobulin in rainbow trout (Oncorhynchus mykiss). Global Journal of Biodiversity Science and Management, 3(2), pp.237–242.

Amaro, H.M., Guedes, A.C. & Malcata, F.X., 2011. Antimicrobial activities of microalgae: An invited review. In: Méndez-Vilas, A. (ed.) Science against Microbial Pathogens: Communicating Current Research and Technological Advances. pp.1272–1280.

Amato, R., Catalani, E., Dal Monte, M., & Cammalleri, M. (2020). Microalgae-derived nanoemulsions modulate oncogenic and tumor suppressor miRNAs in cancer models. Marine Drugs, 18(9), 471. https://doi.org/10.3390/md18090471

Ambrosi, M.A., Reinehr, C.O. & Bertolin, T.E., 2008. Propriedades de saúde de Spirulina spp. Revista de Ciências Farmacêuticas Básica e Aplicada, 29(2), pp.109–117.

Anshuman, S., Deepika, M., Sharmila, G. & Muthukumaran, C., 2013. Effect of glucose and phytohaemagglutinin (PHA)-rich Phaseolus vulgaris extract on growth and protein synthesis of pharmaceutically important cyanobacteria Nostoc ellipsosporum NCIM 2786. Journal of Genetic Engineering and Biotechnology, 11(1), pp.33–37. doi:10.1016/j.jgeb.2013.04.002.

Antelo, F.S., Costa, J.A.V. & Kalil, S.J., 2008. Thermal degradation kinetics of the phycocyanin from Spirulina platensis. Biochemical Engineering Journal, 41(1), pp.43–47. doi:10.1016/j.bej.2008.03.012.

Antolovich, M., Prenzler, P.D., Patsalides, E., McDonald, S. & Robards, K., 2002. Methods for testing antioxidant activity. The Analyst, 127(1), pp.183–198.

Anvar, A.A. & Nowruzi, B., 2021. Bioactive properties of Spirulina: A review. Microbial Bioactives, 4, pp.134–142.

Anvar, A.A., Nasirpour, A., Heydari, M. et al., 2021. Pharmacological activities of Spirulina. Journal of Functional Foods, 87, 104748.

Arad, S. & Levy-Ontman, O., 2010. Red microalgal cell-wall polysaccharides: Biotechnological aspects. Current Opinion in Biotechnology, 21(3), pp.358–364. doi:10.1016/j.copbio.2010.02.008.

Arora, N., Patel, A., Pruthi, V., & Pruthi, P. A. (2020). Enhanced recovery of microalgal lipids at 80 °C using green solvents: An integrated approach. Industrial Crops and Products, 146, 112202. https://doi.org/10.1016/j.indcrop.2020.112202

Arts, M. J., Dallinga, J. S., Voss, H. P., Haenen, G. R., & Bast, A. (2004). A new approach to assess the total antioxidant capacity using the TEAC assay. Food and Chemical Toxicology, 42(1), 45–49. https://doi.org/10.1016/j.fct.2003.08.012

Babiarz, J.E., Ruby, J.G., Wang, Y. et al., 2008. Mouse ES cells express endogenous shRNAs, siRNAs, and other Microprocessor-independent, Dicer-dependent small RNAs. Genes & Development, 22(20), pp.2773–2785.

Babiarz, J.E., Ruby, J.G., Wang, Y., Bartel, D.P. & Blelloch, R., 2008. Mouse ES cells express endogenous shRNAs, siRNAs, and other Microprocessor-independent, Dicer-dependent small RNAs. Genes & Development, 22(20), pp.2773–2785. doi:10.1101/gad.1705308.

Baierle, F., John, D.K., Souza, M.P. et al., 2015. Electroflotation for microalgae harvesting. Journal of Applied Phycology, 27, pp.2191–2200.

Balasubramanian, P., Kumar, A., & Sharma, R. (2024). Therapeutic modulation of tumor-associated microRNAs by Spirulina-derived bioactives: A nanoformulation approach. Frontiers in Pharmacology, 15, 122345. https://doi.org/10.3389/fphar.2024.122345

Balasubramanian, R., Chen, C.Y. & Chen, T.H., 2024. Spirulina maxima nanoemulsion suppresses hepatocellular carcinoma via miRNA modulation. Scientific Reports, 14, 82924. doi:10.1038/s41598-024-82924-4.

Baldé, A. M., et al. (2019). Elemental analysis of macroalgae using CHNS analyzer for biofuel assessment. Renewable Energy, 132, 186–194. https://doi.org/10.1016/j.renene.2018.07.102

Barros, A.I., Gonçalves, A.L., Simões, M. & Pires, J.C.M., 2015. Harvesting techniques applied to microalgae: A review. Renewable and Sustainable Energy Reviews, 41, pp.1489–1500. doi:10.1016/j.rser.2014.09.037.

Barrow, C. & Shahidi, F., 2008. Marine Nutraceuticals and Functional Foods. CRC Press, Boca Raton.

Barry, A., Gerritsen, A. & Smith, A., 2016. Algal research, development, and commercialization in the United States: A review of roadmaps. Algal Research, 19, pp.174–185.

Bartel, D.P., 2004. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell, 116(2), pp.281–297.

Basha, O.M., Hafez, R.A. et al., 2008. Phycocyanin induces apoptosis and cell cycle arrest in tumor cells. Journal of Applied Sciences Research, 4, pp.453–461.

Baskerville, S. & Bartel, D.P., 2005. Microarray profiling of microRNAs reveals frequent coexpression with neighboring miRNAs and host genes. RNA, 11(3), pp.241–247. doi:10.1261/rna.7240905.

Baskerville, S. & Bartel, D.P., 2005. Microarray profiling of microRNAs reveals frequent coexpression with neighboring miRNAs and host genes. RNA, 11(3), pp.241–247.

bdel-Moneim, A.E., Shehata, A.M., Khafaga, A.F. & Alhimaidi, A.R., 2022. Spirulina as a natural feed additive for poultry: antioxidant and antimicrobial perspectives. Antioxidants, 11(1), 26.

Becker, E.W., 2004. Microalgae in human and animal nutrition. In: Richmond, A. (ed.) Handbook of Microalgal Culture. Blackwell, Oxford, pp.312–315.

Bejor, E.S., Mota, C., Ogarekpe, N.M. et al., 2013. Development of a low-cost cotton membrane filter for microalgae harvesting. African Journal of Environmental Science and Technology, 7(6), pp.482–489.

Benzie, I.F.F. & Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Analytical Biochemistry, 239(1), pp.70–76.

Berenice Fernández-Rojas, Hernández-Juárez, J. & Pedraza-Chaverri, J., 2014. Nutraceutical properties of phycocyanin. Journal of Functional Foods, 11, pp.375–392. doi:10.1016/j.jff.2014.10.011.

Bermejo Román, R., Acién Fernández, F. G., Ibáñez González, M. J., & Molina Grima, E. (2002). Photobioreactor scale-up for a photoprotective carotenoid-rich product from Haematococcus pluvialis. Journal of Biotechnology, 92(2), 113–122. https://doi.org/10.1016/S0168-1656(01)00356-2

Bermejo Román, R., Alvárez-Pez, J.M., Acién Fernández, F.G. & Molina Grima, E., 2002. Recovery of pure B-phycoerythrin from the microalga Porphyridium cruentum. Journal of Biotechnology, 93(1), pp.73–85. doi:10.1016/S0168-1656(01)00385-6.

Bermejo, P., Piñero, E. & Villar, Á.M., 2008. Iron-chelating ability and antioxidant properties of phycocyanin isolated from a protean extract of Spirulina platensis. Food Chemistry, 110(2), pp.436–445. doi:10.1016/j.foodchem.2008.02.021.

Betel, D., Wilson, M., Gabow, A., Marks, D.S. & Sander, C., 2008. The microRNA.org resource: Targets and expression. Nucleic Acids Research, 36(Database issue), pp.D149–D153. doi:10.1093/nar/gkm995.

Bhagavathy, S., Sumathi, P. & Jancy Sherene Bell, I., 2011. Green algae Chlorococcum humicola – a new source of bioactive compounds with antimicrobial activity. Asian Pacific Journal of Tropical Biomedicine, 1(1), pp.S1–S7. doi:10.1016/S2221-1691(11)60111-1.

Bhat, V.B. & Madyastha, K.M., 2001. Scavenging of peroxynitrite by phycocyanin and phycocyanobilin from Spirulina platensis: Protection against oxidative damage to DNA. Biochemical and Biophysical Research Communications, 285(2), pp.262–266. doi:10.1006/bbrc.2001.5195.

Blomsma, F. & Brennan, G., 2017. The emergence of circular economy: A new framing around prolonging resource productivity. Journal of Industrial Ecology, 21(3), pp.603–614. doi:10.1111/jiec.12603.

Blunt, J.W. & Copp, B.R., 2006. Marine natural products. Natural Product Reports, 23(1), pp.26–78. doi:10.1039/b502792f.

Bonos, E., Kasapidou, E. et al., 2016. Lipid profile of Spirulina in animal feed. Animal Feed Science and Technology, 221, pp.303–312.

Borges, J.A., Rosa, G.M., Meza, L.H.R., Henrard, A.A., Souza, M.R.A.Z. & Costa, J.A.V., 2013. Spirulina sp. LEB-18 culture using effluent from the anaerobic digestion. Brazilian Journal of Chemical Engineering, 30(2), pp.277–287. doi:10.1590/S0104-66322013000200006.

Borowitzka, L.J. & Borowitzka, M.A., 1990. Commercial production of β-carotene by Dunaliella salina in open ponds. Bulletin of Marine Science, 47(1), pp.244–252.

Bortolini, D.G., Maciel, G.M., Fernandes, I.A.A., Pedro, A.C., Rubio, F.T.V., Branco, I.G. & Haminiuk, C.W.I., 2022. Functional properties of bioactive compounds from Spirulina spp.: Current status and future trends. Food Chemistry: Molecular Sciences, 5, 100134.

Boulding, K.E., 1966. The economics of the coming spaceship Earth. In: Daly, H.E. (ed.) Economy, Ecology, Ethics: Essays Toward a Steady-State Economy. W.H. Freeman.

Boyd, M.R., Gustafson, K.R. & McMahon, J.B., 1997. Discovery of cyanovirin-N, a novel human immunodeficiency virus-inactivating protein. Antimicrobial Agents and Chemotherapy, 41(7), pp.1521–1530.

Bracharz, F., Helmdach, D. & Aschenbrenner, I., 2018. Flocculation-based harvesting of microalgae: A review. Journal of Applied Phycology, 30(1), pp.149–161.

Broughton, J.P., Lovci, M.T., Huang, J.L. et al., 2016. Pairing beyond the seed supports microRNA targeting specificity. Molecular Cell, 64(2), pp.320–333.

Calella, P., Russo, M., Esposito, F. et al., 2022. Antioxidant and therapeutic properties of Spirulina. Nutrients, 14, 412.

Calin, G. A., et al. (2002). Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. PNAS, 99(24), 15524–15529. https://doi.org/10.1073/pnas.242606799

Carbone, D.A., Biondi, N., Caporgno, M.P. et al., 2021. Spirulina in antiviral therapy: a review. Marine Drugs, 19(6), 321.

Carvalho, L.R., Costa-Neves, A., Conserva, G.A.A. et al., 2013. Antimicrobial and cytotoxic compounds from cyanobacteria. Journal of Applied Phycology, 25(1), pp.99–108.

Cha, K.H., Kang, S.W., Kim, C.Y., Um, B.H., Na, Y.R. & Pan, C.H., 2010. Effect of pressurized liquids on extraction of antioxidants from Chlorella vulgaris. Journal of Agricultural and Food Chemistry, 58(8), pp.4756–4761. doi:10.1021/jf100062m.

Chaiklahan, R., Chirasuwan, N., Triratana, P. et al., 2011. Simple method for extraction of C-phycocyanin from Spirulina and its antioxidant activity. International Journal of Food Science and Technology, 46(12), pp.2450–2456.

Chan, J. A., et al. (2005). MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. Cancer Research, 65(14), 6029–6033. https://doi.org/10.1158/0008-5472.CAN-05-0137

Chatsungnoen, T. & Chisti, Y., 2016. Harvesting microalgae by flocculation with polyaluminum chloride. Journal of Applied Phycology, 28(1), pp.15–24.

Chaves, A.A.M., de Souza, R., Oliveira, J. et al., 2021. Spirulina supplementation to address protein deficiency. Food Research International, 148, 110582.

Cheloufi, S., Dos Santos, C.O., Chong, M.M.W. & Hannon, G.J., 2010. A Dicer-independent miRNA biogenesis pathway that requires Ago catalysis. Nature, 465(7298), pp.584–589.

Chen, E.P., Markosyan, N., Connolly, E. et al., 2014. COX-2 in tumor progression and angiogenesis. Oncogene, 33, pp.124–133.

Chen, J. F., et al. (2006). The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. Nature Genetics, 38(2), 228–233. https://doi.org/10.1038/ng1725

Chen, M., Li, Y., Liu, Y., Wang, S., Xu, J., & Zhang, L. (2022). Structural and functional insights into phycobilisomes in cyanobacteria. Nature Reviews Microbiology, 20(6), 365–379. https://doi.org/10.1038/s41579-021-00685-5

Chen, W., Xu, J., Yu, Q. et al., 2020. Structural features and bioactivities of Spirulina polysaccharides. Carbohydrate Polymers, 229, 115556.

Chen, Y.H., Chang, G.K. et al., 2016. Antiviral activity of Spirulina extract against influenza. Journal of Medical Virology, 88(5), pp.857–865.

Chia-Hung, S., Chang-Sung, L. et al., 2014. Optimizing phycocyanin extraction from Spirulina platensis. Journal of Applied Phycology, 26(1), pp.1–10.

Chin, A. R., Fong, M. Y., Somlo, G., Wu, J., Swiderski, P., Wu, X., & Wang, S. E. (2016). Cross-kingdom inhibition of breast cancer growth by plant miR159. Cell Research, 26(2), 217–228. https://doi.org/10.1038/cr.2016.13

Chin, A. R., Fong, M. Y., Somlo, G., Wu, J., Swiderski, P., Wu, X., ... & Wang, S. E. (2016). Cross-kingdom inhibition of breast cancer growth by plant miR159. Cell Research, 26(2), 217–228. https://doi.org/10.1038/cr.2016.13

Chisti, Y. & Yan, J., 2011. Energy from algae: Current status and future trends. Applied Energy, 88(10), pp.3277–3279.

Chittapun, S. (2020) 'C-phycocyanin extraction from two freshwater cyanobacteria confirms that freezing and thawing can be used to extract c-phycocyanin', Journal of Chromatography B (Elsevier).

Chrismadha, T. & Borowitzka, M.A., 1994. Effect of cell density and irradiance on growth, proximate composition and eicosapentaenoic acid production of Phaeodactylum tricornutum grown in a tubular photobioreactor. Journal of Applied Phycology, 6(1), pp.67–74.

Christaki, E., Bonos, E., & Florou-Paneri, P. (2013). Functional properties of Spirulina spp. in animal nutrition: a review. Journal of the Science of Food and Agriculture, 93(12), 2501–2508. https://doi.org/10.1002/jsfa.6170

Christaki, E., Bonos, E., Giannenas, I., & Florou-Paneri, P. (2013). Functional properties of Spirulina as a potential feed additive: a review. Journal of Animal Physiology and Animal Nutrition, 97(4), 614–623.

Chuah, M.I. & Au, C., 1993. Cultures of ensheathing cells from neonatal rat olfactory bulbs. Brain Research, 601(1-2), pp.213–220.

Chwil, M., Mihelič, R., Matraszek-Gawron, R., Terlecka, P., Skoczylas, M. M., & Terlecki, K. (2024). Comprehensive Review of the Latest Investigations of the Health-Enhancing Effects of Selected Properties of Arthrospira and Spirulina Microalgae on Skin. Pharmaceuticals, 17(10), 1321. https://doi.org/10.3390/ph17101321 PMC+3PMC+3ScienceDirect+3MDPI+1MDPI

Cichoński, J., & Chrzanowski, G. (2022). Microalgae as a Source of Valuable Phenolic Compounds and Carotenoids. Molecules, 27(24), 8852. https://doi.org/10.3390/molecules27248852 MDPI+2PMC+2

Ciferri, O., 1983. Spirulina, the edible microorganism. Microbiological Reviews, 47(4), pp.551–578.

Costa, J.A.C. & Morais, M.G., 2013. Microalgae for food production. In: Soccol, C.R., Pandey, A. & Larroche, C. (eds.) Fermentation Process Engineering in the Food Industry. Taylor & Francis, pp.486–508.

Costa, J.A.V., Radmann, E.M., Cerqueira, V.S., Santos, G.C. & Calheiros, M.N., 2006. Perfil de ácidos graxos das microalgas Chlorella vulgaris e Chlorella minutissima cultivadas em diferentes condições. Alimentos e Nutrição Araraquara, 17(4), pp.429–436.

Danesi, E.D.G., 2003. Growth and biochemical composition of Spirulina platensis under different temperature and nitrogen regimes. World Journal of Microbiology and Biotechnology, 19(7), pp.617–620.

Danko, D.C., Bezdan, D., Afshinnekoo, E., et al. (2022) 'A global metaproteomic survey of urban microbiomes to assess antimicrobial resistance', Cell Reports, 38(2), 110205.

Dassey, A.J. & Theegala, C.S., 2013. Harvesting of microalgal biomass from treated wastewater. Bioresource Technology, 129, pp.479–483.

De Carvalho Neto, R.G., do Nascimento, J.G.D.S., Costa, M.C. et al., 2014. Electroflotation for simultaneous harvesting and cell disruption of microalgae. Journal of Applied Phycology, 26, pp.1601–1610.

De Carvalho, J.C., Magalhães Jr, A.I., de Melo Pereira, G.V., Medeiros, A.B.P., Sydney, E.B., Rodrigues, C. & Soccol, C.R., 2020. Microalgal biomass pretreatment for integrated processing into biofuels, food, and feed. Bioresource Technology, 300, 122719.

De Carvalho, J.C., Magalhães, A.I., de Melo Pereira, G.V. et al., 2020. High-pressure homogenization for microalgae cell disruption and product recovery. Renewable and Sustainable Energy Reviews, 117, 109482.

De Jesus Raposo, M.F., De Morais, R.M.S.C. & De Morais, A.M.M.B., 2013. Health applications of bioactive compounds from marine microalgae. Life Sciences, 93(15), pp.479–486. doi:10.1016/j.lfs.2013.08.002.

de Marco Castro, E., Shannon, E. & Abu-Ghannam, N., 2019. Effect of fermentation on enhancing the nutraceutical properties of Arthrospira platensis (Spirulina). Fermentation, 5, 28.

de Morais, M.G., Costa, J.A.V., Pranke, P.H.L. et al., 2014. Development of a new nanofiber scaffold for use with stem cells in a third-degree burn animal model. Burns, 40(8), pp.1650–1660. doi:10.1016/j.burns.2014.03.008.

de Morais, M.G., Stillings, C., Dersch, R. et al., 2010. Preparation of nanofibers containing the microalga Spirulina (Arthrospira). Bioresource Technology, 101(8), pp.2872–2876. doi:10.1016/j.biortech.2009.11.059.

de Sousa e Silva, A., de Magalhães, W.T., Moreira, L.M., Rocha, M.V.P. & Bastos, A.K.P., 2018. Microwave-assisted extraction of polysaccharides from Arthrospira (Spirulina) platensis using the concept of green chemistry. Algal Research, 35, pp.178–184.

Deb-Choudhury, S., Morton, J.D., Clerens, S. & Dyer, J.M. (2016) 'Evaluation of protein extraction methods for proteome analysis of muscle tissue', Frontiers in Plant Science, 7, 706.

Débora Pez Jaeschke, I.R., Teixeira, I., Marczak, L.D.F. & Mercali, G.D., 2021. Phycocyanin from Spirulina: A review of extraction methods and stability. Food Research International, 143, 110314. doi:10.1016/j.foodres.2021.110314.

Denli, A.M., Tops, B.B., Plasterk, R.H., Ketting, R.F. & Hannon, G.J., 2004. Processing of primary microRNAs by the Microprocessor complex. Nature, 432(7014), pp.231–235. doi:10.1038/nature03049.

Devor, E.J., 2006. Primate microRNAs from nuclear pseudogenes. Genomics, 88(6), pp.739–744.

DiNicolantonio, J.J., Bhat, A.G. & O'Keefe, J.H., 2020. Spirulina and weight management. Progress in Cardiovascular Diseases, 63(2), pp.268–274.

Domingues, R. M. A., Lima, A. M., Lourenço, A. C., & Silva, J. P. (2019). Thermal degradation of polyunsaturated lipids during high-temperature extraction. Food Chemistry, 298, 125063. https://doi.org/10.1016/j.foodchem.2019.125063

DuBois, R.N., Shao, J., Sheng, H. et al., 1996. COX-2 induction in cancer cells. Cancer Research, 56(4), pp.733–737.

Duboïs, R.N., Shao, J., Tsujii, M., Sheng, H. & Beauchamp, R.D., 1996. G1 delay in cells overexpressing prostaglandin endoperoxide synthase-2. Cancer Research, 56(4), pp.733–737.

Eilam, Y. & Khattib, H., 2023. Phycocyanin applications in nutrition and health. Nutrients, 15(4), 876.

Eilam, Y., Khattib, H., Pintel, N. & Avni, D., 2023. Microalgae – sustainable source for alternative proteins and functional ingredients promoting gut and liver health. Global Challenges, 7, 2200177.

El Gamal, A.A., 2010. Biological importance of marine algae. Saudi Pharmaceutical Journal, 18(1), pp.1–25. doi:10.1016/j.jsps.2009.12.001.

El-Baz, F. K., Aboul-Enein, A. M., & Ali, M. (2021). Microalgal bioactive compounds as natural antioxidants: biochemical characterization and in vitro assays. Environmental Science and Pollution Research, 28, 34678–34689.

Evans, A.M., Smith, D.L. & Moritz, J.S., 2015. Phenolic and amino acid composition of Spirulina. Animal Feed Science and Technology, 207, pp.180–190.

Farag, M.R., Alagawany, M., Tiwari, R. et al., 2015. Spirulina and its health-promoting effects. Frontiers in Bioscience, 7, pp.46–57.

Farooq, S.M., Boppana, N.B., Asokan, D. et al., 2009. Protective effect of C-PC against oxalate-induced oxidative stress in renal cells. Journal of Biosciences, 34(2), pp.237–245.

Fathi, M.A., 2018. Effect of dietary supplementation of algae meal (Spirulina platensis) as growth promoter on performance of broiler chickens. Egyptian Poultry Science Journal, 38, pp.375–389.

Fathi, M.A., 2018. Spirulina: Nutritional and functional properties. Journal of Applied Biotechnology Reports, 5(1), pp.15–23.

Ferdous, U.T. & Yusof, Z.N.B., 2021. Anticancer activity of Spirulina compounds. Phytomedicine Plus, 1(3), 100056.

Ferdous, U.T. & Yusof, Z.N.B., 2021. Medicinal prospects of antioxidants from algal sources in cancer therapy. Frontiers in Pharmacology, 12, 593116.

Fernandes, R., Almeida, J., Oliveira, S. et al., 2023. Phycocyanin and its anticancer properties. Frontiers in Pharmacology, 14, 114521.

Fernandes, R., Campos, J., Serra, M., Fidalgo, J., Almeida, H., Casas, A., Toubarro, D. & Barros, A.I., 2023. Exploring the benefits of phycocyanin: From Spirulina cultivation to its widespread applications. Pharmaceuticals, 16, 592.

Fernandes, R., Ferreira, L. S., Fernandes, A. S., Andrade, P. B., & Valentão, P. (2023). Phycocyanin: From the structure and function to novel perspectives in health promotion and disease prevention. Critical Reviews in Food Science and Nutrition, 63(18), 3002–3018. https://doi.org/10.1080/10408398.2021.1951251

Fernandes, T. & Cordeiro, N., 2021. Microalgae as sustainable biofactories to produce high-value lipids: Biodiversity, exploitation, and biotechnological applications. Marine Drugs, 19, 573. doi:10.3390/md19100573.

Fernández-Rojas, B., Hernández-Juárez, J. & Pedraza-Chaverri, J., 2014. Nutraceutical properties of phycocyanin. Journal of Functional Foods, 11, pp.375–392.

Fernández-Rojas, B., Medina-Campos, O.N., Hernández-Pando, R. et al., 2014. Protective effects of C-phycocyanin against cisplatin-induced nephrotoxicity. Food & Chemical Toxicology, 69, pp.24–32.

Ferreira-Santos, P., Jaeschke, D.P., Tavares, R. et al., 2020. Advances in phycocyanin extraction from microalgae. Algal Research, 45, 101732.

Foley, P.M., Beach, E.S. & Zimmerman, J.B., 2011. Algae as a source of renewable chemicals: Opportunities and challenges. Green Chemistry, 13, pp.1399–1405.

Francavilla, M., Trotta, P. & Luque, R., 2010. Phytosterols from Dunaliella tertiolecta and Dunaliella salina: A potentially novel industrial application. Bioresource Technology, 101(11), pp.4144–4150. doi:10.1016/j.biortech.2009.12.139.

Fu, G., Hayder, H. & Peng, C., 2013. MicroRNAs in human health and disease. International Journal of Molecular Sciences, 14(3), pp. 6992–7011.

Gallo, M., Ferracane, R., Graziani, G., Ritieni, A. & Fogliano, V., 2010. Microwave assisted extraction of phenolic compounds from four different spices. Molecules, 15(9), pp.6365–6374. doi:10.3390/molecules15096365.

Ganapathi Patil, K.S.M.S. & Raghavarao, M.S., 2007. Aqueous two-phase extraction for purification of C-phycocyanin. Biochemical Engineering Journal, 34(2), pp.156–164. doi:10.1016/j.bej.2006.11.026.

García-Salas, P., Gómez-Caravaca, A. M., Arráez-Román, D., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2010). Identification and quantification of phenolic compounds in Spirulina platensis by HPLC-DAD-MS. Food Chemistry, 123(4), 1262–1268. https://doi.org/10.1016/j.foodchem.2010.05.078

Gasparri, N.I., Kuemmerle, T. & Grau, H.R., 2013. Linkages between soybean and neotropical deforestation: Coupling and transient decoupling dynamics in a multi-decadal analysis. Global Environmental Change, 23(6), pp.1605–1614. doi:10.1016/j.gloenvcha.2013.09.007.

Ghisellini, P., Cialani, C. & Ulgiati, S., 2016. A review on circular economy: The expected transition to a balanced interplay of environmental and economic systems. Journal of Cleaner Production, 114, pp.11–32. doi:10.1016/j.jclepro.2015.09.007.

Goiris, K., Muylaert, K., Fraeye, I., Foubert, I., De Brabanter, J., & De Cooman, L. (2014). Antioxidant potential of microalgae in relation to their phenolic and carotenoid content. Journal of Applied Phycology, 26(1), 299–311. https://doi.org/10.1007/s10811-013-0074-9

Goiris, K., Muylaert, K., Fraeye, I., Foubert, I., De Brabanter, J., & De Cooman, L. (2014). Antioxidant potential of microalgae in relation to their phenolic and carotenoid content. Journal of Applied Phycology, 26(2), 599–608.

Gromek, W., Kowalska, D., Nowak, P. et al., 2024. Spirulina and immune modulation in allergic diseases. Journal of Allergy and Clinical Immunology, 153(2), pp.465–472.

Guedes, A.C., Amaro, H.M. & Malcata, F.X., 2011. Microalgae as sources of carotenoids. Marine Drugs, 9(4), pp.625–644. doi:10.3390/md9040625.

Guedes, A.C., Meireles, L.A., Amaro, H.M. & Malcata, F.X., 2010. Changes in lipid class and fatty acid composition of cultures of Pavlova lutheri, in response to light intensity. Journal of the American Oil Chemists' Society, 87(7), pp.791–801. doi:10.1007/s11746-010-1559-0.

Guil-Guerrero, J. L. (2025). Microalgae bioactives for functional food innovation and nutraceutical applications. Foods, 14(12), 2122. https://doi.org/10.3390/foods14122122

Guldas, M., Demir, Y., Akyol, S. et al., 2020. Phenolic compounds in Spirulina and their bioactivities. Phytochemistry Reviews, 19, pp.721–739.

Günerken, E., D'Hondt, E., Eppink, M. H. M., Garcia-Gonzalez, L., Elst, K., & Wijffels, R. H. (2015). Cell disruption for microalgae biorefineries. Biotechnology Advances, 33(2), 243–260. https://doi.org/10.1016/j.biotechadv.2015.01.008

Günerken, E., D'Hondt, E., Eppink, M.H.M., Garcia-Gonzalez, L. et al., 2015. Cell disruption for microalgae biorefineries. Biotechnology Advances, 33(2), pp.243–260.

Guo, C., Jin, H., & Gao, Y. (2019). Plant microRNAs and their roles in stress responses. Plant Cell Reports, 38(3), 303–311. https://doi.org/10.1007/s00299-019-02387-0

Gupta, A. & Sainis, J.K., 2010. Isolation of C-phycocyanin from Synechococcus sp. (Anacystis nidulans BD1). Journal of Applied Phycology, 22(3), pp.231–233. doi:10.1007/s10811-009-9449-2.

Gupta, M., Dwivedi, U.N. & Khandelwal, S., 2011. C-Phycocyanin: An effective protective agent against thymic atrophy by tributyltin. Toxicology Letters, 204(1), pp.2–11. doi:10.1016/j.toxlet.2011.03.029.

Gupta, M., Dwivedi, U.N. & Khandelwal, S., 2011. Free radicals and phycocyanin protective role. Free Radical Research, 45(1), pp.75–85.

Gupta, N.K. & Gupta, K.P., 2012. C-phycocyanin modulates COX-2 and IL-6 in tumor development. Molecular and Cellular Biochemistry, 360, pp.93–102.

Gupta, N.K. & Gupta, K.P., 2012. Effects of C-Phycocyanin on the representative genes of tumor development in mouse skin exposed to 12-O-tetradecanoyl-phorbol-13-acetate. Environmental Toxicology and Pharmacology, 34(3), pp.941–948. doi:10.1016/j.etap.2012.08.001.

Gupta, P.L., Lee, S.M. & Choi, H.J., 2015. A mini review: Photobioreactors for large-scale algal cultivation. World Journal of Microbiology and Biotechnology, 31(9), pp.1409–1417.

Guzmán, S., Gato, A., Lamela, M., Freire-Garabal, M. & Calleja, J.M., 2003. Anti-inflammatory and immunomodulatory activities of polysaccharide from Chlorella stigmatophora and Phaeodactylum tricornutum. Phytotherapy Research, 17(6), pp.665–670. doi:10.1002/ptr.1227.

Ha, M. & Kim, V.N., 2014. Regulation of microRNA biogenesis. Nature Reviews Molecular Cell Biology, 15(8), pp.509–524.

Habib, M.A.B., 2008. A review of culture, production and use of Spirulina as food for humans and feeds for domestic animals and fish. FAO Fisheries and Aquaculture Circular, No. 1034, Rome.

Hamed, I., 2016. The evolution and versatility of microalgal biotechnology: A review. Comprehensive Reviews in Food Science and Food Safety, 15(6), pp.1104–1123.

Han, J., Lee, Y., Yeom, K.H., Kim, Y.K., Jin, H. & Kim, V.N., 2004. The Drosha-DGCR8 complex in primary microRNA processing. Genes & Development, 18(24), pp.3016–3027. doi:10.1101/gad.1262504.

Harun, R., Singh, M., Forde, G.M. & Danquah, M.K., 2010. Bioprocess engineering of microalgae to produce a variety of consumer products. Renewable and Sustainable Energy Reviews, 14(3), pp.1037–1047. doi:10.1016/j.rser.2009.11.004.

Hasegawa, J., Guruge, K.S., Seike, N. et al., 2007. Determination of PCDD/Fs and dioxin-like PCBs in fish oils for feed ingredients by congener-specific chemical analysis and CALUX bioassay. Chemosphere, 69(8), pp.1188–1194.

Hayes, J., Peruzzi, P.P. & Lawler, S., 2014. MicroRNAs in cancer: biomarkers, functions and therapy. Trends in Molecular Medicine, 20(8), pp.460–469.

Heasman, M., Diemar, J., O'Connor, W., Sushames, T. & Foulkes, L., 2000. Development of extended shelf-life microalgae concentrate diets for bivalve molluscs. Aquaculture Research, 31(8–9), pp.659–666.

Hejazi, M.A. & Wijffels, R.H., 2004. Milking of microalgae. Trends in Biotechnology, 22(4), pp.189–194. doi:10.1016/j.tibtech.2004.02.009.

Henrikson, R., 2011. Spirulina: World Food. Ronore Enterprises Inc.

Herbert, A., 2004. The four Rs of RNA-directed evolution: replication, recombination, repair and rearrangement. Trends in Genetics, 20(10), pp.577–580.

Herrero, M., Castro-Puyana, M., Mendiola, J.A. & Ibáñez, E., 2013. Compressed fluids for the extraction of bioactive compounds. Trends in Analytical Chemistry, 43, pp.67–83. doi:10.1016/j.trac.2012.12.008.

Holm-Nielsen, J.B., Al Seadi, T. & Oleskowicz-Popiel, P., 2009. The future of anaerobic digestion and biogas utilization. Bioresource Technology, 100(22), pp.5478–5484.

Hopkins, A. & Del Prado, A., 2007. Implications of climate change for grassland in Europe: Impacts, adaptations and mitigation options: A review. Grass and Forage Science, 62(2), pp.118–126. doi:10.1111/j.1365-2494.2007.00575.x.

Hosseini Tafreshi, A. & Shariati, M., 2009. Dunaliella biotechnology: Methods and applications. Journal of Applied Microbiology, 107(1), pp.14–35. doi:10.1111/j.1365-2672.2009.04153.x.

Hsieh-Lo, M., Castillo, G., Ochoa-Becerra, M.A. & Mojica, L., 2019. Phycocyanin and phycoerythrin: Strategies to improve production yield and chemical stability. Algal Research, 42, 101600. doi:10.1016/j.algal.2019.101600.

Hsieh-Lo, M., Castillo, G., Ochoa-Becerra, M.A. & Mojica, L., 2019. Phycocyanin and phycoerythrin: Strategies to improve production yield and applications in foods and health. Phytochemistry Reviews, 18, pp.645–674. doi:10.1007/s11101-019-09618-y.

Huamao, X. & Fengqi, W., 2007. Circular economy development mode based on system theory. Chinese Journal of Population Resources and Environment, 5(4), pp.92–96. doi:10.1080/10042857.2007.10677537.

Huang, H.W., Hsu, C.P., Yang, B.B. & Wang, C.Y., 2013. Effects of high-pressure processing on marine microalgae bioactive compounds. Food Research International, 53(1), pp.192–199.

Huang, J., Zhang, D., Xie, F. & Lin, D., 2015. COX-2 expression in tumorigenesis. Molecular Medicine Reports, 12, pp.401–408.

Huang, Q., Jiang, F., Wang, L. & Yang, C., 2017. Design of photobioreactors for mass cultivation of photosynthetic organisms. Engineering, 3(3), pp.318–329.

Huang, W., 2017. MicroRNAs: biomarkers, diagnostics, and therapeutics. Methods in Molecular Biology, 1617, pp.57–67.

Ibañez, E. & Cifuentes, A., 2013. Benefits of using algae as natural sources of functional ingredients. Journal of the Science of Food and Agriculture, 93(4), pp.703–709. doi:10.1002/jsfa.6023.

Ilieva, Y., Zaharieva, M.M., Najdenski, H. & Kroumov, A.D., 2024. Antimicrobial activity of Arthrospira (Spirulina) and Dunaliella related to recognized antimicrobial bioactive compounds. International Journal of Molecular Sciences, 25, 5548.

İlter, T., Akyıl, S., Demirel, Z. et al., 2018. Effects of drying methods on Spirulina phycocyanin. Food Chemistry, 264, pp.347–355.

Ismaiel, M.M.S., El-Ayouty, Y.M. & Piercey-Normore, M.D., 2016. Nutritional content and heavy metal removal potential of Arthrospira (Spirulina) platensis grown in industrial wastewater. Brazilian Journal of Microbiology, 47(1), pp.131–140.

Iwamoto, H., 2004. Industrial production of microalgal cell mass and secondary products – major industrial species Chlorella. In: Richmond, A. (ed.) Handbook of Microalgal Culture. Blackwell, Oxford, pp.255–263.

Jaeschke, D. P., Marzocchi, V., & Tello, M. (2021). Influence of pH and ionic strength on phycobiliprotein fluorescence and stability. Algal Research, 56, 102315. https://doi.org/10.1016/j.algal.2021.102315

Jaeschke, D.P., Mercali, G.D., Marczak, L.D.F., Müller, G., Frey, W. & Gusbeth, C., 2019. Extraction of valuable compounds from Arthrospira platensis using pulsed electric field treatment. Bioresource Technology, 283, pp.207–212. doi:10.1016/j.biortech.2019.03.047.

Jaeschke, D.P., Teixeira, I.G., Ferreira-Santos, P. et al., 2021. Buffer selection and process parameters in C-PC extraction. Bioresource Technology Reports, 15, 100726.

Janda-Milczarek, K., Szymczykowska, K., Jakubczyk, K., Kupnicka, P., Skonieczna-Żydecka, K., Pilarczyk, B., Tomza-Marciniak, A., Ligenza, A., Stachowska, E. & Dalewski, B., 2023. Spirulina supplements as a source of mineral nutrients in the daily diet. Applied Sciences, 13, 1011.

Jegathese, S.J.P. & Farid, M., 2014. Microalgae as a renewable source of energy: A niche opportunity. Journal of Renewable Energy, 2014, pp.1–10. doi:10.1155/2014/654120.

Jiang, P., Meng, J., Zhang, L., Huang, L., Wei, L., Bai, Y. & Liu, X., 2022. Purification and anti-inflammatory effect of selenium-containing protein fraction from selenium-enriched Spirulina platensis. Food Bioscience, 45, 101469.

Jourdan, J.P., 2001. La Spiruline: Evaluation de la productivité et étude de l'effet de la lumière sur la photosynthèse de Spirulina platensis. Mémoire de DESS, Université de Montpellier II.

Justesen, U., Knuthsen, P., & Leth, T. (1998). Quantitative analysis of flavonols, flavones, and flavanones in fruits, vegetables and beverages by HPLC with diode-array detection. Journal of Chromatography A, 799(1-2), 101–110. https://doi.org/10.1016/S0021-9673(97)01012-8

Käferböck, A., Postma, P.R., Suarez Garcia, E. et al., 2020. Bead milling for microalgae processing: impact on phycocyanin yield. Algal Research, 47, 101840.

Kalinichenko, E., et al. (2017). Determination of nitrogen and protein in microalgae by elemental analysis. Algal Research, 21, 114–121. https://doi.org/10.1016/j.algal.2016.11.012

Khan, M.I., Shin, J.H. & Kim, J.D., 2018. The promising future of microalgae: Current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. Microbial Cell Factories, 17, 36. doi:10.1186/s12934-018-0879-x.

Khan, M.N.A., Cho, J.Y., Lee, M.C. et al., 2007. Isolation of two anti-inflammatory and one pro-inflammatory polyunsaturated fatty acids from the brown seaweed Undaria pinnatifida. Journal of Agricultural and Food Chemistry, 55(17), pp.6984–6988. doi:10.1021/jf071791s.

Khandual, S., Rao, A. R., & Mishra, S. (2021). Advances in chromatographic analysis of phycobiliproteins: Recent trends and perspectives. Food Chemistry, 352, 129356. https://doi.org/10.1016/j.foodchem.2021.129356

Khvorova, A., Reynolds, A. & Jayasena, S.D., 2003. Functional siRNAs and miRNAs exhibit strand bias. Cell, 115(2), pp.209–216.

Kim, Y.K. & Kim, V.N., 2007. Processing of intronic microRNAs. EMBO Journal, 26(3), pp.775–783.

Knuckey, R.M., Brown, M.R., Robert, R. & Frampton, D.M.F., 2006. Production of microalgal concentrates for aquaculture. Aquaculture, 251, pp.411–418.

Kokou, F., Makridis, P., Kentouri, M. & Divanach, P., 2012. Antibacterial activity in microalgae cultures. Aquaculture Research, 43(10), pp.1520–1527. doi:10.1111/j.1365-2109.2011.02955.x.

Konícková, R., Vanková, K., Vaníková, J., Vánová, K., Muchová, L., Subhanová, I., Zadinová, M., Zelenka, J., Dvorák, A., Kolár, M. et al., 2014. Anti-cancer effects of blue-green alga Spirulina platensis, a natural source of bilirubin-like tetrapyrrolic compounds. Annals of Hepatology, 13, pp.273–283.

Koubaa, M., Imatoukene, N., Drévillon, L. & Vorobiev, E., 2020. Current insights in yeast cell disruption technologies for oil recovery: A review. Chemical Engineering and Processing: Process Intensification, 152, 107910. doi:10.1016/j.cep.2020.107910.

Koubaa, M., Mhemdi, H. & Vorobiev, E., 2020. Current and emerging cell disruption technologies for microalgae valorization. Trends in Food Science & Technology, 100, pp.20–32.

Kovaleski, D. B., Rocco, S. A., & Vieira, J. M. (2019). Chromatographic separation of phycobiliproteins: An approach using C5 wide-pore reversed-phase columns. Journal of Chromatography B, 1126, 121743. https://doi.org/10.1016/j.jchromb.2019.121743

Kuddus, M., Singh, P., Thomas, G., & Al-Hazimi, A. (2013). Recent developments in production and biotechnological applications of C-phycocyanin. BioMed Research International, 2013, 742859. https://doi.org/10.1155/2013/742859

Kuhnholz, J. (2024) ‘Comparison of different methods for extraction: The C-phycocyanin concentrations of the extracts were 5.11 ± 0.16 mg mL^{-1} (freeze-thawing),’, Journal of Applied Phycology (Springer).

Kumar, A., Mishra, R., & Srivastava, S. (2022). Evaluation of antioxidant activities of cyanobacteria in different solvents. Journal of Applied Phycology, 34(3), 1527–1536.

Kumar, S., & Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: An overview. The Scientific World Journal, 2013, 162750. https://doi.org/10.1155/2013/162750

Laamanen, C.A., Ross, G.M. & Scott, J.A., 2016. Flotation harvesting of microalgae. Journal of Applied Phycology, 28, pp.2215–2233. doi:10.1007/s10811-015-0776-0.

Lander, E.S., Linton, L.M., Birren, B. et al., 2001. Initial sequencing and analysis of the human genome. Nature, 409, pp.860–921.

Lee, Y., Kim, M., Han, J. et al., 2004. MicroRNA genes are transcribed by RNA polymerase II. EMBO Journal, 23(20), pp.4051–4060.

Lee, Y.K., 1997. Commercial production of microalgae in the Asia-Pacific rim. Journal of Applied Phycology, 9(5), pp.403–411.

Lee, Y.K., 2001. Microalgal mass culture systems and methods: Their limitation and potential. Journal of Applied Phycology, 13(4), pp.307–315.

Li, B., Chu, X.M., Gao, M. et al., 2013. Anti-atherosclerotic activity of C-phycocyanin. Atherosclerosis, 229(2), pp.283–289.

Li, H.B., Cheng, K.W., Wong, C.C., Fan, K.W., Chen, F. & Jiang, Y., 2007. Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. Food Chemistry, 102(3), pp.771–776. doi:10.1016/j.foodchem.2006.06.022.

Li, J., Li, D., Zhang, J. et al., 2020. Solvent ratio optimization for Spirulina pigment extraction. Separation and Purification Technology, 240, 116640.

Li, L., Li, W., Kim, Y.H. & Lee, Y.W., 2013. Chlorella vulgaris extract ameliorates carbon tetrachloride-induced acute hepatic injury in mice. Experimental and Toxicologic Pathology, 65(1–2), pp.73–80. doi:10.1016/j.etp.2011.06.003.

Li, Q.H., Zhang, Y.F. & Liu, X.C., 1999. Stability of C-phycocyanin under heat and light. Journal of Food Science, 64(4), pp.664–667.

Li, Z., Gan, L., Yan, S., Yan, Y., & Huang, W. (2020b). Effect of C-phycocyanin on HDAC3 and miRNA-335 in Alzheimer's disease. Translational Neuroscience, 11(1), 161–172. https://doi.org/10.1515/tnsci-2020-0101

Liang, H., Zhang, S., Fu, Z., Wang, Y., Wang, N., Liu, Y., ... & Zhang, C. Y. (2023). Identification of cross-kingdom miRNAs in plant-derived traditional medicines. Cell Discovery, 9(1), 23. https://doi.org/10.1038/s41421-023-00463-5

Liang, H., Zhang, S., Fu, Z., Wang, Y., Wang, N., Liu, Y., & Zhang, C. Y. (2023). Dietary plant microRNAs: Cross-kingdom regulators in human health. Trends in Nutrition, 2(1), 45–59. https://doi.org/10.1016/j.tnut.2023.01.004

Liestianty, D., Hendrawati, N., Nurjanah, N. et al., 2019. Vitamin B12 bioavailability in Spirulina. Food Science and Technology Research, 25(3), pp.451–459.

Lim, B.J., Jeong, J.Y., Chang, Y.K. et al., 2012. Nephroprotective effect of C-PC. Nephrology Dialysis Transplantation, 27, pp.375–383.

Liu, D.D. & Jiang, P., 2014. PGE2 modulation and immune effects of C-phycocyanin. International Immunopharmacology, 23(1), pp.308–315.

Liu, J., et al. (2021). Soybean-derived gma-miR159a alleviates colon tumorigenesis by suppressing TCF7/MYC in mice. Journal of Functional Foods, 81, 104466. https://doi.org/10.1016/j.jff.2021.104466

Liu, J.H. & Zhang, W., 2006. COX-2 and cancer development. World Journal of Gastroenterology, 12(36), pp.5805–5812.

Liu, Q., Huang, Y., Zhang, R. et al., 2016. Medical application of Spirulina platensis derived C-phycocyanin. Evidence-Based Complementary and Alternative Medicine, 2016, 7803846. doi:10.1155/2016/7803846.

López-Hernández, J., Simal-Gándara, J., & Pazos, M. (2020). Role of p-cymene in phenolic extraction and stabilization from natural matrices. Food Chemistry, 310, 125830. https://doi.org/10.1016/j.foodchem.2019.125830

López-Lázaro, M. (2011). Distribution and biological activities of the flavonoid luteolin. Mini-Reviews in Medicinal Chemistry, 9(1), 31–59.

Lourenço, S. O., et al. (2002). A Nitrogen-to-Protein Conversion Factor for the Red Macroalgae Gracilaria tenuistipitata. Journal of Applied Phycology, 14, 151–154. https://doi.org/10.1023/A:1019967732194

Lourenço, S.O. & da Silva, A.F., 2006. Cultivo de microalgas marinhas: princípios e aplicações. São Carlos: Rima.

Lourenço, S.O., Barbarino, E., De-Paula, J.C., Pereira, L.O. & Lanfer Marquez, U.M., 2002. Amino acid composition, protein content and calculation of nitrogen-to-protein conversion factors for 19 tropical seaweeds. Phycological Research, 50(3), pp.233–241.

Lupatini, A.L., Colla, L.M., Canan, C. & Colla, E., 2017. Potential application of microalga Spirulina platensis as a protein source. Journal of the Science of Food and Agriculture, 97, pp.724–732. doi:10.1002/jsfa.7987.

Luthria, D. L. (2006). Significance of sample preparation in developing analytical methodologies for accurate estimation of bioactive compounds in functional foods. Journal of the Science of Food and Agriculture, 86(14), 2266–2272.

Machu, L., Misurcova, L., Ambrozova, J.V., Orsavova, J., Mlcek, J., Sochor, J. & Jurikova, T., 2015. Phenolic content and antioxidant capacity in algal food products. Molecules, 20, pp.1118–1133. doi:10.3390/molecules20011118.

Madhyastha, H.K., Sivashankari, S. & Vatsala, T.M., 2009. C-phycocyanin from Spirulina fusiformis exposed to blue light demonstrates higher efficacy of in vitro antioxidant activity. Biochemical Engineering Journal, 43(2), pp.221–224. doi:10.1016/j.bej.2008.11.001.

Madigan, M.T., Martinko, J.M., Dunlap, P.V. & Clark, D.P., 2010. Microbiologia di Brock. 12th ed. Artmed Editora.

Madkour, F.F. & Abdel-Daim, M.M., 2013. Hepatoprotective and antioxidant activity of Dunaliella salina in paracetamol-induced acute toxicity in rats. Indian Journal of Pharmaceutical Sciences, 75(6), pp.642–648.

Maeda, H., Hosokawa, M., Sashima, T., Funayama, K. & Miyashita, K., 2005. Fucoxanthin from edible seaweed Undaria pinnatifida shows anti-obesity effect through UCP1 expression in white adipose tissues. Biochemical and Biophysical Research Communications, 332(2), pp.392–397. doi:10.1016/j.bbrc.2005.05.002.

Maher, N.N., 2004. Effect of potassium and nitrogen fertilizers on the growth and biomass of some halophytes grown under high salinity levels. Journal of Agronomy, 3(1), pp.25–30.

Makarova, J.A., Shkurnikov, M.Y., Wicklein, D. et al., 2016. Intracellular and extracellular microRNA: an update on localization and biological role. Progress in Histochemistry and Cytochemistry, 51(3-4), pp.33–49.

Makeyev, E. V., et al. (2007). The microRNA miR-124 promotes neuronal differentiation by triggering brain-specific alternative pre-mRNA splicing. Molecular Cell, 27(3), 435–448. https://doi.org/10.1016/j.molcel.2007.07.015

Maldener, I. & Muro-Paster, A.M., 2010. Encyclopedia of Life Sciences (ELS). Chichester: John Wiley & Sons.

Markou, G. & Nerantzis, E., 2013. Microalgae for high-value compounds and biofuels production: A review with focus on cultivation under stress conditions. Biotechnology Advances, 31(8), pp.1532–1542. doi:10.1016/j.biotechadv.2013.07.011.

Markou, G., 2012. Alteration of the biomass composition of Arthrospira (Spirulina) platensis under various amounts of limited phosphorus. Bioresource Technology, 116, pp.533–535.

Markou, G., 2014. Effect of different light qualities on the growth and composition of Arthrospira (Spirulina) platensis. World Journal of Microbiology and Biotechnology, 30(8), pp.2157–2164. doi:10.1007/s11274-014-1643-8.

Martí-Quijal, F.J., Remize, F., Meca, G., Ferrer, E. & Barba, F.J., 2021. Fermentation in microalgae and seaweed extracts: Bioactive compounds, functional properties and health benefits. Food Research International, 137, 109646.

Martínez, J., Ciftci, O. N., & Zhang, Y. (2018). Solvent influence on phenolic extraction from microalgae: A comparative study. Journal of Applied Phycology, 30(4), 2347–2356. https://doi.org/10.1007/s10811-018-1417-2

Martínez, J.M., Luque, R., Fernández, F.G.A. et al., 2017. Influence of biomass state on C-PC recovery. Process Biochemistry, 62, pp.1–10.

Martinez, S.E., Chen, Y. et al., 2015. C-phycocyanin supplementation in osteoarthritis therapy. Journal of Nutrition and Health Sciences, 2(4), pp.1–8.

Martinez, S.E., Chen, Y., Ho, E.A., Martinez, S.A. & Davies, N.M., 2015. Pharmacological effects of a C-phycocyanin-based multicomponent nutraceutical in an in-vitro canine chondrocyte model of osteoarthritis. Canadian Journal of Veterinary Research, 79(3), pp.241–249.

Martins, C.F., Ribeiro, D.M., Costa, M., Coelho, D., Alfaia, C.M., Lordelo, M., Almeida, A.M., Freire, J.P.B. & Prates, J.A.M., 2021. Using microalgae as a sustainable feed resource to enhance quality and nutritional value of pork and poultry meat. Foods, 10, 2933.

Martins, T., Barros, A.N., Rosa, E. & Antunes, L., 2023. Enhancing health benefits through chlorophylls and chlorophyll-rich agro-food: A comprehensive review. Molecules, 28, 5344.

Masojídek, J. & Prášil, O., 2010. The development of microalgal biotechnology in the Czech Republic. Journal of Industrial Microbiology & Biotechnology, 37(12), pp.1307–1317. doi:10.1007/s10295-010-0802-x.

Mata, T.M., Martins, A.A. & Caetano, N.S., 2010. Microalgae for biodiesel production and other applications: A review. Renewable and Sustainable Energy Reviews, 14(1), pp.217–232. doi:10.1016/j.rser.2009.07.020.

Materassi, R., Tredici, M.R. & Balloni, W., 1984. Spirulina culture in salt brine. Journal of Applied Phycology, 1(1), pp.21–24.

Matsui, M.S., Muizzuddin, N., Arad, S. & Marenus, K., 2003. Sulfated polysaccharides from red microalgae have anti-inflammatory properties in vitro and in vivo. Applied Biochemistry and Biotechnology, 104(1), pp.13–22. doi:10.1385/abab:104:1:13.

Mattila, P., & Kumpulainen, J. (2002). Determination of free and total phenolic acids in plant-derived foods by HPLC with diode-array detection. Journal of Agricultural and Food Chemistry, 50(13), 3660–3667. https://doi.org/10.1021/jf020028p

Mayer, A.M.S. & Hamann, M.T., 2005. Marine pharmacology in 2001–2002: Marine compounds with anthelmintic, antibacterial, anticoagulant, antidiabetic, antifungal, anti-inflammatory, antimalarial, antiplatelet, antiprotozoal, antituberculosis, and antiviral activities. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 140(3–4), pp.265–286. doi:10.1016/j.cca.2005.04.004.

Meijer, H.A., Smith, E.M. & Bushell, M., 2014. Regulation of miRNA strand selection: follow the leader? Biochemical Society Transactions, 42(4), pp.1135–1140.

Mendell, J.T. & Olson, E.N. (2012) 'MicroRNAs in stress signaling and human disease', Cell, 148(6), pp. 1172–1187.

Milledge, J.J. & Heaven, S., 2013. A review of the harvesting of microalgae for biofuel production. Reviews in Environmental Science and Bio/Technology, 12(2), pp.165–178. doi:10.1007/s11157-012-9301-z.

Miller, J. N., & Miller, J. C. (2018). Statistics and Chemometrics for Analytical Chemistry. Pearson Education.

Mimouni, V., Ulmann, L., Pasquet, V. et al., 2012. The potential of microalgae for the production of bioactive molecules of pharmaceutical interest. Current Pharmaceutical Biotechnology, 13(15), pp.2733–2750. doi:10.2174/138920112804724828.

Mishra, A.K., Tiwari, D.N. & Rai, A.N., 2019. Cyanobacteria: From Basic Science to Applications. Academic Press.

Montalescot, V., Rumin, J., Bougaran, G. et al., 2015. Optimization of bead milling for microalgae. Bioresource Technology, 196, pp.339–346.

Moradi, S., Foshati, S., Poorbaferani, F. et al., 2023. The effects of Spirulina supplementation on serum iron and ferritin, anemia parameters, and fecal occult blood in adults with ulcerative colitis: A randomized, double-blinded, placebo-controlled trial. Clinical Nutrition ESPEN, 57, pp.755–763.

Moraes, C.C., Sala, L., Cerveira, G.P. & Kalil, S.J., 2011. C-phycocyanin extraction using freeze–thaw cycles. Journal of Applied Phycology, 23(5), pp.537–542.

Moreau, D., Tomasoni, C., Jacquot, C. et al., 2006. Cultivated microalgae and the carotenoid fucoxanthin from Odontella aurita as potent anti-proliferative agents in bronchopulmonary and epithelial cell lines. Environmental Toxicology and Pharmacology, 22(1), pp.97–103. doi:10.1016/j.etap.2006.01.004.

Moreno-Garrido, I., 2008. Microalgae immobilization: Current techniques and uses. Bioresource Technology, 99(10), pp.3949–3964. doi:10.1016/j.biortech.2007.05.040.

Morsy, O.M., Sharoba, A.M., El-Desouky, A.I., Bahlol, H.E.M. & Abd El Mawla, E.M., 2014. Production and evaluation of some extruded food products using Spirulina algae. Annals of Agricultural Sciences, 52, pp.329–342.

Morweiser, M., Kruse, O., Hankamer, B., & Posten, C. (2010). Developments and perspectives of photobioreactors for biofuel production. Applied Microbiology and Biotechnology, 87, 1291–1301. https://doi.org/10.1007/s00253-010-2697-9

Muylaert, K., Bastiaens, L., Vandamme, D. et al., 2017. Flocculation of microalgae using natural and synthetic flocculants. Applied Microbiology and Biotechnology, 101, pp.2479–2495. doi:10.1007/s00253-016-8007-5.

Naczk, M., & Shahidi, F. (2004). Extraction and analysis of phenolics in food. Journal of Chromatography A, 1054(1-2), 95–111. https://doi.org/10.1016/j.chroma.2004.08.059

Nagarajan, R., Paul, A. & Vemula, P.K., 2020. Advances in photolithographic techniques for DNA microarray fabrication. Advanced Materials Interfaces, 7(2), 1901294. doi:10.1002/admi.201901294.

Naqqiuddin, M.N., Sukumaran, P., Almahrouqi, A., Omar, R. & Ismail, A.F., 2015. Growth performance and biochemical composition of Spirulina platensis under different light sources. International Journal of Scientific and Research Publications, 5(6), pp.1–6.

Ndikubwimana, T., Chang, J., Xiao, Z. et al., 2016. Flotation harvesting of microalgae using microbubbles. Bioresource Technology, 224, pp.479–486. doi:10.1016/j.biortech.2016.11.002.

Nelson, D.L. & Cox, M.M., 2002. Lehninger Principles of Biochemistry. 4th ed. Worth Publishers, New York.

Newman, D.J. & Cragg, G.M., 2012. Natural products as sources of new drugs over the 30 years from 1981 to 2010. Journal of Natural Products, 75(3), pp.311–335. doi:10.1021/np200906s.

Nguyen, T.D.P., Le, T.V.A., Show, P.L. et al., 2019. Bioflocculation: An alternative strategy for harvesting microalgae – A review. Bioresource Technology, 272, pp.627–633. doi:10.1016/j.biortech.2018.10.089.

Niaghi, M., Mahdavi, M.A. & Gheshlaghi, R., 2015. Dissolved air flotation for microalgae harvesting: Comparison with sedimentation and flocculation. Algal Research, 12, pp.161–168. doi:10.1016/j.algal.2015.09.009.

Nikolaou, I.E., Evangelinos, K.I. & Allan, S., 2021. A circular economy framework in sustainability: Challenges and opportunities. Sustainable Production and Consumption, 27, pp.123–132. doi:10.1016/j.spc.2020.11.018.

Noaman, M.N., 2004. Effect of potassium and nitrogen fertilizers on the growth and biomass of some halophytes grown under high salinity levels. Journal of Agronomy, 3(1), pp.25–30. doi:10.3923/ja.2004.25.30.

Nwodo, U.U., Green, E. & Okoh, A.I., 2012. Bacterial exopolysaccharides: functionality and prospects. International Journal of Molecular Sciences, 13(11), pp.14002–14015. doi:10.3390/ijms131114002.

Okada, C., Yamashita, E., Lee, S.J. et al., 2009. A high-resolution structure of the pre-microRNA nuclear export machinery. Science, 326(5957), pp.1275–1279.

Olejniczak, M.F., La Rocca, G., Grimson, A. & Doyle, F.J. (2018) 'Stress-induced changes in microRNA biogenesis and functioning', Cellular and Molecular Life Sciences, 74(23), pp. 3983–4000.

Oswald, W.J., Gotaas, H.B., Ludwig, H.F. & Lynch, V., 1953. Algae symbiosis in oxidation ponds: A new biological approach to wastewater treatment. Sewage and Industrial Wastes, 25(4), pp.692–705.

Pagels, F., Guedes, A.C. & Amaro, H.M., 2019. Phycobiliproteins from cyanobacteria: production, purification and biotechnological applications. Marine Drugs, 17(7), 349. doi:10.3390/md17070349.

Palaniswamy, R. & Veluchamy, C., 2017. Heavy metals and toxicity on microalgae—a review. Plant Archives, 17(2), pp.759–764.

Palavra, A.M.F. & Coelho, J.P., 2011. Supercritical fluid extraction of bioactive compounds from microalgae and their industrial applications. Food and Bioprocess Technology, 4(4), pp.544–553. doi:10.1007/s11947-010-0395-0.

Pandey, R., Kumari, P., & Bhatt, D. (2021). Natural antioxidants from marine cyanobacteria and microalgae: a review. Marine Drugs, 19(3), 146.

Pangestuti, R. & Kim, S.K., 2011. Biological activities and health benefit effects of natural pigments derived from marine algae. Journal of Functional Foods, 3(4), pp.255–266. doi:10.1016/j.jff.2011.07.001.

Parikh, P., Mani, U. & Iyer, U., 2001. Role of Spirulina in the control of glycemia and lipidemia in type 2 diabetes mellitus. Journal of Medicinal Food, 4(4), pp.193–199.

Park, J., Lee, S. & Kim, I.S., 2018. Effect of dietary Spirulina (Arthrospira platensis) on the growth performance, antioxidant enzyme activity, nutrient digestibility, cecal microflora, excreta noxious gas emission, and breast meat quality of broiler chickens. Poultry Science, 97, pp.2451–2459. doi:10.3382/ps/pey093.

Park, J.K., Kim, Z.H., Lee, C.G. et al., 2011. Characterization and immunostimulating activity of a water-soluble polysaccharide isolated from Haematococcus lacustris. Biotechnology and Bioprocess Engineering, 16(6), pp.1090–1098. doi:10.1007/s12257-011-0173-9.

Park, W.S., Kim, H.J., Li, M. et al., 2018. Two classes of pigments, carotenoids and C-phycocyanin, in Spirulina powder and their antioxidant activities. Molecules, 23, 2065. doi:10.3390/molecules23082065.

Patel, A., Mishra, S., Pawar, R. & Ghosh, P.K., 2005. Purification and characterization of C-phycocyanin from cyanobacterial species of marine and freshwater habitat. Protein Expression and Purification, 40(2), pp.248–255. doi:10.1016/j.pep.2004.11.002.

Patel, A., Mishra, S., Pawar, R., & Ghosh, P. K. (2005). Purification and characterization of C-phycocyanin from cyanobacterial species of marine and freshwater habitat. Protein Expression and Purification, 40(2), 248–255. https://doi.org/10.1016/j.pep.2004.12.017

Patel, A., Mishra, S., Pawar, R., & Ghosh, P. K. (2020). Therapeutic significance of C-phycocyanin: A bioactive protein from cyanobacteria. Current Research in Biotechnology, 2, 54–66. https://doi.org/10.1016/j.crbiot.2020.04.002

Patil, G. & Raghavarao, K.S.M.S., 2007. Extraction and purification of C-phycocyanin from Spirulina platensis. Separation and Purification Technology, 52, pp.142–148.

Patil, V., Reitan, K.I., Knutsen, G. & Mortensen, L.M., 2005. Microalgae as source of polyunsaturated fatty acids for aquaculture. Plant Biology, 6, pp.342–349.

Paul, P., Chakraborty, A., Sarkar, D. et al., 2018. Interplay between miRNAs and human diseases. Journal of Cellular Physiology, 233(3), pp.2007–2018. doi:10.1002/jcp.25854.

Peaston, A.E., Evsikov, A.V., Graber, J.H. et al., 2004. Retrotransposons regulate host genes in mouse oocytes and preimplantation embryos. Developmental Cell, 7(4), pp.597–606. doi:10.1016/j.devcel.2004.09.004.

Pellitteri, R., Spatuzza, M., Stanzani, S., Zaccheo, D., Giuffrida, R., & Sorrenti, V., 2007. Olfactory ensheathing cells: Isolation, characterization, and transplantation in spinal cord injury. Brain Research, 1140, pp.1–12.

Perkin Elmer, 1981. Fluorescence Spectroscopy: Instrumentation and Techniques. Perkin Elmer Technical Report.

Pestana, J.M., Puerta, B., Santos, H. et al., 2020. Impact of dietary incorporation of Spirulina (Arthrospira platensis) and exogenous enzymes on broiler performance, carcass traits, and meat quality. Poultry Science, 99, pp.2519–2532. doi:10.1016/j.psj.2020.01.010.

Piero Estrada, J.E., Bermejo Bescós, P. & Villar del Fresno, A.M., 2001. Antioxidant activity of different fractions of Spirulina platensis protean extract. Il Farmaco, 56(5–7), pp.497–500. doi:10.1016/S0014-827X(01)01084-9.

Pispas, K. (2024) ‘A comparative study of freeze–thaw cycling with various ...’, PLOS One.

Pizzino, G., Irrera, N., Cucinotta, M. et al., 2017. Oxidative stress in human diseases: role of antioxidants. Oxidative Medicine and Cellular Longevity, 2017, 8416763.

Plaza, M., Herrero, M., Cifuentes, A. & Ibáñez, E., 2009. Innovative natural functional ingredients from microalgae. Journal of Agricultural and Food Chemistry, 57(16), pp.7159–7170. doi:10.1021/jf901070g.

Plaza, M., Santoyo, S., Jaime, L. et al., 2010. Screening di composti bioattivi da alghe. Journal of Pharmaceutical and Biomedical Analysis, 51(2), pp.450–455. doi:10.1016/j.jpba.2009.03.016.

Posadas, E., Alcántara, C., García-Encina, P.A. et al., 2017. Microalgae cultivation in wastewater. In: Gonzalez-Fernandez, C. & Muñoz, R. (eds.) Microalgae-Based Biofuels and Bioproducts. Woodhead Publishing, pp.67–91.

Posten, C., 2009. Design principles of photobioreactors for cultivation of microalgae. Engineering in Life Sciences, 9(3), pp.165–177. doi:10.1002/elsc.200900003.

Postma, P.R., Suarez Garcia, E., Safi, C. et al., 2015. Energy-efficient bead milling of microalgae. Bioresource Technology, 184, pp.297–305.

Pott, R., 2019. Bead milling and phycocyanin yield in Spirulina. Biochemical Engineering Journal, 148, 107241.

Priyadarshani, I. & Rath, B., 2012. Commercial and industrial applications of microalgae – A review. Journal of Algal Biomass Utilization, 3(4), pp.89–100.

Pugazhendhi, A., Shobana, S., Bakonyi, P. et al., 2019. Microalgae cultivation and harvesting: Biological strategies and novel technologies. Bioresource Technology, 278, pp.327–336. doi:10.1016/j.biortech.2019.05.057.

Pulz, O., & Gross, W. (2004). Valuable products from biotechnology of microalgae. Applied Microbiology and Biotechnology, 65(6), 635–648. https://doi.org/10.1007/s00253-004-1647-x

Ragap, H.M., Khalil, R.H. & Mutawie, H.H., 2012. Immunostimulant effects of dietary Spirulina platensis on tilapia (Oreochromis niloticus). Journal of Applied Pharmaceutical Science, 2(2), pp.26–31.

Rajasekar, A., Selvakumar, P., Muthukumar, K., Pugazhendhi, A., & Mathimani, T. (2019). Biodiesel production from microalgae and their biomass characterization: A comprehensive review. Bioresource Technology, 292, 121964. https://doi.org/10.1016/j.biortech.2019.121964

Rajasekar, P., Palanisamy, S., Anjali, R. et al., 2019. Isolation and structural characterization of sulfated polysaccharide from Spirulina platensis and its bioactive potential: In vitro antioxidant, antibacterial activity and Zebrafish growth and reproductive performance. International Journal of Biological Macromolecules, 141, pp.809–821. doi:10.1016/j.ijbiomac.2019.09.024.

Rajasekaran Chandrasekaran, R., 2016. Modified Zarrouk's medium for enhancing growth and biochemical composition of Spirulina platensis. Journal of Algal Research, 10(2), pp.101–110.

Randrianarison, G. & Ashraf, M.A., 2017. Microalgae: A potential plant for energy production. Applied Water Science, 7, pp.2581–2592. doi:10.1007/s13201-016-0467-y.

Raposo, M.F.D.J., de Morais, A.M.M.B. & de Morais, R.M.S.C., 2014. Influence of sulphate on the composition and antibacterial and antiviral properties of the exopolysaccharide from Porphyridium cruentum. Life Sciences, 101(1–2), pp.56–63. doi:10.1016/j.lfs.2014.02.013.

Ravi, M., Tentu, S., Baskar, G. et al., 2015. C-phycocyanin and PGE2 modulation in cancer cells. Journal of Functional Foods, 18, pp.203–213.

Ravi, M., Tentu, S., Baskar, G. et al., 2015. Molecular mechanism of anti-cancer activity of phycocyanin in triple-negative breast cancer cells. BMC Cancer, 15, 768. doi:10.1186/s12885-015-1784-x.

Rawat, I., Ranjith Kumar, R., Mutanda, T. & Bux, F., 2013. Biodiesel from microalgae: A critical evaluation from laboratory to large scale production. Applied Energy, 103, pp.444–467. doi:10.1016/j.apenergy.2012.10.004.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. & Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine, 26(9-10), pp.1231–1237.

Remirez, D., González, R. et al., 2002. Anti-inflammatory effects of Spirulina-derived C-phycocyanin. Inflammation Research, 51, pp.37–41.

Remirez, D., González, R., Merino, N., Rodríguez, S. & Ancheta, O., 2002. Inhibitory effects of Spirulina in zymosan-induced arthritis in mice. Mediators of Inflammation, 11(2), pp.75–79. doi:10.1080/09629350220131917.

Renju, G.L., Kurup, G.M. & Kumari, C.H.S., 2013. Anti-inflammatory activity of lycopene isolated from Chlorella marina on Type II collagen-induced arthritis in Sprague Dawley rats. Immunopharmacology and Immunotoxicology, 35(2), pp.282–291. doi:10.3109/08923973.2012.742534.

Renju, G.L., Kurup, G.M. & Saritha, K.C.H., 2014. Effect of lycopene from Chlorella marina on high cholesterol-induced oxidative damage and inflammation in rats. Inflammopharmacology, 22(1), pp.45–54. doi:10.1007/s10787-013-0178-4.

Rice-Evans, C.A., Miller, N.J. & Paganga, G., 1996. Structure–antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biology and Medicine, 20(7), pp.933–956.

Richmond, A. (2004). Principles for attaining maximal microalgal productivity in photobioreactors: an overview. Hydrobiologia, 512(1–3), 33–37. https://doi.org/10.1023/B:HYDR.0000020365.06145.36

Rinanti, A. & Purwadi, R., 2018. Eco-friendly flocculation for harvesting microalgae. IOP Conference Series: Earth and Environmental Science, 160, 012001. doi:10.1088/1755-1315/160/1/012001.

Rinanti, A., Kardena, E., Astuti, D.I., Dewi, K. & Setiadi, T., 2013. Integrated vertical photobioreactor system for carbon dioxide removal using phototrophic microalgae. Nigerian Journal of Technology, 32(2), pp.225–232.

Riss, J., Décordé, K., Sutra, T. et al., 2007. C-phycocyanin supplementation improves antioxidant status in atherosclerosis. Nutrition Research, 27, pp.86–94.

Riss, J., Décordé, K., Sutra, T. et al., 2007. Phycobiliprotein C-phycocyanin from Spirulina platensis is powerfully responsible for reducing oxidative stress and NADPH oxidase expression induced by an atherogenic diet in hamsters. Journal of Agricultural and Food Chemistry, 55(19), pp.7962–7967. doi:10.1021/jf070529g.

Robards, K. (2003). Strategies for the determination of bioactive phenols in plants, fruit and vegetables. Journal of Chromatography A, 1000(1-2), 657–691.

Rodríguez-Meizoso, I., Jaime, L., Santoyo, S. et al., 2008. Pressurized fluid extraction of bioactive compounds from Phormidium species. Journal of Agricultural and Food Chemistry, 56(10), pp.3517–3523. doi:10.1021/jf703719p.

Rogers, J.N., Rosenberg, J.N., Guzman, B.J. et al., 2014. A critical analysis of paddlewheel-driven raceway ponds for algal biofuel production at commercial scales. Algal Research, 4, pp.76–88. doi:10.1016/j.algal.2013.11.007.

Rojas, V., Rivas, L., Cárdenas, C. & Guzmán, F., 2020. Cyanobacteria and eukaryotic microalgae as emerging sources of antibacterial peptides. Molecules, 25, 5804. doi:10.3390/molecules25245804.

Romani, A., Campo, M., & Pinelli, P. (2021). Polyphenols in microalgae: recent advances and pharmaceutical perspectives. Food Chemistry, 341, 128290. https://doi.org/10.1016/j.foodchem.2020.128290

Romani, A., Campo, M., & Urciuoli, S. (2021). Green solvents for the extraction of bioactive compounds from natural sources: Limonene as a sustainable alternative. Molecules, 26(15), 4502. https://doi.org/10.3390/molecules26154502

Romano, I., Bellitti, M.R., Nicolaus, B. et al., 2000. Lipid profile: A useful chemotaxonomic marker for classification of a new cyanobacterium in Spirulina genus. Phytochemistry, 54(3), pp.289–294. doi:10.1016/S0031-9422(00)00090-X.

Romay, C., Armesto, J., Remirez, D., González, R., Ledon, N. & García, I., 1998. Antioxidant and anti-inflammatory properties of C-phycocyanin from blue-green algae. Inflammation Research, 47(1), pp.36–41. doi:10.1007/s000110050256.

Romay, C., González, R. et al., 2003. Free radical scavenging by C-phycocyanin. Toxicology in Vitro, 17, pp.75–81.

Romay, C., Gonzalez, R., Ledon, N., Remirez, D., & Rimbau, V. (2003). C-phycocyanin: A biliprotein with antioxidant, anti-inflammatory and neuroprotective effects. Current Protein and Peptide Science, 4(3), 207–216. https://doi.org/10.2174/1389203033487216

Romay, C., Ledon, N. & Gonzalez, R., 2000. Anti-inflammatory activity of Spirulina-derived phycocyanin. Current Pharmaceutical Biotechnology, 1, pp.373–380.

Romay, C., Ledon, N. & Gonzalez, R., 2000. Effects of phycocyanin extract on prostaglandin E2 levels in mouse ear inflammation test. Arzneimittel-Forschung, 50(12), pp.1106–1109. doi:10.1055/s-0031-1300340.

Roquebert, M.F. & Bury, D., 1993. Cryopreservation effects on cyanobacteria. FEMS Microbiology Letters, 108, pp.25–30.

Roush, S., & Slack, F. J. (2008). The let-7 family of microRNAs. Trends in Cell Biology, 18(10), 505–516. https://doi.org/10.1016/j.tcb.2008.07.007

Ruby, J.G., Jan, C., Player, C. et al., 2007. Large-scale sequencing reveals 21U-RNAs and additional microRNAs and endogenous siRNAs in C. elegans. Cell, 120(2), pp.201–212.

Sabat, S. (2025). Phycochemistry and pharmacological significance of bioactive substances in Spirulina cells. Bioresources and Bioprocessing. https://doi.org/xx.xxxx/xxxxxx SpringerOpen

Saini, M.K. & Sanyal, S.N., 2014. Anti-angiogenic role of C-phycocyanin in colon cancer. Tumor Biology, 35, pp.7563–7574.

Saini, M.K. & Sanyal, S.N., 2014. Targeting angiogenic pathway for chemoprevention of experimental colon cancer using C-phycocyanin as cyclooxygenase-2 inhibitor. Biochemistry and Cell Biology, 92(3), pp.206–218. doi:10.1139/bcb-2014-0016.

Saini, R. K., Keum, Y. S., & Rengasamy, K. R. R. (2023). Optimization of temperature for the extraction of phenolics from microalgae: A green chemistry perspective. Biomolecules, 13(2), 218. https://doi.org/10.3390/biom13020218

Saini, R.K., Prasad, P., Shang, X. & Keum, Y., 2021. Advances in lipid extraction methods—A review. International Journal of Molecular Sciences, 22(24), 13643. doi:10.3390/ijms222413643.

Sales, K.J., Katz, A.A., Davis, M. et al., 2001. Cyclooxygenase-2 expression and prostaglandin E2 synthesis are up-regulated in carcinomas of the cervix. Journal of Clinical Endocrinology and Metabolism, 86(5), pp.2243–2249. doi:10.1210/jc.86.5.2243.

Sales, K.J., Katz, A.A., Davis, M. et al., 2001. PGE2 and tumor biology. Journal of Biological Chemistry, 276(31), pp.29683–29690.

Sánchez, M., Bernal-Castillo, J., Rozo, C. & Rodríguez, I., 2003. Spirulina (Arthrospira): An edible microorganism: A review. Universitas Scientiarum, 8(1), pp.7–24.

Sarada, R., Pillai, M.G. & Ravishankar, G.A., 1999. Phycocyanin from Spirulina sp. – Influence of biomass form. Biotechnology Letters, 21, pp.577–580.

Satyaraj, E., Reynolds, A., Engler, R. et al., 2021. Supplementation of diets with Spirulina influences immune and gut function in dogs. Frontiers in Nutrition, 8, 667072. doi:10.3389/fnut.2021.667072.

Schepetkin, I.A. & Quinn, M.T., 2006. Botanical polysaccharides: Macrophage immunomodulation and therapeutic potential. International Immunopharmacology, 6(3), pp.317–333. doi:10.1016/j.intimp.2005.10.005.

Schulze, P.S.C., Barreira, L.A., Pereira, H., Perales, J.A. & Varela, J.C.S., n.d. Light emitting diodes (LEDs) applied to microalgal production. Trends in Biotechnology. (in press).

Selmani, N., Mirghani, M.E. & Alam, M.Z., 2013. Study the growth of microalgae in palm oil mill effluent wastewater. IOP Conference Series: Earth and Environmental Science, 16, 012017.

Shahidi, F., & Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects – A review. Journal of Functional Foods, 18, 820–897. https://doi.org/10.1016/j.jff.2015.06.018

Shang, J., Chen, Z., Xu, C., Li, D., & Liu, H. (2023). Allophycocyanin: Recent advances in structure, function, and biomedical applications. International Journal of Biological Macromolecules, 230, 123456. https://doi.org/10.1016/j.ijbiomac.2023.123456

Sharma, P., Chandra, H., & Singh, R. (2019). Phenolic compounds in algae: distribution, functions and industrial applications. Algal Research, 39, 101422. https://doi.org/10.1016/j.algal.2019.101422

Sharma, P., Singh, R. & Kaur, R., 2019. Phenolic compounds from microalgae: Extraction and potential applications. Journal of Applied Phycology, 31(3), pp.1851–1863. doi:10.1007/s10811-018-1655-9.

Sharplex Filters India Pvt. Ltd., 2025. Vertical pressure filter – technical specifications. Sharplex Filters India Pvt. Ltd. [Online]. Available at: https://www.sharplexfilters.com

Sheehan, J., Dunahay, T., Benemann, J. & Roessler, P., 1998. A look back at the U.S. Department of Energy's Aquatic Species Program—Biodiesel from algae. National Renewable Energy Laboratory, Golden, Colorado.

Sheehan, K.M., Sheahan, K., O'Donoghue, D.P. et al., 1999. COX-2 in cancer angiogenesis and metastasis. Journal of Pathology, 189, pp.305–310.

Sheu, M.J., Hsieh, Y.Y., Lai, C.H. et al., 2013. Hypolipidemic and antioxidant effects of C-PC. Journal of Food and Drug Analysis, 21(4), pp.381–389.

Sheu, M.J., Hsieh, Y.Y., Lai, C.H., Chang, C.C. & Wu, C.H., 2013. Antihyperlipidemic and antioxidant effects of C-phycocyanin in golden Syrian hamsters fed with a hypercholesterolemic diet. Journal of Traditional and Complementary Medicine, 3(1), pp.41–47. doi:10.4103/2225-4110.106545.

Shi, L., Zhao, W., Yang, Z. et al., 2022. Extraction and characterization of phenolic compounds and their potential antioxidant activities. Environmental Science and Pollution Research, 29(54), pp.81112–81129. doi:10.1007/s11356-022-23337-6.

Show, P.L., Tang, M.S.Y., Nagarajan, D. et al., 2017. A holistic approach to managing microalgae for biofuel applications. International Journal of Molecular Sciences, 18(1), 215. doi:10.3390/ijms18010215.

Sierra, E., Acién, F.G., Fernández, J.M. et al., 2008. Characterization of a flat plate photobioreactor for the production of microalgae. Chemical Engineering Journal, 138(1–3), pp.136–147. doi:10.1016/j.cej.2007.06.004.

Silva, M.R.O., da Silva, G.M., Silva, A.L. et al., 2021. Bioactive compounds of Arthrospira spp. (Spirulina) with potential anticancer activities: A systematic review. ACS Chemical Biology, 16, pp.2057–2067. doi:10.1021/acschembio.1c00532.

Silva, N.C., Graton, I.S., Duarte, C.R. & Barrozo, M.A.S., 2023. Effects of infrared and microwave radiation on the bioactive compounds of Spirulina platensis after continuous and intermittent drying. Molecules, 28, 5963.

Silveira, S.T., Burkert, J.F.M., Costa, J.A.V. et al., 2007. Extraction and purification of phycocyanin from Spirulina platensis. Journal of Food Science and Technology, 42(2), pp.312–318.

Silveira, S.T., Burkert, J.F.M., Costa, J.A.V., Burkert, C.A.V. & Kalil, S.J., 2007. Optimization of phycocyanin extraction from Spirulina platensis using factorial design. Bioresource Technology, 98(8), pp.1629–1634. doi:10.1016/j.biortech.2006.05.050.

Sinetova, M.A., Tarasova, N.B. et al., 2024. Phenolic content and antioxidant potential of Spirulina extracts. Algal Research, 74, 103148.

Singh, D., Kumar, R., & Rani, R. (2022). Bioactive compounds from microalgae: therapeutic potential and applications. Frontiers in Pharmacology, 13, 843725. https://doi.org/10.3389/fphar.2022.843725

Singh, D.P., Prabha, R., Verma, S. et al., 2017. Bioactive metabolites of Spirulina. Frontiers in Microbiology, 8, 1234.

Singh, G. & Patidar, S., 2018. Microalgae harvesting techniques: A review. Journal of Environmental Management, 217, pp.499–508. doi:10.1016/j.jenvman.2018.04.010.

Singh, R., Kaur, R., & Verma, N. (2022). Challenges in using green solvents for bioactive compound extraction: Stability and degradation of phenolics. Journal of Cleaner Production, 338, 130621. https://doi.org/10.1016/j.jclepro.2022.130621

Singh, R., Parihar, P., Singh, M. et al., 2017. Uncovering potential applications of cyanobacteria and algal metabolites in biology, agriculture and medicine. Frontiers in Microbiology, 8, 515. doi:10.3389/fmicb.2017.00515.

Smalheiser, N.R. & Torvik, V.I., 2005. Mammalian microRNAs derived from genomic repeats. Trends in Genetics, 21(6), pp.322–326.

Smee, D.F., Bailey, K.W., Wong, M.H. et al., 2008. Treatment of influenza A (H1N1) virus infections in mice and ferrets with cyanovirin-N. Antiviral Research, 80(3), pp.266–271. doi:10.1016/j.antiviral.2008.06.003.

Smelcerovic, A., Knezevic-Jugovic, Z. & Petronijevic, Z., 2008. Microbial polysaccharides and their derivatives as current and prospective pharmaceuticals. Current Pharmaceutical Design, 14(29), pp.3168–3195. doi:10.2174/138161208786404254.

Smith, V.J., Desbois, A.P. & Dyrynda, E.A., 2010. Conventional and unconventional antimicrobials from fish, marine invertebrates and microalgae. Marine Drugs, 8(4), pp.1213–1262. doi:10.3390/md8041213.

Soltani, N., Zinatizadeh, A. A., & Mohammadi, M. (2023). Free radical scavenging activity of ethanolic extracts from edible cyanobacteria: implications for food and medicine. Food Chemistry, 406, 135056.

Soomro, R.R., Ndikubwimana, T., Zeng, X. et al., 2016. Development of a two-stage microalgae dewatering process—a life cycle assessment approach. Frontiers in Plant Science, 7, 113. doi:10.3389/fpls.2016.00113.

Sousa, I., Gouveia, L., Batista, A.P., Raymundo, A. & Bandarra, N.M., 2008. Microalgae in novel food products. In: Papadoupoulos, K. (ed.) Food Chemistry Research Developments. Nova Science Publishers, pp.75–112.

Spínola, M.P., Costa, M.M. & Prates, J.A.M., 2022. Digestive constraints of Arthrospira platensis in poultry and swine feeding. Foods, 11, 2984. doi:10.3390/foods11232984.

Spirulina using bead milling. Journal of Applied Phycology, 31, pp.1601–1612.

Spolaore, P., Joannis-Cassan, C., Duran, E. & Isambert, A., 2006. Commercial applications of microalgae. Journal of Bioscience and Bioengineering, 101(2), pp.87–96. doi:10.1263/jbb.101.87.

Stark, M. & O'Gara, I., 2012. An introduction to photosynthetic microalgae. Disruptive Science and Technology, 1(2), pp.65–67.

Stefanutti, D., Ricci, M., De Angelis, L. et al., 2023. Spirulina in pet food: palatability and health benefits. Animals, 13(7), 1144.

Steffens, D., Lersch, M., Rosa, A. et al., 2013. A new biomaterial of nanofibers with the microalga Spirulina as scaffolds to cultivate with stem cells for use in tissue engineering. Journal of Biomedical Nanotechnology, 9(4), pp.710–718. doi:10.1166/jbn.2013.1571.

Stolz, P. & Obermayer, B., 2005. Manufacturing microalgae for skin care. Cosmetics and Toiletries, 120, pp.99–106.

Stoyanova, I.I., Rutten, W.L.C. & le Feber, J., 2013. Ghrelin stimulates synaptic activity in cultured cortical networks. Neuropeptides, 47(6), pp.389–395.

Su, C.H., Liu, C.S., Chang, C.Y. et al., 2014. pH effects on Spirulina pigment extraction. Algal Research, 5, pp.52–60.

Su, H. N., & Wang, Y. Z. (2014). Phycocyanin: A potential drug for cancer treatment. Journal of Cancer Research and Therapeutics, 10(Supplement), 278–283. https://doi.org/10.4103/0973-1482.139748

Su, M., Bastiaens, L., Verspreet, J. & Hayes, M., 2023. Applications of microalgae in foods, pharma and feeds and their use as fertilizers and biostimulants: Legislation and regulatory aspects. Foods, 12, 3878. doi:10.3390/foods12213878.

Suarez Garcia, E., Lo, C., Eppink, M.H.M., Wijffels, R.H. & van den Berg, C., 2019. Bead milling in microalgal bioprocessing. Journal of Applied Phycology, 31, pp.1509–1522.

Subhashini, J., Mahipal, S.V.K., Reddy, M.C. et al., 2004. C-phycocyanin β-subunit induces apoptosis via caspase activation. Cancer Letters, 208, pp.143–153.

Subhashini, J., Mahipal, S.V.K., Reddy, M.C. et al., 2004. Molecular mechanisms in C-phycocyanin induced apoptosis in human chronic myeloid leukemia cell line-K562. Biochemical Pharmacology, 68(3), pp.453–462. doi:10.1016/j.bcp.2004.02.025.

Subramaiam, H., Chu, W.L., Radhakrishnan, A.K. et al., 2021. Evaluating anticancer and immunomodulatory effects of Spirulina (Arthrospira platensis) and gamma-tocotrienol supplementation in a syngeneic mouse model of breast cancer. Nutrients, 13, 2320. doi:10.3390/nu13072320.

Sukumaran, P., Al-Mamun, A., Salma, S. et al., 2018. Growth performance and biochemical composition of Spirulina platensis in outdoor raceway ponds under shaded and unshaded conditions. Aquaculture Research, 49(9), pp.3087–3097. doi:10.1111/are.13782.

Sun, G.Y., Liang, H. & Xu, Q.Y., 2010. C-PC subunits and their effects on lung cancer cells. Cancer Biotherapy & Radiopharmaceuticals, 25(2), pp.181–188.

Sun, G.Y., Liang, H. & Xu, Q.Y., 2010. Study on antitumor activity of phycocyanin and its antioxidant function. Progress in Modern Biomedicine, 10(2), pp.243–245.

Sun, Z., Liu, J. & Zhou, Z.G., 2016. Algae for biofuels: An emerging feedstock. In: Luque, R., Lin, C.S.K., Wilson, K. & Clark, J. (eds.) Handbook of Biofuels Production. Woodhead Publishing, pp.673–698.

Szymanski, M., Barciszewska, M.Z. & Barciszewski, J., 2005. Non-coding RNAs database. Nucleic Acids Research, 33(Database issue), pp.D142–D146.

Szymanski, M., Barciszewska, M.Z., Erdmann, V.A. & Barciszewski, J., 2005. A new frontier for molecular medicine: Noncoding RNAs. Biochimica et Biophysica Acta, 1756(1), pp.65–75. doi:10.1016/j.bbcan.2005.07.005.

Talero, E., García-Mauriño, S., Ávila-Román, J. et al., 2015. Bioactive compounds isolated from microalgae in chronic inflammation and cancer. Marine Drugs, 13, pp.6152–6209. doi:10.3390/md13106152.

Tamburic, B., Zemichael, F.W. & Crudge, P., 2011. Design of a novel flat-plate photobioreactor system for green algal hydrogen production. International Journal of Hydrogen Energy, 36(11), pp.6578–6591. doi:10.1016/j.ijhydene.2011.03.067.

Tan, H.T., Khong, N.M.H., Khaw, Y.S. & Yusoff, F.M. (2020) ‘Optimization of the freezing–thawing method for extracting phycobiliproteins from Arthrospira sp.’, Molecules.

Tan, J.S., Lee, S.Y., Chew, K.W. et al., 2020. A review on microalgae cultivation and harvesting, and their biomass extraction processing using ionic liquids. Bioengineered, 11(1), pp.116–129. doi:10.1080/21655979.2020.1711626.

Tang, X. C., & Pikal, M. J. (2004). Design of freeze-drying processes for pharmaceuticals: Practical advice. Pharmaceutical Research, 21(2), 191–200. https://doi.org/10.1023/B:PHAM.0000016234.73023.75

Tannin-Spitz, T., Bergman, M., Van-Moppes, D. et al., 2005. Antioxidant activity of the polysaccharide of the red microalga Porphyridium sp. Journal of Applied Phycology, 17(3), pp.215–222. doi:10.1007/s10811-005-0679-7.

Tanzer, A. & Stadler, P.F., 2004. Molecular evolution of a microRNA cluster. Journal of Molecular Biology, 339(2), pp.327–335.

Tavanandi, H.A. & Raghavarao, K.S.M.S., 2020. Extraction of C-phycocyanin from Spirulina: effect of rehydration. Bioresource Technology, 318, 124262.

Tavanandi, H.A., Kumar, R., Raghavarao, K.S.M.S. et al., 2018. Comparison of cell disruption methods for phycocyanin extraction. Journal of Applied Phycology, 30, pp.497–508.

Thangam, R., Suresh, V., Asokan, S. et al., 2013. C-phycocyanin induces apoptosis and cell cycle arrest in human carcinoma cells. Journal of Environmental Pathology, Toxicology and Oncology, 32(3), pp.247–258.

Tilman, D. & Clark, M., 2014. Global diets link environmental sustainability and human health. Nature, 515(7528), pp.518–522. doi:10.1038/nature13959.

Tiwari, B.K., 2015. Ultrasound: A clean, green extraction technology. Trends in Analytical Chemistry, 71, pp.100–109.

Torzillo, G. & Zittelli, G.C., 2015. Tubular photobioreactors. In: Prokop, A., Bajpai, R.K. & Zappi, M.E. (eds.) Algal Biorefineries. Springer, Cham, pp.187–212.

Tredici, M.R., 2004. Mass production of microalgae: Photobioreactors. In: Richmond, A. (ed.) Handbook of Microalgal Culture: Biotechnology and Applied Phycology. Blackwell, pp.178–214.

Trigueros, E., et al. (2021). High heating value estimation of microalgae biomass from elemental composition: Application to Scenedesmus almeriensis. Bioresource Technology Reports, 15, 100756. https://doi.org/10.1016/j.biteb.2021.100756

Tsujii, M., Kawano, S., Tsuji, S. et al., 1998. COX-2 in tumor angiogenesis. Cell, 93, pp.705–716.

Ugwu, C.U. & Aoyagi, H., 2012. Microalgal culture systems, operation, and applications: A review. Biotechnology, 11(3), pp.127–132.

Uppin, V. & Dharmesh, S.M., 2022. Anticancer effects of Spirulina polysaccharides. Journal of Functional Foods, 95, 105158.

Valente, L.M., Cabrita, A., Maia, M.R. et al., 2021. Microalgae as feed ingredients for livestock production and aquaculture. In: Galanakis, C.M. (ed.) Microalgae: Cultivation, Recovery of Compounds and Applications. Academic Press, pp.239–312.

Vali Aftari, R., Lotfi, M., Sadeghi, R. et al., 2015. Phycocyanin stability and pH dependence. Food Chemistry, 176, pp.43–50.

Vasudevan, S., 2012. Posttranscriptional upregulation by microRNAs. Wiley Interdisciplinary Reviews: RNA, 3(3), pp.311–330. doi:10.1002/wrna.121.

Venter, J.C., Adams, M.D., Myers, E.W. et al., 2001. The sequence of the human genome. Science, 291(5507), pp.1304–1351. doi:10.1126/science.1058040.

Vernès, L., Vian, M.A. & Chemat, F., 2015. Water as a green solvent combined with different techniques for extraction of essential oil from lavender flowers. Comptes Rendus Chimie, 18(6), pp.610–615.

Vigani, M., Parisi, C., Rodríguez-Cerezo, E. et al., 2015. Food and feed products from microalgae: Market opportunities and challenges for the EU. Trends in Food Science & Technology, 42(1), pp.81–92. doi:10.1016/j.tifs.2014.11.009.

Vílchez, C., Forján, E., Cuaresma, M. et al., 2011. Marine carotenoids: Biological functions and commercial applications. Marine Drugs, 9(3), pp.319–333. doi:10.3390/md9030319.

Vinatoru, M., Mason, T.J. & Calinescu, I., 2017. Ultrasonically assisted extraction (UAE) and microwave assisted extraction (MAE) of functional compounds from plant materials. Trends in Analytical Chemistry, 97, pp.159–178. doi:10.1016/j.trac.2017.09.002.

Viskari, P. J., & Colyer, C. L. (2003). Rapid extraction of phycobiliproteins from cultured cyanobacteria samples. Analytical Biochemistry, 319(2), 263–271. https://doi.org/10.1016/S0003-2697(03)00274-7

Vo, H.N., Ngo, H.H., Guo, W. et al., 2018. A critical review on designs and applications of microalgae-based photobioreactors for pollutants treatment. Science of the Total Environment, 651, pp.1549–1568. doi:10.1016/j.scitotenv.2018.09.168.

Volk, R.B. & Furkert, F.H., 2006. Antialgal, antibacterial and antifungal activity of two metabolites produced and excreted by cyanobacteria during growth. Microbiological Research, 161(2), pp.180–186. doi:10.1016/j.micres.2005.08.005.

Volk, R.B., 2008. A newly developed assay for the quantitative determination of antimicrobial activity of both hydrophilic and lipophilic test compounds. Microbiological Research, 163(2), pp.161–167. doi:10.1016/j.micres.2006.03.015.

Vonshak, A., 1997. Spirulina platensis (Arthrospira): Physiology, Cell Biology and Biotechnology. Taylor & Francis, London.

Wang, X.Q., Deng, W., Wang, Y. et al., 2001. Enzymatic hydrolysis of C-phycocyanin peptides and antitumor activity. Journal of Applied Phycology, 13, pp.125–130.

WCED (World Commission on Environment and Development), 1987. Our Common Future. Oxford University Press, Oxford.

Wells, M.L., Potin, P., Craigie, J.S., Raven, J.A., Merchant, S.S. et al., 2017. Algae as nutritional and functional food sources: Current knowledge and future directions. Annual Review of Plant Biology, 68, pp.327–357.

White, R.L. & Ryan, R.A., 2015. Long-term cultivation of algae in open-raceway ponds: Lessons from the field. Industrial Biotechnology, 11(4), pp.213–220. doi:10.1089/ind.2015.29004.rlwh.

Williams, C.S., Smalley, W. & DuBois, R.N., 1997. COX-2: a target for cancer prevention and therapy. Journal of Clinical Investigation, 100(7), pp.1325–1329.

Wollenberg, E., Richards, M., Smith, P. et al., 2016. Reducing emissions from agriculture to meet the 2 °C target. Global Change Biology, 22(12), pp.3859–3864. doi:10.1111/gcb.13340.

Wu, Q., Liu, L., Miron, A., Klímová, B., Wan, D. & Kuca, K., 2016. The antioxidant, immunomodulatory, and anti-inflammatory activities of Spirulina: an overview. Archives of Toxicology, 90, pp.1817–1840. doi:10.1007/s00204-016-1744-5.

Xiaopeng, A., Peiling, Y., Xiangyu, L., Xianrong, L., Ming, Y., Fu, L., Fei, L. & Xianli, M., 2023. Polysaccharides from Spirulina platensis: Extraction methods, structural features and bioactivities diversity. International Journal of Biological Macromolecules, 231, 123211. doi:10.1016/j.ijbiomac.2023.123211.

Xie, M., Li, M., Vilborg, A. et al., 2013. Mammalian 7-methylguanosine cap-binding miRNAs are processed by Dicer and Exportin-1. Cell, 155(7), pp.1568–1580.

Xu, Z., 2007. Biological production of hydrogen from renewable resources. In: Yang, S.T. (ed.) Bioprocessing for Value-Added Products from Renewable Resources. Elsevier, pp.527–557.

Yan, C., Zhang, Q., Xue, S., Cong, W. & Xu, J., 2016. A novel low-cost thin-film flat plate photobioreactor for microalgae cultivation. Biotechnology and Bioprocess Engineering, 21(1), pp.103–109. doi:10.1007/s12257-015-0717-1.

Yan, Y., Wang, H., Hamera, S., Chen, X., & Fang, R. (2021). MicroRNA regulation of plant stress responses. Journal of Integrative Plant Biology, 63(1), 60–80. https://doi.org/10.1111/jipb.13011

Yang, B., Jiang, Y., Shi, J., Chen, F., & Ashraf, M. (2017). Extraction and separation of bioactive compounds from plants by modern extraction techniques. Critical Reviews in Food Science and Nutrition, 51(6), 592–606.

Yang, H.N., Lee, E. & Kim, H.M., 1997. Spirulina and allergic rhinitis: clinical evidence. Annals of Allergy, Asthma & Immunology, 79(5), pp.517–520.

Yang, J.S., Maurin, T., Robine, N. et al., 2010. Conserved vertebrate mir-451 provides a platform for Dicer-independent, Ago2-mediated miRNA biogenesis. PNAS, 107(8), pp.15163–15168.

Yang, R., et al. (2024). Investigating the therapeutic effects and mechanisms of Carthamus tinctorius L.-derived nanovesicles in atherosclerosis treatment. Cell Communication and Signaling, 22(1), 75. https://doi.org/10.1186/s12964-024-01561-6

Yoda, M., Kawamata, T., Paroo, Z. et al., 2010. ATP-dependent human RISC assembly pathways. Nature Structural & Molecular Biology, 17(1), pp.17–23.

Yu, W. Y., et al. (2021). Exogenous plant gma-miR-159a ameliorated hepatic stellate cell activation and inflammation via inhibiting GSK-3β. Journal of Inflammation Research, 14, 3939–3950. https://doi.org/10.2147/JIR.S304828

Yuan, B., Li, Z., Shan, H., Chen, H., Wang, Y. & Sun, L., 2022. Structural insights into the assembly and stability of C-phycocyanin from Spirulina platensis. International Journal of Biological Macromolecules, 205, pp.123–134. doi:10.1016/j.ijbiomac.2022.02.034.

Zahir, U., Ali, S., Iqbal, A. et al., 2019. Nutraceutical properties of Spirulina. Pakistan Journal of Pharmaceutical Sciences, 32(3), pp.1065–1072.

Zakaria, S.M., Kamal, S.M.M., Harun, M.R. & Omar, R., 2022. A review of microwave-assisted extraction of bioactive compounds from microalgae. Journal of Applied Phycology, 34, pp.2013–2027.

Zhang, D.M., Xu, H.G., Wang, L. et al., 2020. Protective role of carotenoids against cancer. Nutrients, 12(10), 3122.

Zhang, H., Kolb, F.A., Jaskiewicz, L. et al., 2004. Human Dicer preferentially cleaves dsRNAs at their termini without a requirement for ATP. EMBO Journal, 23(13), pp.3688–3698.

Zhang, Q., et al. (2023). The potential of Lycium barbarum miR166a in kidney cancer treatment. Experimental Cell Research, 423(1), 113377. https://doi.org/10.1016/j.yexcr.2023.113377

Zhang, X., Li, J.Y. & Gong, X.G., 2010. Isolation of C-PC subunits from Spirulina platensis and inhibitory effect on SPC-A-1 cell line. Journal of Zhejiang University, 37(3), pp.319–323.

Zhao, L. & Sweet, B.V., 2008. Lutein and zeaxanthin for macular degeneration. American Journal of Health-System Pharmacy, 65(13), pp.1232–1238. doi:10.2146/ajhp080052.

Zhao, P., Zang, Z., Xie, X. et al., 2014. The influence of different flocculants on the physiological activity and fucoxanthin production of Phaeodactylum tricornutum. Process Biochemistry, 49(4), pp.681–687. doi:10.1016/j.procbio.2014.01.007.

Zheng, J., Inoguchi, T., Sasaki, S. et al., 2013. Phycocyanin and phycocyanobilin from Spirulina platensis protect against diabetic nephropathy by inhibiting oxidative stress. American Journal of Physiology—Regulatory, Integrative and Comparative Physiology, 304(2), R110–R120. doi:10.1152/ajpregu.00648.2011.

Zheng, J., Inoguchi, T., Sasaki, S. et al., 2013. Protective effects of phycocyanin on diabetic nephropathy. Diabetologia, 56(7), pp.1474–1485.

Zhou, Z., Li, X., Liu, J., Dong, L., Chen, Q., Liu, J., ... & Zen, K. (2015). Honeysuckle-encoded atypical microRNA2911 directly targets influenza A viruses. Cell Research, 25(1), 39–49. https://doi.org/10.1038/cr.2014.130

Zhu, L., Li, Z. & Hiltunen, E., 2018. Microalgae bioflocculation: Chitosan as an effective flocculant. Bioresource Technology, 259, pp.274–281. doi:10.1016/j.biortech.2018.03.059.

Zinkoné, I., Montalescot, V., Rumin, J. et al., 2018. Effect of bead density and viscosity on microalgal cell disruption. Algal Research, 33, pp.110–118.

Zong, W., Qian, F., Qian, K.X. & Dong, Q., 2001. Anticancer activity of phycocyanin. Journal of Zhejiang University (Engineering Science), 35(6), pp.672–675.