CHAPTER II

LITERATURE REVIEWS

2.1 Spirulina Cultivation and Environmental Influences

Spirulina is a multicellular and filamentous cyanobacterium whose open helix cylindrical shape undergoes binary fission. It is classified within the phylum known as blue-green algae. It has been reported to exist in lakes, ponds, and lagoons in Kenya, Egypt, India, Thailand, France, etc. (Rich 1931; Vareschi 1982; El-Bestawy et al., 1996).

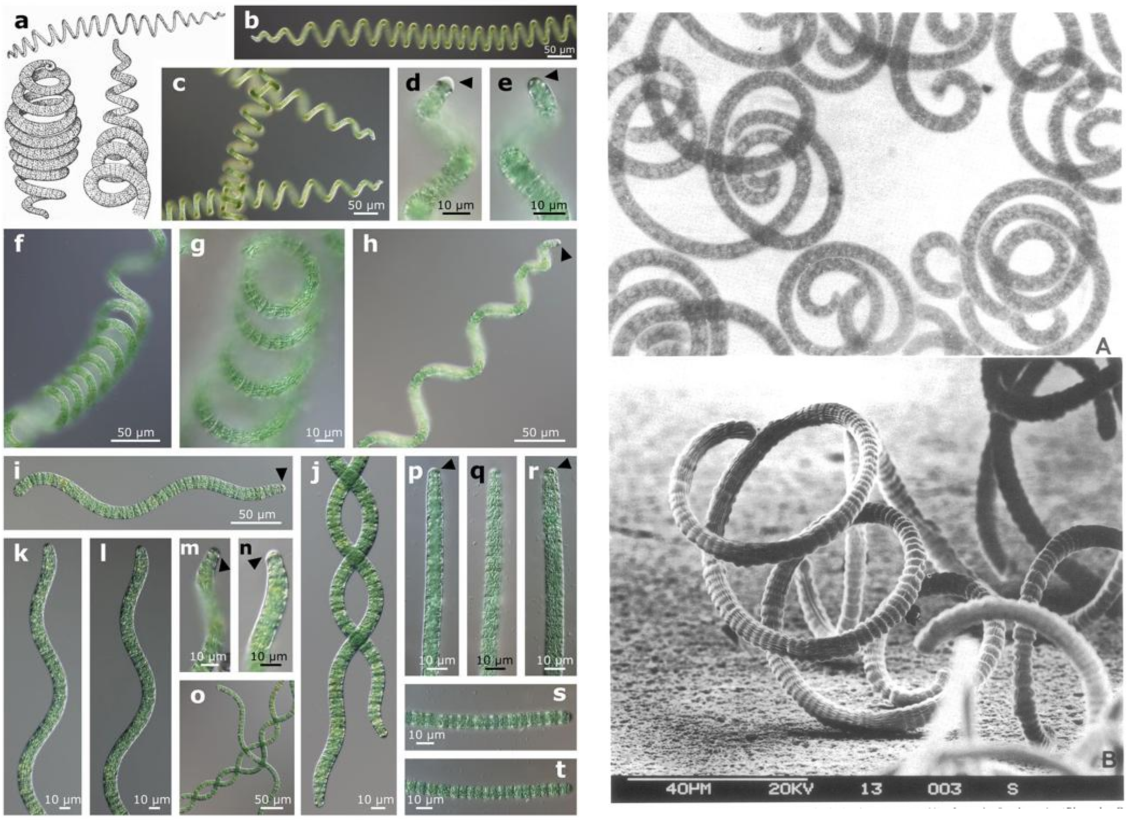

Figure 2.1 Microscopic examination of Spirulina under light microscope (Nowicka-Krawczyk, et al 2019) and scanning electron microscope (Aissaoui, O., et al 2017)

The growth conditions for Spirulina depend on nitrogen, potassium and bicarbonate concentrations, an optimal pH and culture salinity. Furthermore, since Spirulina is autotrophic, it has a very high content of macro and micronutrients, essential amino acids, proteins, carbohydrates, fats, fiber, vitamins, minerals, and antioxidants (Aouir, A. et al., 2017), and produces various pigments such as phycocyanin, lutein, and beta-carotene (Leema et al., 2010).

Table 2.1 Typical analysis of the composition of Spirulina (per 3 grams of dry biomass) (Shao, W., et al 2019)

Items	Amount	Items	Amount
General Composition		**Phytonutrients**	
Carbohydrates	17-25%	Chlorophyll	30 mg
Protein	53-62%	Total carotenoids	15 mg
Lipids	4-6%	β-carotene	6.8 mg
Minerals	8-13%	Total phycocyanins	519 mg
Vitamins		C-phycocyanin	240 mg
Vitamin A	11,250 IU	Zeaxanthin	9 mg
Vitamin B1	3.5 μg	Superoxide dismutase	1080 units
Vitamin B2	140 μg	**Minerals**	
Vitamin B3	400 μg	Calcium	10 mg
Vitamin B6	30 μg	Magnesium	15 mg
Vitamin B12	9.0 μg	Iron	6.5 mg
Vitamin E	285 μg	Phosphorus	33 mg
Inositol	1.7 μg	Potassium	60 mg
Biotin	0.5 μg	Sodium	30 mg
Folic acid	6.2 μg	Manganese	400 μg
Pantothenic acid	4.5 μg	Zinc	90 μg
Vitamin K1	60 μg	Boron	22 μg
Vitamin K2	15 μg	Copper	20 μg
		Selenium	0.9 μg
		Iodine	15 μg

Therefore, Spirulina has been used in food for a long time and is thought to be safe for human consumption based on the latest scientific findings. Additionally, to meet their protein needs, African tribes incorporate Spirulina biomass into their local cuisine (Beshi, M. 2021). It has been selected as one of the main meals for extended space travel by the National Aeronautics and Space Administration (NASA) and the European Space Agency (ESA). Spirulina is exported as a nutrition product in Bangladesh, Spain, France and Ireland just to name a few (Ihsanullah et al., 2017). Spirulina has gained popularity in the aquaculture, food, and health sectors due to its ease of harvesting, processing, and water-based growth. It has a high market value for use in the creation of value-added products and supplemental foods because of its high protein content and nutrient bioavailability. The growth of Spirulina is affected by various environmental factors. Physical factors are necessary for the industrial growth and production of Spirulina. Such as pH, temperature, light, and nutrients, which directly affect the photosynthesis process. (Abu et al., 2007; Colla et al., 2007; Madkour et al., 2012; Costa et al., 2003). Another significant factor influencing cell size, growth rate, and biochemical makeup is temperature. Low temperatures, however, have an impact on CO_2 synthesis, leading to photoinhibition, which lowers PSII efficiency and protein D1 repair (Vonshak, A., et al 2014). It also influences the production of carotenoid pigments. This is in charge of absorbing light energy and preventing damage to chlorophyll. When it comes to light quality and intensity, red light has the greatest effects on growth and protein accumulation because it directly absorbs chlorophyll a, phycocyanin, and allophycocyanin (Wang et al., 2007). Compared to other light conditions, blue light results in lower growth rates but encourages high protein production. Furthermore, the dry weight production efficiency is in the following order: Green > Yellow > Blue > White > Red. Excessive light levels (greater than 2500 μmol/($m^2 \cdot s$)) can cause ROS production and harm the PSII system. (Y. Zhang et al., 2024). Chlorophyll a and protein accumulation are increased by prolonged exposure to light (Posten, 2009). In addition to temperature and light levels, the makeup of nutrients and exposure to heavy metals have a variety of effects on cells. According to Muwafq and Bernd (2006), copper (Cu^{2+}) is extremely toxic, destroys chlorophyll, and promotes the production of ROS. Although it has less of an impact than copper, zinc (Zn^{2+}) promotes the production of plant paints (El-Maghrabi, 2002). Although it has less of an impact than copper and zinc, nickel (Ni^{2+}) stimulates the formation of partial pigmentation. Magnesium (Mg^{2+}): Enhances pH and encourages the synthesis of proteins and chlorophyll (Nyabuto et al., 2015). Macronutrients (N, P, C): At 2.5% salinity, formulas with $NaNO_3$, K_2HPO_4, and $NaHCO_3$ produce the highest yield (Nyabuto et al., 2015). Salinity influences these differences, affecting the growth, productivity, and size structure of Spirulina strains (Lao & Edullantes, 2025).

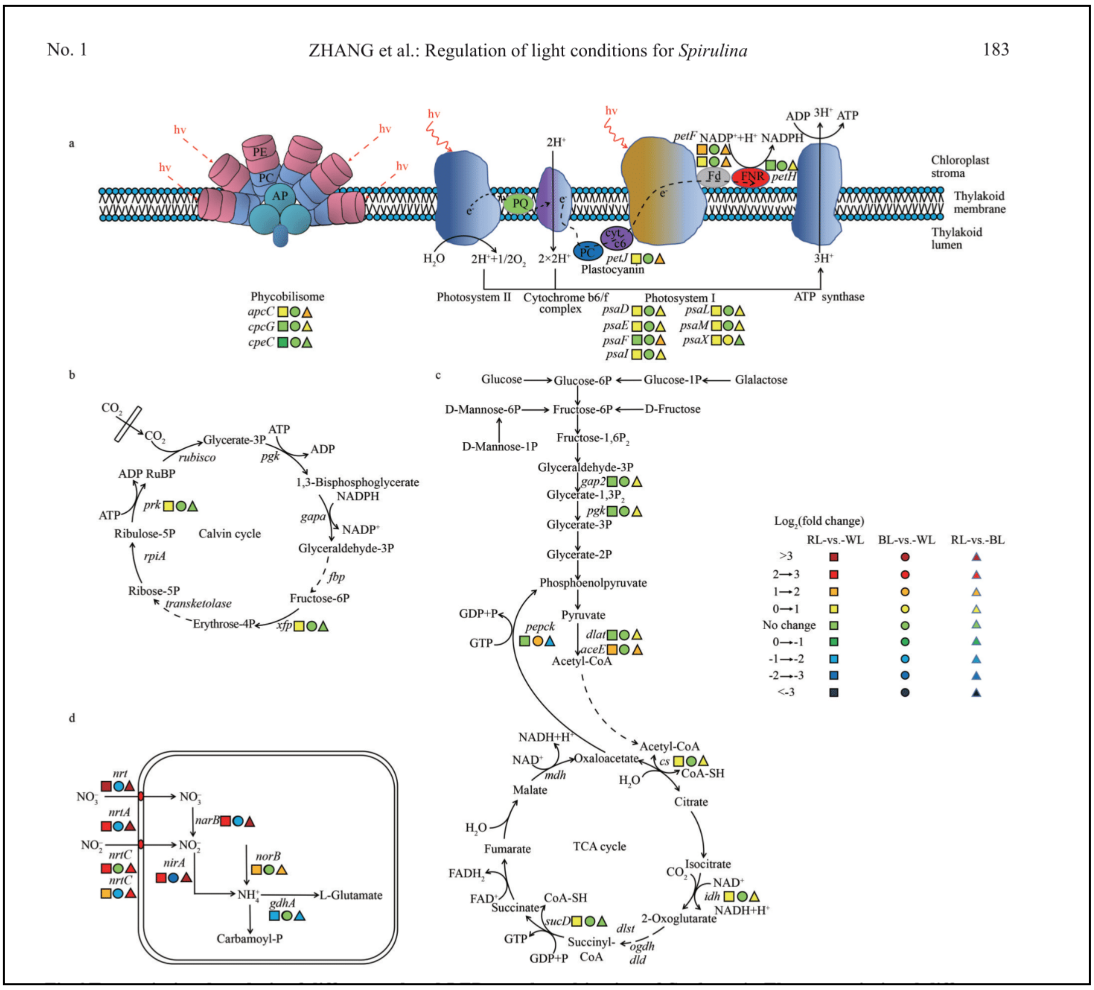

Figure 2.2 Regulation of light conditions for Spirulina **Source**: Zhang, Y., et al. (2024)

Spirulina can be grown in open systems, such as lakes, lagoons, and ponds, or in closed systems, like vertical column photobioreactors (PBRs) and polybags (Singh and Sharma, 2012). Although minor, space requirement has measurable effects on growth efficiency. Limited space can lead to uneven light distribution and reduced growth efficiency in dense cultures. And overcrowding limits light penetration and nutrient availability. The evaporation in open systems or high-temperature environments, water loss through evaporation can alter medium concentration. This may lead to increased salinity, affecting Spirulina’s metabolic activity and osmotic balance. Carbon dioxide (CO_2) is essential for photosynthesis. But it decreases via surface exchange or degassing reduces carbon availability. In case of Temperature (Weather Dependence). Outdoor systems are highly sensitive to daily and seasonal temperature fluctuations. Hydrodynamic Stress may be caused by mixing, aeration, or turbulence in reactors. Moderate agitation promotes gas exchange and prevents settling, but excessive stress can damage filaments and reduce productivity (Sili, C., et al 2012). A closed system (e.g., vertical column PBR, poly bags, etc.) or an open system (e.g., raceway pound, attached culture, etc.) can be used to cultivate industrial Spirulina. An open system has the advantages of being ideal for mass algae cultivation, reasonably priced, and simple to clean up after cultivation. Open culture systems have several drawbacks, including less control over culture conditions, trouble maintaining algal cultures for extended periods of time, low productivity, taking up a lot of land, being limited to a small number of strains of algae, and being easily contaminated. High mass transfer, good mixing with low shear stress, a high potential for scalability, ease of sterilization, good immobilization of algae, decreased photoinhibition and photo-oxidation, and the convenience of gas supply are the benefits of a closed system (Soni et al., 2021).

However, even in most controlled culture systems, differences between and within Spirulina strains continue to occur. The most recent research on Spirulina found both intra- and interspecific differences in chemical composition between *Arthospira* *platensis* and *Arthospira maxim*a, when cultivated under different light conditions (Milia et al., 2022)

However, even in the most controlled culture systems, differences between and within Spirulina strains continue to occur. Recent research has confirmed both intraspecific and interspecific variations in chemical composition and morphology, for example between *Arthrospira platensis* and *Arthrospira maxima* under different light conditions (Milia et al., 2022; Chaiyasitdhi, 2018). More recent publications also report increasing evidence of strain-specific differences, including taxonomic reclassification of commercially grown taxa into the new genus *Limnospira* (Nowicka-Krawczyk et al., 2019; Sinetova et al., 2024). These findings emphasize that both genetic background and cultivation conditions contribute to variability among Spirulina strains.

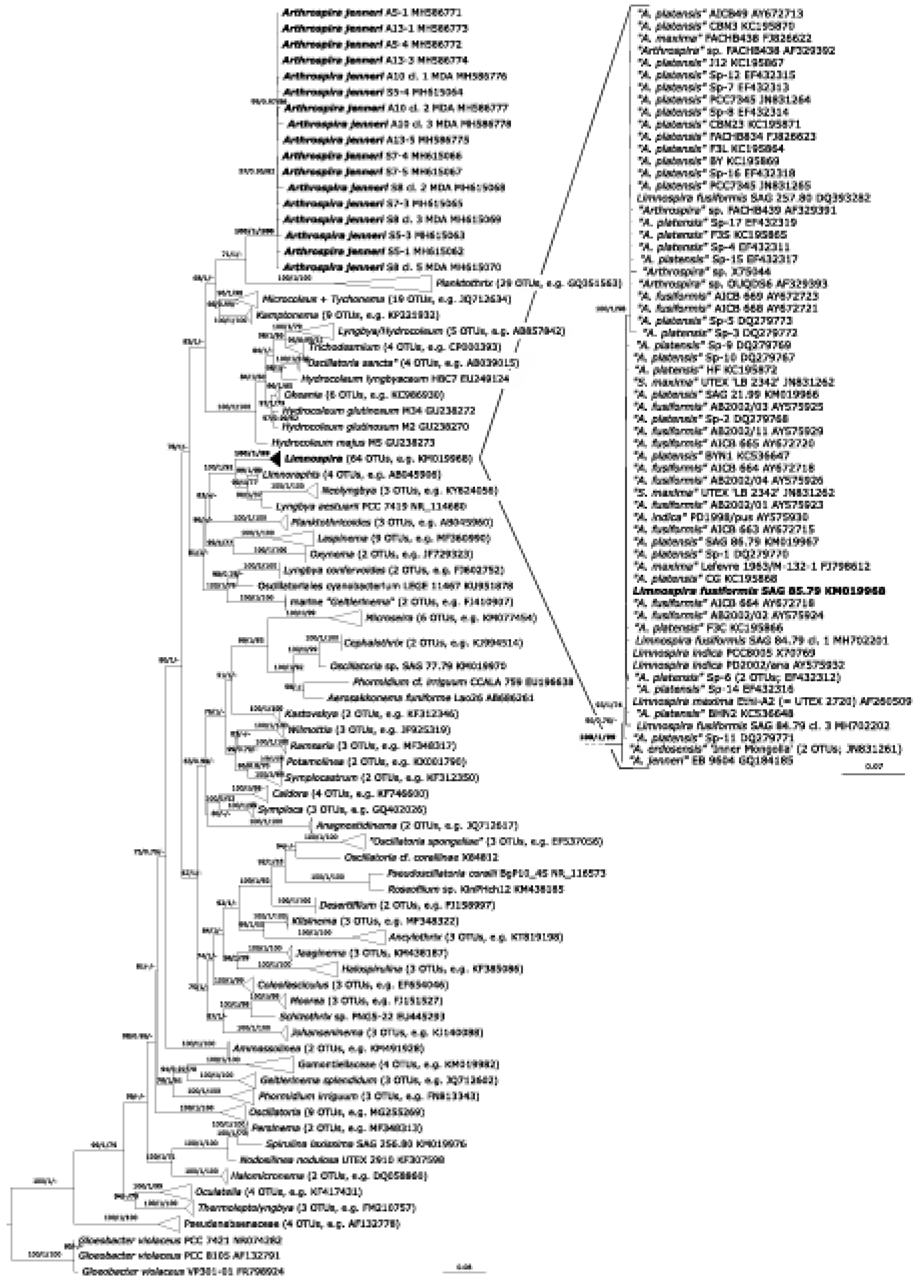

Figure 2.3 Phylogenetic tree of *Arthrospira* and *Limnospira*. Source: Nowicka-Krawczyk, P., et al 2019

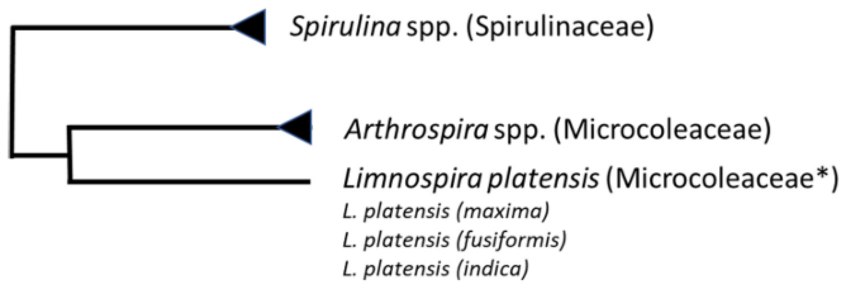

Figure 2.4 A simplified scheme of taxonomic positioning of Spirulina. Source: Sinetova, M. A., et al 2024

Beyond its biological structure, the increasing demand for Spirulina has led to widespread commercial production and innovation across the global topics that are explored in the following section.

2.1.1 Trends in the uses of Spirulina microalgae

Commercialized Spirulina is increasingly being grown and used as food and feed globally, including in Europe, due to its high nutritional value and health benefits as previous information. Regional Production and Consumption Patterns: Commercialized Spirulina is increasingly being grown and used as food and feed globally, including in Europe, due to its high nutritional value and health benefits. The FAO estimates that annual biomass production levels have reached roughly 30,000 tons, and its use has nearly doubled globally over the last 20 years. Spirulina is one well-known, secure, and nutritious food item. It has been officially classified as "Marine Algae" since 2017 and is included in the EU Food Catalog. As a result, it is now governed by EU Organic Products Regulation RCE 889/2008.

France's Paris. In 1974, Mr. Durand Chastel, the French manager of Sosa Texcoco, established the first large-scale commercial Spirulina biomass production plant after determining that Spirulina was a resource that was hindering the carbonate evaporation process at the carbonate production site in Mexico owned by the Sosa Texcoco Company. Microalgae cultivation has a long history, dating back to early international research conducted in the US, Japan, Germany, and other countries. Bur

Lew's 1953 book "Algal culture, from laboratory to pilot plant" went into great detail about this. The first pilot plant was established in Japan as a result of this research, and in 1960 a commercial *Chlorella* production facility was constructed there. In order to develop microalgae cultivation for protein products in Germany, a global effort was gathered at that time (Soeder and Binsack 1978).

Large-scale Spirulina production has also expanded to several nations, most notably the US and China, which currently account for more than half of global production. A global average of 20,000 tons annually is frequently reported, but Asian production volumes, which can range greatly from 10,000 tons annually to more, are frequently overlooked. (Wurmann et al., 2016; Virginin et al., 2022; Vieira et al., 2025).

Table 2.2 Trends in the uses of Spirulina microalgae (Soeder and Binsack 1978:Wurmann, C., et al 2016:Virgin, I., et al 2022.:Vieira, V. V., et al 2025)

Region	Major product	Consumption Trend	Market Characteristics
North America	USA, Mexico	High supplement consumption, growing natural food coloring market	Premium product positioning, well-developed distribution
Europe	France, Spain, Germany	Strong demand for natural ingredients, strict regulations on synthetic additives	Focus on sustainability, organic certification
Asia	China, India, Thailand, Japan	Rapidly growing production, increasing domestic consumption	Cost-effective production, traditional usage history
Latin America	Brazil, Chile	Growing production capacity, export-oriented	Favorable cultivation conditions
Africa	Kenya, Ethiopia, South Africa	Emerging production, focus on nutritional applications	Addressing malnutrition, development initiatives

2.1.2 Commercial Applications

Pigments in Spirulina gained attention in the field of food and human nutrition due to their health benefits and fluorescence under infrared light. As a result, they have been increasingly incorporated into various food and beverage products, and the market for this segment is anticipated to grow significantly by 2030, although the nutraceutical segment held the largest market share in 2021 (Desai, S. S., & Mane, V. K. 2024). Among other things, they have been added to ice creams, snacks, muffins, crackers, bars, cookies, bread, pasta and noodles, yoghurts, jelly gums, and smoothies and other drinks (Alfadhly, N.K.Z. et al 2022). Spirulysat® and Spirugrass® have been successfully registered by Algo Source. The first is a well-known extract with a high phycocyanin content that is enhanced with amino acids, polysaccharides, and other substances. However, Spirugrass®, a biorefining byproduct of Spirulina, is distinguished by its high content of beta-carotenes, iron, vitamin K, and amino acids. (T. Dalmonte, 2024)

Aquarists and Aquaculture The total production of aquaculture animals was 87.5 million tons in 2020, valued at USD 264.8 billion, and is projected to grow to 106 million tons in 2030. To meet the rising demand for aquatic animal foods, aquaculture development must continue to be innovative and sustainable. Microalgae can be added to aquaculture in two different ways: first, as food for zooplankton, which in turn provides food for fish and their larvae; second, as a component of feed for adult fish, replacing fish meal or fish oil. Aquaculture companies usually have their own microalgae production systems for feeding zooplankton.

Additionally, fish fed copepod fortified with *Chlorella* sp. and Spirulina (*Arthrospira*) sp. showed the highest growth and survival rates of *Betta splendens*. These copepods have the potential to replace fish oil and fish meals in diets, improving meat quality and growth, boosting immunity, and improving pigmentation in a variety of fish species. (Ahmad, M.T. et al 2020)

Bioactive compounds and lipids obtained from microalgae are becoming more widely acknowledged as viable substitutes for traditional synthetic ingredients in the cosmetic and skin care industries. A variety of cosmetic products, such as eyeliners, lipsticks, eye shadows, moisturizers, face cleansers, shampoos, sunscreens, and beauty masks, can be made using microalgae or particular bioactive compounds. The anti-aging and wrinkle-reducing properties of bioactive compounds from *Chlorella* sp. and Spirulina (*Arthrospira*) sp., which have antioxidant and free radical scavenging properties, make them useful in skincare and cosmetic products. In creams and sunscreens, carotenoids and peptides provide superior UV protection, while polysaccharides are best suited for moisturizing and supporting the reservation of the skin's oil balance and water barrier. Triacylglycerides, waxes, ceramides, phospholipids, sterols, as well as hydrogenated, esterified, and oxidized lipids, are varieties of microalgal lipids that are frequently utilized in cosmetics. With the growing demand for safe and environmentally friendly cosmetics and skin care products, ingredients derived from *Chlorella* sp. and Spirulina (*Arthrospira*) sp. are expected to play a significant role in the industry. Each of these lipids contributes distinct properties to cosmetic formulations, making them valuable and versatile ingredients. their capacity to offer sustainable and effective substitutes. Algenist used Spirulina to create the Blue Algae Vitamin C™, an active blue form of L-ascorbic acid. Spiruderm®, a liquid Spirulina extract with a high phycocyanin concentration, was created by AlgoSource and is used as an active ingredient to moisturize, re-densify, and smooth out fine lines on the skin. (Zhuang, D. et al. 2022: Ragusa, I. et al. 2021).

Feed for Animals Following extensive web research, it was discovered that there are very few animal feed products. Adding Spirulina to the diets of pigs and poultry has the potential to replace less sustainable protein sources like fishmeal while having no discernible effect on animal productivity or product quality. Spirulina has also been shown to have a number of positive effects on poultry, such as lowered cholesterol, better growth, and strengthened immunity. Additionally, it raises glutathione peroxidase, oxidant capacity, oxidative stability, and high-density lipoproteins in chickens. (B. A. Altmann & S. Rosenau, 2022)

Farming Global agricultural practices today heavily rely on synthetic pesticides and fertilizers, which has negative effects on the environment and human health. The growth of green gram (*Vigna radiata*), including the length of its shoots and roots as well as its weight at the flowering stage, was enhanced by biofertilizer extracts of *C. vulgaris* and Spirulina. Along with improving the plant's physical traits like its ability to absorb water and oil, these treatments also had a positive impact on the pH, EC, and mineral content of the soil. *Arthrospira* sp., or Spirulina, has gained interest recently as a sustainable source of agricultural biostimulants. This microalgae is probably the result of a combination of bioactive substances, including vitamins, amino acids, polysaccharides, and phytohormones. Thus, biostimulants derived from Spirulina may offer a sustainable substitute for chemical pesticides, fertilizers, and growth regulators. Furthermore, six of the nine fungal pathogen strains tested were successfully inhibited by the Spirulina-based biostimulants, which also decreased the growth of the pathogens. When the effects of two biostimulants Spirulina and Egyptian clover (*Trifolium alexandrinum*) on the soil characteristics, growth, and yield of a pea (*Pisum sativum* L.) plant were assessed, it was discovered that applying these biofertilizers separately or in combination greatly enhanced plant growth and yield. (A. L. Gonçalves, 2021).

Pharmaceutical Substances Numerous bioactive substances with a variety of biological characteristics, such as anti-inflammatory, anticoagulant, antioxidant, antimicrobial, anticancer, and neuroprotective effects, are produced by microalgae. The bioactive substances found in Spirulina (*Arthrospira*) sp. and *Chlorella* sp. may aid in the creation of novel medications for both humans and animals. (A. P. Abreu et al., 2023)

Biopolymers The rapid rate of global industrialization, which is currently endangering global stability, necessitates urgent attention to the replacement of petroleum-based products and polymers. The ability of Spirulina to produce PHA through induced nitrogen deficiency has been investigated. Coelho et al. and Costa et al., for example, demonstrated yields of 30.7% PHA (w/w dry biomass) and 12.0% PHA (w/w dry biomass), respectively. A study by Corrêa et al. from 2021 also used Spirulina to produce PHA. (S. G. Mastropetros et al., 2022).

Since *Chlorella* sp. and Spirulina (*Arthrospira*) sp. can treat a wide range of wastewater types, including municipal, aquaculture, swine, olive oil milling, distillery, confectionary, brine tapioca, tofu, rubber, paper mill, dye wastewaters, agricultural runoffs, and groundwaters, wastewater treatment by these two species has been documented. *Chlorella* sp. and Spirulina (*Arthrospira*) sp. biomass can be generated and then valorized during wastewater treatment. It should be mentioned that microalgae-based wastewater systems are different from traditional wastewater treatment methods because they are inexpensive and offer two main advantages: (1) wastewater purification and (2) simultaneous biomass harvesting. Furthermore, a recent study demonstrated that the wastewater treated by microalgae could be used again for irrigation, which is thought to be the primary use of freshwater and is highly advantageous for the environment and economy. (A. P. Abreu et al., 2023)

Table 2.3 Industrial Applications and Cultivation (Ahmad,M.T. et al 2020: Gonçalves, A. L. 2021: Ragusa, I. et al 2021: Altmann, B. A., & Rosenau, S. 2022: Mastropetros, S. G., et al 2022:Zhuang, D. et al 2022:Alfadhly, N.K.Z. et al 2022: Abreu, A. P., et al 2023: Desai, S. S., & Mane, V. K. 2024: Dalmonte, T. 2024)

Industry Sector	Applications	Growth Driver
Food & Beverages	Natural food colorant (blue), nutritional supplements, protein-enriched foods, bakery products, beverages, confectionery, dairy	Clean-label trend, plant-based diet growth, natural colorant demand
Nutraceuticals	Dietary supplements (tablets, capsules, powders), functional ingredients, protein supplements	Health consciousness, preventive healthcare, aging population

Personal Care & Cosmetics	Natural pigments, antioxidant formulations, anti-aging products, skincare, hair care	Clean beauty trend, demand for natural ingredients
Feed for Animals	Aquaculture feed, poultry feed, pet food supplements	Sustainable farming practices, demand for natural feed additives
Agriculture	Biofertilizers, biostimulants, plant growth enhancers	Organic farming growth, sustainable agriculture
Pharmaceuticals & Biotechnology	Therapeutic proteins, bioactive peptides, genetic engineering platforms	Research advancements, biomedical applications
Environmental Applications	Wastewater treatment, carbon capture, bioremediation	Climate change mitigation, environmental regulations

Cultivation	Open raceway ponds, Photobioreactors, Thin-layer cascade systems	Commercial biomass production
Harvesting	Filtration, Centrifugation, Flocculation	Biomass recovery
Drying	Spray drying, Sun drying, Freeze drying	Powder production
Extraction	Ultrasound-assisted, Enzymatic, Supercritical CO_2	High-value compound isolation
Formulation	Encapsulation, Tablet compression, Blending	Product development
Biorefinery	Fractionation, Sequential extraction	Multiple product streams

Table 2.4 Product Forms and Their Industrial Utilization (Payne, E. 2023: Sabat, S., et al 2025)

Product Form	**Primary Industries**
Powder	Food, Nutraceuticals, Cosmetics
Tablets & Capsules	Nutraceuticals, Pharmaceuticals
Phycocyanin	Food coloring, Cosmetics, Biotechnology
Liquid Extract	Beverages, Skincare
Flakes & Granules	Food, Animal Feed

The integration of exogenous genes into the Spirulina chromosome through markerless homologous recombination and the stable, high-level expression of therapeutic proteins, such as bioactive peptides, single-chain antibodies, enzymes, signaling proteins, and vaccine antigens, are examples of genetic engineering techniques for Spirulina. (B. W. Jester et al., 2022).

According to recent research, algae hold promise as a biomass source for the production of bioplastics because they provide a number of benefits, including lower carbon dioxide emissions, less food waste, and lower energy consumption. The high protein content of microalgae is a key component of the polymer's advantageous characteristics. Numerous studies on microalgae have taken advantage of their biomass potential for the production of biofuel, biochemicals, and substitute foods. (W. Y. Cheah et al., 2023).

Biorefineries based on Spirulina are a good way to improve both environmental sustainability and economic viability. The current review examines the difficulties and potential future developments related to the biorefining of Spirulina to produce protein and c-phycocyanin, with the goal of advancing a circular bioeconomy. (B. Thevarajah et al., 2022).

The potential health benefits of certain bioactive peptides derived from Spirulina, including their antimicrobial, antiallergic, antihypertensive, antitumor, and immunomodulatory qualities, are being investigated. (C. A. Ovando et al. 2018).

The most prevalent organic carbon sources that increase the biomass yield in Spirulina are glucose and acetate, as has been extensively documented. Therefore, it is possible that the organic materials (such as lactose, fats, proteins, and perhaps other additives) commonly found in wastewater from dairy processing could promote Spirulina cell growth. Furthermore, research has shown that the organic carbon content may help cells grow during the later stages of a growth cycle when the light-restricted cultures' photosynthetic activity is at its lowest. (W. T. Chang and others, 2013)

Table 2.5 Recent Industrial Innovations and Emerging Applications (Chang, W. T., et al 2013: Ovando, C. A., et al 2018: Thevarajah, B., et al 2022: Jester, B. W., et al 2022: Cheah, W. Y., et al 2023)

Innovation	**Industrial Sector**
Genetic engineering of Spirulina for therapeutic proteins	Biotechnology, Pharmaceuticals
Spirulina-based bioplastics	Packaging, Materials
Spirulina biorefinery systems	Multiple industries
Spirulina as therapeutic protein production platform	Pharmaceuticals
Spirulina-derived peptides as functional ingredients	Food, Cosmetics
Carbon-capture utilization with Spirulina	Environmental, Energy

2.1.3 Assessment of Spirulina Quality

Microbiological testing is an essential procedure for preventing and identifying health, safety, and well-being crises. It can identify contaminants like pesticides and heavy metals (cadmium, lead, arsenic, and mercury) as well as harmful bacteria like *Salmonella*, mold, yeast, and *E. coli*. (M. A. Pfaller et al., 2004).

In contrast, physical quality testing is a more accessible method, as it involves evaluating external characteristics such as the color, odor, and texture of Spirulina. In recent years, several physical quality testing methods have been further developed, particularly through the application of CAPP (Cold Atmospheric Pressure Plasma): Boosts lipid production in *Chlorella vulgaris* and lowers pH from 7.8 to 2.4 in 6 minutes (J.Q.M. Almarashi et al., 2020). Multi-needle gas-liquid hybrid discharge reactor that uses a gas stream combined with oxygen and air to inhibit algae (N.N. Aye et al., 2012). And non-thermal plasma: Disrupting the cell wall of Nannochloropsis gaditana for better lipid extraction (A.P. Matos et al., 2019) Cold plasma oxidation: Removal of harmful algae and BMAA toxins in water bodies while reducing pH to 2.5–3.5 (B. Nisol et al., 2019)

In commercial production, production standards is symbols displayed on the product to ensure the quality and standards that the product has passed the inspection in accordance with the requirements of the responsible authority such as, Certified by USDA Organic, Non-GMO Project, Naturland, FDA, ANSI, and more. GMP standard and HACCP, ISO system Environmental Monitoring Water quality, pH, temperature, light, and nutrient levels. It is found that product quality standards often focus mainly on preventing potential harm to consumers, such as the HACCP system that emphasizes risk control from the production process to consumption. However, if the product does not meet the claim, such as components or properties that do not match the stated product, the product will not be used as a product for the first time. FDA standards will play a role in consumer protection. Especially in the field of supervision, advertising and health references of new dietary supplements and food products (novel foods). While the validation of species used in the food industry can be done with biomolecular techniques such as SDS-PAGE, which is used to specifically identify green seaweed species Ulva and Enteromorpha that are allowed to be used in the food industry (Grobbelaar, J. U. 2003). Nutritional Analysis, there are a variety of analysis methods by technique, with destructive methods and experimental examples including HPLC, Macro-Bradford Assay, Phenol sulfuric acid method, and Bigogno method. Non-destructive is a technique that performs the analysis without much deterioration of the sample. Chemical analysis will be ICP-AES, ICP-MS techniques in the case of mineral samples, XRD techniques, surface analysis will be XPS, ToF-SIMS techniques, and molecular units, valences, and coordination environments will be Raman, FTIR, Mossbauer, XAFS techniques.

FTIR and Raman will be popular. Through samples at lower concentrations or weaker signals, such as the band at 2713 cm^{-1} attributed to the aldehyde (O=) C-H stretch and the band at 1702 cm^{-1} attributed to the carbonyl group, FTIR will yield more sensitive data than Raman in terms of quick analysis and less preparation (Yan, B., Gremlich, H. U., et al 1999). However, FTIR has been shown in numerous studies to be a rapid indicator of the content of biomolecules (Sharma, V. et al. 2020: AlShikaili, T. Y., et al. 2022). No difficult sample preparation Consequently, the expense of analysis is reduced. The sample won't deteriorate if it is analyzed again during the process. It is therefore the best option for production or industries that need constant quality control, like rapidly expanding microorganism production lines. However, the technique and the specifics of fat cannot be analyzed by FTIR. destructive techniques like GC-MS or HPLC, but they can also be used as an effective biochemical trend indicator, particularly when tracking changes in the composition of biomolecules.

2.2 FTIR (Fourier Transform Infrared Spectroscopy)

In FTIR, it shoots IR radiation from a Glowbar source through the sample material and then to a beam splitter, which splits half of the beam of radiation that has passed through the sample material to a rotating glass. Both the reflected beam and the transmitted beam are reflected back to the beam splitter, which stores all the wavenumber data at once, and because there are so many data points, a computer must be used as an important component in the FTIR spectrometer to analyze the data

The FTIR has several advantages: first, it has a high signal-to-noise value, which helps to provide a clear signal. Therefore, multiple scans can be done quickly and the data is combined. The signal from the true peak is positive, and it usually occurs at the same frequency when it shines. The peak of the noise is both positive and negative and occurs at a random number of waves, so the signal of the true peak is repeated many times, but the signal of the interference wave is canceled, so we can obtain a clear IR spectrum and use a small sample (1 milligram or less) and obtain a spectrum of good quality as used. Because the frequency is compared to the He-Ne laser's peak, it also has the benefit of being accurate.

Absorption band (cm^{-1})	Assignment
~3,010	trans-CH=CH-
~2,955	υ_{asym} CH_3 in lipids
~2,920	υ_{asym} CH_2 in lipids
~2,875	υ_{sym} CH_3 in lipids
~2,850	υ_{sym} CH_2 in lipids
~1,740	C=O stretching in lipids and fatty acids
~1,650	Amide I: C=O vibration
~1,546	Amide II: N–H and C–N vibration
~1,455	CH_2/CH_3 in lipids and proteins
~1,240	υ_{asym} PO_2^- in nucleic acids or phospholipids
~1,200–900	υ(C–O–C) of polysaccharides

υ_{asym}, asymmetric stretch; υ_{sym}, symmetric stretch.

Figure 2.5 Assignments of the main absorption bands in the FTIR spectrum of Spirulina. Source: Liu, J., et al 2022

Biochemical analyses, however, can identify species- or even strain-specific composition differences. Raman spectroscopy for instance is an inelastic light scattering technique that is workable with liquid samples. In contrast, this technique is expensive and the information collected is often biased by machine noise. The HPLC technique is commonly used in the separation of substances and can clearly identify the type and quantity of given organic compounds. but this technique requires to prepare chemicals and solvents and is even more expensive.

An alternative, far less expensive method for determining a sample's infrared spectrum of absorption, emission, and photoconductivity is FTIR/UV-Vis. It can also be used to identify various molecular functional groups. The biochemical profiles of chicken, grassland plant species, yeast, and *Cannabis sativa* L. have all been examined using this technique in the past (Shapaval et al., 2019; Rana et al., 2018; Katemala, S., et al 2022; Siano et al., 2018).

Moreover, FTIR/UV-Vis is inexpensive, quick, and requires little sample preparation. It is therefore an appropriate technique in terms of determining species-specific or standard culture compounds, both in the industry and in start-ups that require quality control measurements without the need of elaborate and expensive equipment.

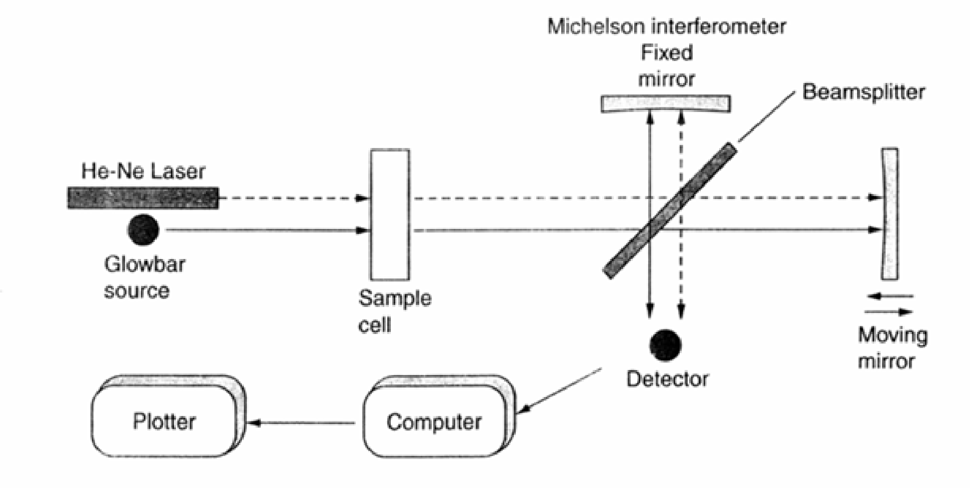

Figure 2.6 Infrared absorption frequencies of various functional groups

Source: http://old-book.ru.ac.th/e-book/c/CM328/CM328-10.pdf

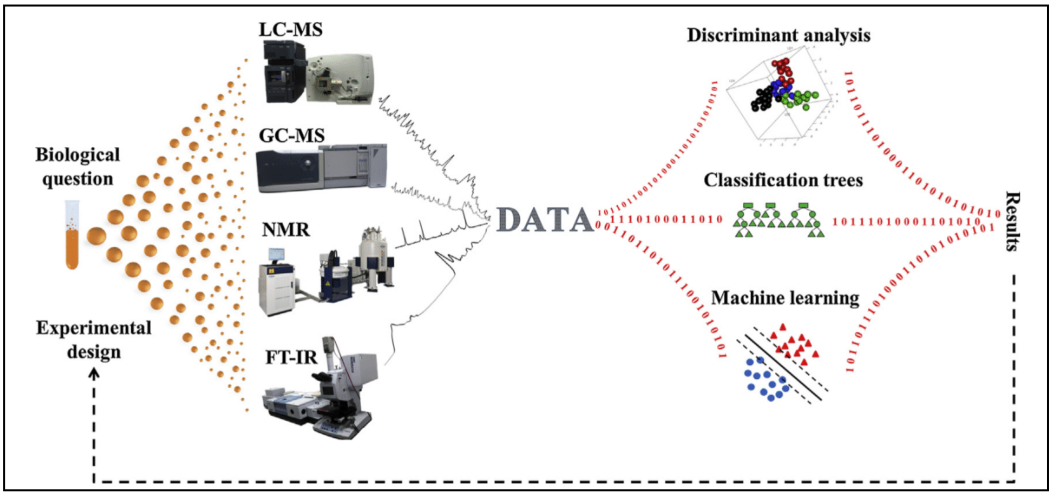

Figure 2.7 A graphical representation of the different analytical approaches and informatics techniques employed in metabolomics studies. Source: Gromski, P. S., et al 2015

2.3 Partial Least Squares (PLS-DA)

One of the most widely used classification methods in chemometrics, and particularly in the metabolomics category, is PLS-DA. However, PLS-DA is still frequently a subject of debate and interpretation in spite of the Metabolomics Standards Initiative's (MSI) guidelines for reporting research findings. The need for effective and trustworthy data analytics tools is growing as the bioinformatics (such as genomics, molecular phylogenetics, metabolomics, proteomics, chemoinformatics, and drug design) market is predicted to reach US$12.48 billion in 2020. The field of metabolomics has seen a sharp rise in research publications over the last ten years due to its recognition as a valuable tool for choosing attributes and classifications, particularly in data sets with high and complex data dimensions, like data from chemometrics and omics. Discriminant analysis using partial least squares (PLS-DA) A chemometrics method called PLS-DA is used to identify differences between sample groups, which are the relationship between two matrix data, X (raw data) and Y (groups, class membership, etc.).

Through the identification of plot scores with few dimensions, this technique makes complex data sets visually interpretable and illustrates the division between the groups. as depicted in the figure. Validation data predictions should be near 1, and if they are near 0.5, the model is not accurate enough and should not be used for predictions (Gromski, P. S., et al 2015).

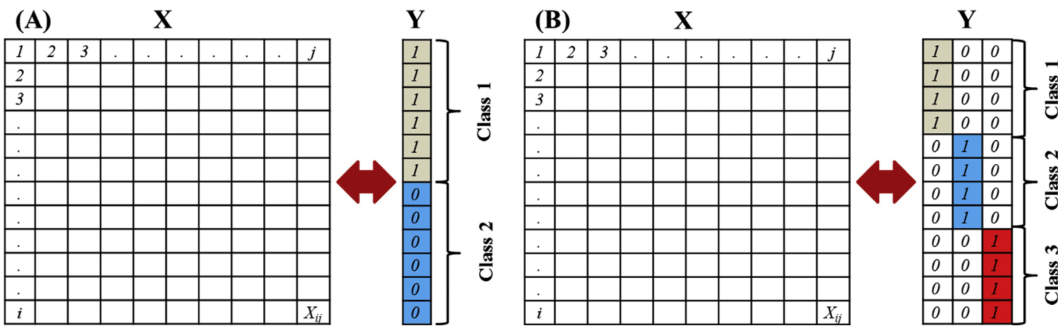

Figure 2.8 An illustration of partial least squares-discriminant analysis (PLS-DA)

Source: Gromski, P. S., et al 2015

2.4 Quasar

Quasar is an open-source software tool designed to make machine learning accessible for analyzing biospectroscopy data, particularly infrared spectroscopy. In biomedical and biochemical spectroscopy research, data quantities are often too large to interpret effectively, particularly when multiple replicates and analytical techniques are required for reliable results. The commercial software users face the fact that currently available data analysis tools suffer from poor user-friendliness, limited capabilities, and difficult access, while problem-specific software or scripts are often highly specialized or simply too hard to use. As machine learning techniques increasingly involve data analysis in natural sciences, there is a growing demand for user-friendly and flexible tools that can effectively combine machine learning with spectroscopy datasets. The open-source software with strong community engagement is the way forward to counter these problems. Quasar was developed as a user-friendly solution that promotes reproducibility and community contribution while remaining flexible enough to work with various machine learning techniques. Currently, 35 scientific publications have successfully used Quasar. (Toplak, M., et al 2021).