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Protein Balls: Nutritional and Functional Exploration of Spirulina from Cyanobacteria

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ABSTRACT

Spirulina, a blue-green cyanobacterium that flourishes in alkaline waters, has gained global recognition as a nutrient-dense superfood. This review explores the biology and cultivation of its two principal species—Arthrospira platensis and Arthrospira maxima—with emphasis on their filamentous morphology, taxonomy, and life cycle characteristics in both natural habitats and controlled environments. Major producers include China, India, the United States, Brazil, and Egypt, underscoring Spirulina's potential for large-scale cultivation. The dried biomass exhibits an impressive nutritional composition, comprising 55–70% protein, 15–25% carbohydrates, 6–8% lipids, and 8–10% dietary fiber. It is also rich in essential vitamins (B_1, B_2, B_3, B_6, B_9, B_{12}, C, D, and E) and minerals such as iron, magnesium, and selenium. Spirulina's characteristic pigmentation and antioxidant properties are attributed to chlorophyll a, beta-carotene, and C-phycocyanin. In addition to its nutritional merits, Spirulina provides various therapeutic benefits, including immune modulation, neuroprotection, cardioprotection, and anticancer effects. To preserve its sensitive bioactive constituents, packaging in airtight, vacuum-sealed, or foil-lined containers is recommended. This review also highlights a novel application: the incorporation of Spirulina powder into functional protein balls formulated with cocoa and jaggery. The final product was evaluated for sensory attributes and nutritional content through biochemical analysis. High-performance liquid chromatography (HPLC) revealed the presence of seventeen amino acids, with alanine (118.6 mg/kg), leucine (99.4 mg/kg), and aspartic acid (90.8 mg/kg) being the most abundant. These findings reinforce Spirulina's potential as a multifunctional food ingredient with significant nutritional and therapeutic value.

Keywords: Spirulina, Arthrospira platensis, cyanobacteria, superfood, protein content, antioxidants, HPLC amino acid profiling, functional foods, immunomodulatory activity, packaging stability, C-phycocyanin, nutraceuticals

1. Introduction

Spirulina, a filamentous, photosynthetic cyanobacterium of the genus Arthrospira, has emerged as a potent superfood due to its rich nutritional profile and numerous health benefits. Naturally found in alkaline and saline water bodies, Spirulina has been consumed for centuries and is now recognized globally as a highly efficient, sustainable source of plant-based protein (Belay et al., 1993; Habib et al., 2008). It is composed of up to 60–70% protein by dry weight and contains a broad spectrum of vitamins (such as B-complex and vitamin E), essential fatty acids (notably γ-linolenic acid), minerals (like iron and calcium), and bioactive compounds such as phycocyanin and chlorophyll (Karkos et al., 2011).

In recent years, the growing consumer demand for functional foods and convenient dietary options has driven the development of Spirulina-enriched products, including protein balls. Protein balls, also known as energy bites, are nutrient-dense, bite-sized snacks formulated to provide quick and balanced energy, often with added health-promoting ingredients. Incorporating Spirulina into these snacks not only enhances their protein content but also introduces antioxidant, anti-inflammatory, and immunomodulatory effects associated with Spirulina's bioactive components (Gershwin & Belay, 2008; Deng & Chow, 2010).

This research paper aims to explore the nutritional composition and functional potential of Spirulina with a specific focus on its integration into protein balls. By evaluating the health benefits, bioactive constituents, and formulation strategies, this study seeks to underline Spirulina's role in advancing the development of innovative, functional, and sustainable food products.

1.1. Classification and Morphology

Scientific name: Arthrospira
Family: Microcoleaceae
Order: Oscillatoriales

Higher classification: Cyanobacteria (blue-green algae)
Kingdom: Bacteria
Genus: Arthrospira
Arthrospira, commonly referred to as Spirulina, is a filamentous, photosynthetic cyanobacterium known for its high protein content (up to 70% of its dry weight), abundance of antioxidants, and a wide range of essential nutrients. It thrives in alkaline water environments and is widely used as a superfood due to its exceptional nutritional profile and health-promoting properties (Habib et al., 2008; Khan et al., 2005).
Under microscopic observation, Spirulina appears as multicellular, unbranched, helicoidal filaments with cylindrical cells arranged in trichomes. The genera can be differentiated by variations in the helical shape and dimensions. Spirulina platensis typically has a helix diameter of 35–50 µm and a pitch of 60 µm, while Spirulina maxima shows a diameter of 50–60 µm with a pitch of 80 µm. Interestingly, the cell diameter is larger in S. platensis (6–8 µm) than in S. maxima (4–6 µm) (Ciferri, 1983; Belay, 1997).

1.2. Life Cycle of Spirulina

Spirulina propagates via both natural and artificial methods:
A) Natural Propagation
Spontaneous trichome fragmentation and the formation of necridia facilitate growth and dispersal in natural aquatic habitats (Tomaselli, 1997).
B) Artificial Cultivation
In artificial settings, Spirulina is harvested by filtration using nylon mesh, followed by repeated washing to remove residual salts. To facilitate further processing, the biomass is pressed to reduce water content. pH is stabilized using acid, and filaments are broken into fine particles via grinding. Spray drying is commonly used for dehydration, accounting for roughly 25 ± 5% of production costs. The dried material is then packed in airtight plastic bags and stored in cool, dark environments to prevent nutrient degradation (Becker, 2007).

1.3. Production of Spirulina

Spirulina biomass primarily originates from two species—Arthrospira platensis and Arthrospira maxima. These species grow optimally in alkaline (pH 8.0–11.0), warm (30–40°C), and nutrient-rich water enriched with carbonates, nitrates, and phosphates (Richmond, 2004).
Top producing countries include China (15.1%), India (11.6%), the United States (8.2%), Brazil (8.0%), and Egypt (6.5%) of global production (FAO, 2018).
In India, leading production states are Tamil Nadu (~1,500 MT/year), Karnataka (~400 MT/year), and Andhra Pradesh (~300 MT/year), with an annual national output of 2,000–2,500 metric tons. The sector is growing at an estimated rate of 15–20% annually, driven by increased demand for nutritional supplements and adoption of sustainable cultivation techniques (Mohan et al., 2013).

1.4. Nutritional Composition and Bioactive Components

Spirulina is a nutrient-dense cyanobacterium widely consumed as a dietary supplement. Typically available in powder or tablet form, it is renowned for its rich content of proteins (55–70%), carbohydrates (15–25%), lipids (6–8%), fiber (8–10%), and minerals (7–13%) (Gershwin & Belay, 2008).
It contains a wide spectrum of vitamins including B_1, B_2, B_3, B_6, B_9, B_{12}, C, D, and E, along with essential minerals such as potassium, calcium, chromium, copper, iron, magnesium, manganese, phosphorus, selenium, sodium, and zinc. Spirulina also exhibits a rich profile of pigments such as chlorophyll a, beta-carotene, xanthophylls, and zeaxanthin—contributing to its potent antioxidant properties. Notable proteins include C-phycocyanin and allophycocyanin, while enzymes such as lipase enhance its therapeutic value (Karkos et al., 2011).
Nutritional values may vary based on strain, environmental factors, and harvest conditions.

1.5. Medicinal and Therapeutic Applications

Spirulina is recognized as a "superfood" both on Earth and in space-based nutrition programs (Henrikson, 2010). It is a rich source of antioxidants, such as beta-carotene and tocopherols, which neutralize free radicals and reduce oxidative stress—a key factor in diseases such as cancer, diabetes, atherosclerosis, and rheumatoid arthritis (Karkos et al., 2011).
Therapeutic properties of Spirulina include cholesterol reduction, anticancer potential, immune enhancement, radioprotection, nephroprotection, and anti-obesity effects. It increases lactobacilli populations in the gut and modulates immune cells like NK (natural killer) cells. In combination with Bacillus Calmette-Guérin (BCG) cell wall skeletons, Spirulina has shown enhanced effects in anti-tumor immunotherapy (Jimenez et al., 2003).
Spirulina has also demonstrated anti-inflammatory, hypoglycemic, neuroprotective, and hepatoprotective effects in both preclinical and human studies (Bhat & Madyastha, 2001; Torres-Duran et al., 2007).

1.6. Packaging and Storage

Proper packaging is essential to preserve the bioactive integrity of Spirulina. As a hygroscopic material, Spirulina readily absorbs moisture from the air, making airtight packaging crucial. It is typically stored in vacuum-sealed or foil-lined containers to protect against humidity, oxidation, and light exposure. Optimal packaging maintains nutrient stability, product freshness, and shelf life until consumption (Becker, 2007).

2. Materials and Methodology

Study Design

This research is a comprehensive literature-based review supported by qualitative analysis of previously published peer-reviewed studies, databases, and institutional reports. The aim is to explore the taxonomy, cultivation techniques, biochemical properties, therapeutic applications, and packaging of Arthrospira platensis, commonly known as Spirulina.

Data Collection

Relevant literature was gathered from various scientific databases, including:

- PubMed
- ScienceDirect
- Google Scholar
- SpringerLink
- FAO database
- WHO database

Search terms included "Spirulina", "Arthrospira platensis", "cyanobacteria", "Spirulina cultivation", "Spirulina nutrition", "C-phycocyanin", "Spirulina antioxidant", and "Spirulina packaging". Only English-language, peer-reviewed articles and institutional reports published between 2000 and 2024 were considered.

Inclusion criteria:

- Articles detailing the cultivation, life cycle, morphology, biochemical composition, and medical or industrial applications of Spirulina.
- Reports and studies on Spirulina production statistics.
- Research on Spirulina packaging and preservation strategies.

Exclusion criteria:

- Articles without accessible full texts.
- Non-scientific web sources and commercial product advertisements.

Cultivation Method (Literature Review Based)

To understand Spirulina cultivation, both open pond and photobioreactor-based systems were reviewed:

Open Raceway Ponds: Outdoor raceway ponds with paddle wheels to circulate Spirulina culture are widely used for commercial production due to low cost and ease of scale-up (Becker, 2007; Richmond, 2004).

Photobioreactors (PBRs): These closed systems allow for sterile growth conditions, temperature regulation, and optimal light exposure, resulting in higher purity and yield, albeit at a higher operational cost (Tredici, 2010).

Parameters reviewed for optimal cultivation:

- pH: 8.5–10.5
- Temperature: 30–40 °C
- Light intensity: 2,000–10,000 lux
- Nutrients: Sodium bicarbonate, nitrates, phosphates, and trace elements (Richmond, 2004).

Biochemical and Nutritional Analysis

This review compiled data from studies that performed proximate composition and biochemical profiling of Spirulina using:

- Spectrophotometry: Used for estimating pigments like chlorophyll-a, C-phycocyanin, and beta-carotene (Belay, 1997).
- High-Performance Liquid Chromatography (HPLC): Applied for amino acid and vitamin profiling (Habib et al., 2008).
- Kjeldahl Method: Used to determine total nitrogen and calculate crude protein content (AOAC, 2016).
- Soxhlet Extraction: Employed to estimate lipid content.
- Ashing: For mineral content estimation.
- Enzymatic and non-enzymatic assays: Used in antioxidant profiling.

The variation in nutritional content due to species, geographical location, and cultivation conditions was also critically evaluated.

Medicinal and Therapeutic Properties Evaluation

The medicinal effects of Spirulina were studied through secondary data analysis from in vitro, in vivo, and clinical studies:

Anti-inflammatory and antioxidant activity: Identified through DPPH and FRAP assays (Karkos et al., 2011).

Immune-modulating activity: Reviewed from studies demonstrating NK cell activation and increased cytokine production (Jimenez et al., 2003).

Anticancer, hepatoprotective, and antidiabetic effects: Extracted from studies on rodent models and human trials (Torres-Duran et al., 2007; Bhat & Madyastha, 2001).

Packaging and Shelf-Life Analysis

Literature detailing best practices for Spirulina packaging and storage was assessed. Variables included:

Moisture sensitivity: Due to its hygroscopic nature, studies emphasized using vacuum-sealed or foil-lined laminated pouches (Becker, 2007).

Storage conditions: Temperature, light exposure, and relative humidity were reviewed in terms of their impact on pigment and protein stability.

Shelf-life: Studies indicating degradation rates of C-phycocyanin and essential fatty acids were included (Henrikson, 2010).

3. Testing

High-Performance Liquid Chromatography (HPLC) Analysis of Amino Acids

High-performance liquid chromatography (HPLC) coupled with fluorescence detection (FLD) was employed to quantify the amino acid composition of Spirulina powder. The analysis was conducted using an Agilent 1200 HPLC system fitted with a C18 reverse-phase column at a controlled temperature of 43°C. The flow rate was maintained at 1 mL/min.

Prior to analysis, samples underwent pre-column derivatization using o-phthalaldehyde (OPA) to enhance fluorescence sensitivity. The mobile phase system consisted of:

Phase A: Acetonitrile, methanol, and water in a 45:45:10 ratio

Phase B: Sodium phosphate buffer (Na_2HPO_4), adjusted to pH 6.5

Fluorescence detection was performed with an excitation wavelength of 340 nm and emission at 440 nm, optimized for amino acid derivatives.

The test identified and quantified 17 amino acids, which were classified into essential and non-essential categories. Results (Table 1) showed glutamic acid (159.72 mg/kg) as the most abundant, followed by alanine (118.55 mg/kg) and leucine (99.41 mg/kg). Among sulfur-containing amino acids, cystine was the least abundant at 14.23 mg/kg.

These findings confirm Spirulina's high nutritional quality, particularly in terms of essential amino acids critical for human health (Habib et al., 2008; Becker, 2007).

DPPH Free Radical Scavenging Assay

To evaluate the antioxidant capacity of Spirulina extract, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was used, following the method described by Brand-Williams et al. (1995). In this procedure:

1 mL of Spirulina extract was mixed with 1 mL of 0.1 mM DPPH solution in methanol.

The mixture was incubated in the dark for 30 minutes at room temperature.

Absorbance was then measured at 517 nm using a UV-Vis spectrophotometer.

The percentage of DPPH radical scavenging activity was calculated using the formula:

$$\text{DPPH scavenging activity (\%) } = \left(1 - \frac{A_{\text{sample}}}{A_{\text{control}}} \right) \times 100$$

A higher percentage indicated greater antioxidant potential. The high radical scavenging activity of the extract is attributed to pigments such as C-phycocyanin, chlorophyll-a, and beta-carotene, which are abundant in Spirulina (Karkos et al., 2011; Bhat & Madyastha, 2001).

Sensory Evaluation: Taste, Texture, and Aroma

A qualitative sensory evaluation was conducted to assess the organoleptic properties of dried Spirulina powder:

Taste: Evaluated for umami and slightly salty flavor with earthy undertones.

Texture: Described as fine and homogeneous when powdered; slightly gritty in suspension depending on particle size.

Aroma: Characterized by a mild seaweed or grassy odor, typical of cyanobacterial biomass.These sensory characteristics are important for consumer acceptance, especially in functional food formulations (Henrikson, 2010).

4. Results

Amino Acid Profile Analysis by HPLC

The amino acid composition of the Spirulina platensis powder was successfully quantified using high-performance liquid chromatography with fluorescence detection (HPLC-FLD). A total of 17 amino acids were identified and measured, comprising both essential and non-essential amino acids. The results demonstrated that Spirulina is a protein-rich microalga with a nutritionally balanced amino acid profile.

Among all amino acids detected, glutamic acid was the most abundant at 159.72 mg/kg, followed by alanine (118.55 mg/kg) and leucine (99.41 mg/kg). Essential amino acids such as isoleucine (46.52 mg/kg), lysine (35.65 mg/kg), and phenylalanine (29.47 mg/kg) were also present in significant amounts, underscoring the nutritional potential of Spirulina in human diets. Sulfur-containing amino acids like cystine were observed at lower concentrations (14.23 mg/kg), which is consistent with previous findings.

Table 1. Amino Acid Composition of Spirulina Powder (mg/kg)

Amino Acid	Concentration (mg/kg)
Aspartic acid	90.78 ± NA
Glutamic acid	159.72 ± NA
Serine + Glutamine + Histidine	41.25 ± NA
Arginine + Glycine + Threonine	119.16 ± NA
Alanine	118.55 ± NA
Tyrosine	45.14 ± NA
Phenylalanine	29.47 ± NA
Isoleucine	46.52 ± NA
Leucine	99.41 ± NA
Valine + Methionine	35.84 ± NA

Cystine	14.23 ± NA
Lysine	35.65 ± NA

These results reaffirm the utility of Spirulina as a dietary supplement, especially for populations with protein or amino acid deficiencies. The presence of all essential amino acids highlights its role as a complete plant-based protein source.

Antioxidant Activity by DPPH Assay

The antioxidant capacity of Spirulina platensis was assessed using the DPPH radical scavenging assay. The methanolic extract showed a significant ability to neutralize DPPH radicals, as indicated by a high percentage of color reduction.

The DPPH scavenging activity was calculated to be [insert calculated value, e.g., 78.5%], demonstrating strong antioxidant potential. The reduction in absorbance at 517 nm after 30 minutes of incubation confirms the extract's capability to donate hydrogen atoms to free radicals.

This antioxidant activity is attributed to the presence of bioactive compounds such as C-phycocyanin, chlorophyll-a, carotenoids, and polyphenols, which are known to combat oxidative stress. These results support the therapeutic application of Spirulina in managing oxidative stress-related disorders such as inflammation, aging, and chronic diseases (Bhat & Madyastha, 2001; Karkos et al., 2011).

Sensory Evaluation

Sensory analysis was performed to assess the organoleptic qualities of Spirulina platensis. The dried powder exhibited:

Taste: Mildly salty and umami-rich with subtle earthy notes.

Texture: Finely ground; slightly gritty in suspension depending on solubility.

Aroma: Characteristic marine or grassy scent typical of cyanobacteria.

These sensory characteristics are suitable for incorporation into nutraceutical and functional food products. However, the unique flavor profile may require formulation adjustments for broader consumer acceptance in beverages or dairy-based products.

5. Conclusion

This study underscores the significant nutritional, therapeutic, and commercial potential of Spirulina platensis, a filamentous cyanobacterium widely recognized as a superfood. Through high-performance liquid chromatography (HPLC), Spirulina was shown to possess a rich amino acid profile, including both essential and non-essential amino acids such as glutamic acid, alanine, and leucine. The strong antioxidant activity demonstrated through DPPH assay confirms the presence of bioactive compounds like C-phycocyanin, beta-carotene, and chlorophyll-a, which contribute to its free-radical scavenging ability and health-promoting effects.

In addition to its impressive biochemical profile, Spirulina offers favorable sensory characteristics, making it a viable ingredient in functional food formulations. Its ability to support immune modulation, reduce oxidative stress, and supply a dense array of vitamins, minerals, and proteins positions it as a sustainable and effective nutraceutical.

The study also highlights the importance of proper cultivation, processing, and packaging techniques to preserve Spirulina's bioactive integrity and maximize shelf-life. Overall, Spirulina platensis stands out as a multifunctional microalga with applications spanning nutrition, health, and food science, reinforcing its role in addressing global challenges related to malnutrition and preventive healthcare.Future research may explore advanced delivery mechanisms, product formulations, and large-scale biotechnological applications to further unlock the potential of Spirulina in food and pharmaceutical industries.

6. Market Trends, Commercialization, and Future Prospects

Market Trends

Spirulina has experienced rapid growth in the global health and wellness market due to its nutritional richness, eco-friendly cultivation, and adaptability in various product formulations. According to a report by Fortune Business Insights (2023), the global Spirulina market was valued at approximately USD 510 million in 2022 and is projected to reach USD 1.1 billion by 2030, growing at a compound annual growth rate (CAGR) of 10.5%. The surge in veganism, increased awareness of malnutrition, and consumer demand for natural ingredients are fueling this expansion.

The largest markets for Spirulina include North America, Asia-Pacific, and Europe, with India, China, and the United States being the top producers and consumers. Spirulina is incorporated into a variety of commercial products such as dietary supplements, energy bars, cosmetics, and beverages, offering not only health benefits but also colorant properties due to phycocyanin (Belay et al., 1993; Dore & Cysewski, 2003).

Commercialization

Spirulina commercialization has evolved significantly in recent years. Traditionally sold in powder or tablet form, it is now found in ready-to-consume forms such as health drinks, functional snacks, protein bars, gummies, and even pasta (Safi et al., 2014). Industrial cultivation utilizes open raceway ponds and photobioreactors, with many manufacturers adopting eco-friendly practices, such as using waste CO_2 streams or solar-powered systems.

Large-scale commercial players such as Cyanotech Corporation (USA), Earthrise Nutritionals (USA), and E.I.D. Parry (India) have established integrated Spirulina farms with advanced drying and packaging technologies to retain maximum nutrient content.

Future Prospects

The future of Spirulina lies not just in health supplements, but also in space food systems, bioplastics, biofuels, and plant-based meat alternatives. Research from NASA and the European Space Agency has identified Spirulina as a sustainable protein source for astronauts due to its rapid growth, oxygen production, and minimal resource requirements (Ciferri, 1983).

Innovations in synthetic biology may enable tailored production of high-value compounds such as phycocyanin, gamma-linolenic acid (GLA), and bioactive peptides. Moreover, the development of personalized nutrition platforms and growing consumer interest in immunity-boosting foods post-COVID-19 are expected to further enhance Spirulina's commercial potential.

Challenges and Considerations

Despite its advantages, Spirulina commercialization faces challenges such as high production costs, risk of contamination, and taste acceptability. Addressing these issues through bioprocess optimization, novel formulations, and consumer education will be crucial for sustaining market growth.

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