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REVIEW

Health aspects of *Spirulina* (*Arthrospira*) microalga food supplement

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Abstract: *Spirulina*, now named *Arthrospira*, is a microscopic and filamentous cyanobacterium that has a long history of use as a safe food, lacking toxicity. It is commercially produced in large outdoor ponds under controlled conditions. The aim of this review article is to summarize the recent available information concerning the human clinical potential and applications of *Spirulina*, as well as clinical data related to the safety and side effects of *Spirulina*. Potential health benefits of *Spirulina* are mainly due to its chemical composition, which includes proteins (the highest protein content of any natural food, 55–70 %), carbohydrates, essential amino acids, minerals (especially iron), essential fatty acids, vitamins and pigments. In this respect, three major bioactive components of *Spirulina*, the protein phycocyanin (a biliprotein pigment), sulfated polysaccharides and gamma linolenic acid seem to play significant roles in imparting improved human body functions. Furthermore, new experimental evidence supports the immunomodulation and antiviral effects of *Spirulina* supplementation. According to the Dietary Supplements Information Expert Committee of the United States Pharmacopeial Convention, the available clinical evidence does not indicate a serious risk to health or other public health concerns due to *Spirulina*. However, a few cases of severe side effects have been reported.

Keywords: cyanobacterium; phycocyanin; phycocyanobilin; sulfated polysaccharides.

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1. INTRODUCTION

Agriculture is faced with multiple challenges in the 21st century: *i*) increase in food production for a growing world population, which is expected to increase by about 2.3 billion people over the next 40 years, mostly in developing countries, *ii*) insufficient fresh water supply together with land degradation, which causes losses in agricultural productivity, *iii*) increase of the production of feedstock for bioenergy, *iv*) adoption of more efficient and sustainable production methods and *v*) adaptation to climate change.[1,2]. Furthermore, it is presently accepted that malnutrition is a silent massacre. According to United Nations sources, millions of children every year die either from malnutrition or are victims of malnutrition and micronutrient deficiency – the lack of key vitamins and minerals – with severe consequences on their physical and intellectual development.[3,4] Moreover, both overweight and underweight people may suffer either from a deficiency or an excess of the intake of nutrients needed for healthy living.[5]

Taking into account the above challenges, a future increase of the global production of food and food protein and a combat of malnutrition could be addressed through the development of non-traditional farm products by biotechnological intervention. Such a solution seems today economically viable by supporting the mass cultivation of microalgae rich in protein, vitamins and other functional nutrients known to benefit health. In this respect, *Spirulina* microalga seems to offer the perfect solution.

Spirulina, now named *Arthrospira*, are microscopic photosynthetic and filamentous cyanobacteria (blue–green algae) that have a long history of use as food. Cyanobacteria are believed to have evolved 3.5 billion years ago and they are the first group of bacteria that evolved that could fix atmospheric carbon dioxide into organic carbon compounds using water with the simultaneous evolution of oxygen. Before Columbus, Mexicans (Aztecs) exploited this microorganism as human food, while presently the African Kanembou tribe in the Lake Chad area (Republic of Chad) employs it for the same purpose. The name *Spirulina* derives from the spiral or helical nature of its filaments. *Arthrospira* is the scientific name of a cyanobacteria genus comprising a whole group of edible cyanobacteria sold under the name *Spirulina*. Among the various *Arthrospira* species, *A. platensis* and *A. maxima* are the most important. *Arthrospira* trichomes (filaments), which contain cylindrical cells aligned together in spirals or in straight lines. These filaments have variable length (usually 100–200 μm) and a diameter close to 6–12 μm, but the cell dimensions, degree of coiling and length of filaments

vary with species. The cell organization of *Spirulina* is typical of a prokaryote gram-negative bacterium with a lack of membrane-bound organelles. The cell wall constitutes a weak envelope that is composed of a number of layers, mostly of a peptidoglycan and lipopolysaccharide nature. The *Spirulina* cells have a number of inclusions, such as thylacoid membranes with phycobilisomes, carboxysomes, ribosomes, DNA fibrils and gas vacuoles, as well as polyglycan, polyphosphate and cyanophycin granules.[6–10]

Spirulina grows naturally in alkaline lakes but is commercially produced in large outdoor or greenhouse ponds under controlled conditions. Microalgal cultivation is based on a photosynthetic process using sunlight, nutrition elements and CO_2 contained in a fresh water culture medium under a relatively high temperature (optimum temperature: 35–38 °C). Commercial culture of *Spirulina* is followed by harvesting the biomass, drying and packaging.[9,11]

World market evolution of *Spirulina* involves mainly dried *Spirulina* whole biomass used as a human health food supplement, assuming that its consumption may benefit, prevent, help, or cure common diseases and malnutrition. Other commercial products containing *Spirulina* biomass or *Spirulina* extracts or active ingredients include protein supplements in animal feed, products for the improvement of pet health, natural colors for foods and cosmetics and purified biomolecules for medicine and biotechnology.[10] The objectives of this paper are to review recent literature on various health and safety aspects of *Spirulina* food supplements and their possible side effects.

2. CHEMICAL COMPOSITION

Chemical analysis of *Spirulina* showed that it is an excellent source of proteins, vitamins, dietary minerals and pigments. The biochemical composition depends upon the specific *Arthrospira* source, culture conditions and season of production.[9,10,12–14]

The protein content of *Spirulina* (50–70 % of the dry weight) exceeds that of meat, dried milk, eggs, soybeans or grains. *Spirulina* proteins are complete, since all the essential amino acids are present. The highest values for the essential amino acids are those for leucine, valine and isoleucine. When compared to standard alimentary proteins (from meat, eggs or milk), it is somewhat deficient in methionine, cysteine, and lysine, but is superior to all plant proteins including proteins from legumes.[6,10,13]

Spirulina proteins with significant health effects are the phycobiliproteins phycocyanin C and allophycocyanin at an approximately 10:1 ratio, which are proteins with linear tetrapyrrole prosthetic groups (phycocyanobilin) that in their functional state are covalently linked to specific cysteine residues of the proteins and they form light-harvesting antenna complexes of the cyanobacteria. *Spirulina*

is the only food containing phycocyanins, which represent about 15–25 % of the dry biomass of the microalga.[15,16]

Half of the total *Spirulina* lipids are fatty acids.[12,14] A detailed analysis of *Spirulina* fatty acids showed the presence of essential fatty acids (mostly ω-6). The rare polyunsaturated fatty acid γ-linolenic acid (GLA) with putative medicinal properties represents 10–20 % of the fatty acids in *A. maxima*, compared to 49 % in *A. platensis* and can be considered one of the best known source of GLA after human milk and some little used vegetable oils such as evening primrose, borage, blackcurrant seed and hemp oil. 10 g of *Spirulina* provide over 100 mg of GLA (which corresponds to more than two capsules of evening primrose oil).[9] Other major fatty acids present are the unsaturated oleic and linoleic acids as well as the saturated palmitic acid, which forms more than 60 % of lipids in *A. maxima*. Monogalactosyl- and sulfoquinovosyl-diacylglycerol as well as phosphatidylglycerol are the major *Spirulina* lipids (20–25 % each).[17] It is important that sulfolipids from cyanobacteria are active against the AIDS virus.[10,12]

Virtually all the assimilable *Spirulina* carbohydrates consist of polymers containing glucose. The major polymeric component in *A. platensis* is a branched polysaccharide, structurally similar to glycogen.[6] High molecular weight anionic polysaccharides with antiviral and immunomodulating activities (see below) have been isolated from *Spirulina*.[18] A sulfated polysaccharide fraction with antiviral action (calcium spirulan) was extensively purified and shown to be composed of rhamnose, 3-*O*-methylrhamnose (acofriose), 2,3-di-*O*-methylrhamnose, 3-*O*-methylxylose, uronic acids and sulfates.[19,20] Recently, an acidic polysaccharide fraction has also been isolated from *A. platensis*, which induces the synthesis of TNF-α in RAW macrophages.[18]

Spirulina is claimed to be the richest whole-food source of provitamin A (β-carotene), with 20 g of *Spirulina* also fulfilling the significant body requirements of vitamins B1 (thiamine), B2 (riboflavin) and B3 (niacin).[10,12,13,21] Its mineral content varies depending on the culture medium. The most interesting minerals in *Spirulina* are iron, calcium, phosphorus and potassium.[10,12] The analytical data for the chemical composition of *Spirulina* are presented in Table I.

Whole-genome sequences of several *Spirulina* strains have already appeared in the literature. *A. platensis* NIES-39 genome structure is estimated to be a single circular chromosome of 6.8 Mb, yielding 6,630 protein-coding genes, two sets of rRNA genes and 40 tRNA genes.[22] Whole-genome sequencing of the *Arthrospira* PCC 8005 strain, which was selected by the European Space Agency (ESA) as a nutritional product and an oxygen producer of the Micro-Ecological Life Support System Alternative (MELiSSA) for long-term manned space missions, showed the presence of 6,279,260 bases with an average G+C content of 44.7 %, 5,856 protein-coding sequences and 176 genes encoding RNA were also predicted.[23] Recently, the draft whole-genome shotgun sequencing of *A. maxima*

was obtained. The draft genome was approximately 6.0 Mb in total, with 5,690 protein-coding sequences.[24]

TABLE I. Analytical composition of *Spirulina* (*Arthrospira*)

Component	Relative dry weight, %	Reference
Proteins	50–70	12
Carbohydrates	15–25	10,13
Lipids	6–13	14
Nucleic acids	4.2–6	12
Iron	0.058–0.18	12
Calcium	0.13–1.4	12
Phosphorus	0.67–0.9	12
Potassium	0.64–1.54	12
Carotenoids	0.37–0.59	10,18
Chlorophyll α	0.66–1.2	10,13,18
Ash	3–11	9
Moisture	4–9	9

3. HEALTH ASPECTS

Although historically *Spirulina* was used as a food component, it has been thoroughly investigated using *in vitro* and *in vivo* experiments, including cell and tissue culture, animal testing as well as human clinical trials, for its role in human health management. A huge number of publications in peer reviewed scientific journals and book chapters covering health aspects of *Spirulina* have appeared during the last three decades. These articles described experimental approaches involving whole cell *Spirulina* preparations, various cell extracts and purified biomolecules, aiming at elucidating the potential health benefits of the consumption of this microalga, so far with exciting results. Potential health effects included: immunomodulation, antioxidant, anticancer, antiviral and antibacterial activities, as well as positive effects against malnutrition, hyperlipidemia, diabetes, obesity, inflammatory allergic reactions, heavy metal/chemical-induced toxicity, radiation damage and anemias.[10,12,25–30] In this respect, the most promising active *Spirulina* constituents appeared to be the protein phycocyanin,[30] sulfated polysaccharide fractions,[18] GLA[31] and certain sulfolipids.[32] While these medicinal claims may be based on experimental observations, more research is needed, especially with larger scale randomized studies in humans, in order to rate the effectiveness of *Spirulina* as a nutraceutical and source of potential pharmaceuticals, and to understand the mechanisms of action of specific *Spirulina* biomolecules, their short-term and long-term effects, and the safety of their use in functional foods.

Available new information or data not covered by previous review articles concerning human clinical potential of *Spirulina*, and data related to the safety and side effects of *Spirulina* are summarized below.

3.1. Immunomodulation

Forty volunteers of both sexes with an age of 50 years or older took a *Spirulina* supplement (3 g per day) for 12 weeks. A steady increase in the average values of the mean corpuscular hemoglobin in subjects of both sexes was recorded. An increase of indoleamine 2,3-dioxygenase enzyme activity (a sign of immune function) and white blood cell count were also observed for the majority of subjects.[33]

In a recent clinical trial involving two studies, a pilot study with 11 individuals and a double-blind placebo controlled study with 12 individuals, healthy volunteers supplemented their diet with 200 or 400 mg day^{-1}, respectively, for seven days with Immulina® (a commercial extract of *A. platensis*, which is known to activate THP-1 monocytes and CD+ T cells *in vitro* and enhance immunological functions in mice). An enhancement of natural killer cell activity following administration of Immulina® was observed. Evidence was presented that Braun-type lipoproteins of the *Spirulina* commercial extract were responsible for the major portion of the *in vitro* monocyte activation.[34]

3.2. HIV-infected and undernourished patients

In a randomized study to compare the effect of *A. platensis vs*. soybean as food supplements on insulin-resistant HIV-infected patients, 33 patients received 19 g of supplement (*Spirulina* or soybean) daily for 8 weeks. It was concluded that the insulin sensitivity in HIV patients improved more when *Spirulina* rather than soybean was used as nutritional supplement.[35] Furthermore, when HIV-infected or HIV-negative undernourished children and HIV-infected adults were treated with *Spirulina* supplementation, clinical improvement was always observed, including weight increase, improvement of hematological parameters and decrease in the HIV viral load.[36–39]

3.3. Clinical potential of phycocyanobilin

The chromophore phycocyanobilin (PCB) of *Spirulina*, was found to strongly inhibit NADPH oxidase activity, since in mammalian cells it is reduced to phycocyanorubin, a close homolog of bilirubin, which shows a potent inhibitory activity of this enzyme complex (observed in nanomolar intracellular concentrations). Due to the central roles of NADPH oxidase activation in pathology, PCB supplementation may induce prevention and therapy of various diseases in part mediated by NADPH oxidase overactivity in affected tissues. Medical conditions associated with or linked to NADPH oxidase activity include among others: cardiovascular diseases, metabolic syndrome, diabetic complications, Parkinson's disease, Alzheimer’s disease, rheumatoid arthritis, allergic reactions and cancer. Administration of PCB may be achieved by any desirable route including ingestion of whole *Spirulina*, phycocyanin protein or isolated tetrapyrrole chromo-

phore. PCB represents about 4.7 % of the mass of phycocyanin. Thus, about 0.66 % of the dry mass of *Spirulina* is PCB, or about 15 g of *Spirulina* can be expected to provide about 100 mg of PCB.[40,41]

3.4. Safety aspects

Spirulina is regulated as a food and as a dietary supplement.[13] The Food and Drug Administration (FDA) of the USA has categorized several *Arthrospira* dried biomass products as "generally recognized as safe" (GRAS) for human consumption.[42] *Spirulina* has typically been studied in daily doses of 1 to 10 g and a recommended dosage for adults is usually in the range of 3–10 g day^{-1}.[9,10,12]

The amount of iodine contained in 10 g of dried *Spirulina* biomass is only 3 μg[44] or less (not detected).[44] Since the upper safe level for total daily intake of iodine (for a 60 kg bodyweight adult), established by the Scientific Committee on Food (SCF) and the European Food Safety Authority (EFSA), is 600 μg, while the corresponding value suggested by the US Institute of Medicine is 1100 μg,[45] there is no risk of a consumer taking in excessive iodine by *Spirulina* consumption.

Spirulina contains substantial amounts of several B12 analogues (corrinoid forms, pseudovitamin B12) which do not fulfill specific functional roles of vitamin B12 for humans.[46] However, vitamin B12 bioavailability experiments with animals fed with *Spirulina* showed that *Spirulina* intake does not interfere with mammalian B12 metabolism, thus showing that the B12 analogues do not present inhibitory actions.[47]

Spirulina total nucleic acid contents of 4.2-6 % of microalga dry matter have been reported[12] (the RNA content is about 3–4 times higher than that of DNA),[6] which are higher than those of animal meat and various plant foods,[48] similar to those of unicellular algae but lower than those of bacteria and yeast.[6] Dietary nucleic acids highly influence serum uric acid levels because of purine metabolism. A maximum safe limit of RNA in the diet of 2 g day^{-1} has been suggested[48] since high levels of uric acid may result in pathological conditions.[49] Accordingly, a safety margin of 30 g maximum daily intake of *Spirulina* was recommended.[49]

3.5. Side effects

The Dietary Supplements Information Expert Committee (DSI-EC) reviewed recent information from human clinical trials, animal studies, and regulatory and pharmacopeial sources and analyzed adverse event reports regarding *Spirulina* to assess potential health concerns. The DSI-EC concluded that the available evidence does not indicate a serious risk to health or other public health concerns and assigned a Class A safety rating for *A. maxima* and *A. platensis*, thereby

permitting the admission of quality monographs for these dietary supplement ingredients in the United States Pharmacopoeia and National Formulary.[50] However, information is limited concerning interactions with pharmaceutical compounds or other dietary supplements. A few side effects have been reported from the ingestion of *Spirulina*, including headache, stomach ache, muscle pain, flushing of the face, sweating and concentration difficulties.[51] A few cases of severe side-effects have also been reported, including hepatotoxicity[52] and rhabdomyolysis.[51] Since *Spirulina* is an immunomodulatory supplement, it might affect disease severity in patients with autoimmune diseases. These patients have to avoid the consumption of *Spirulina*.[53,54] A case of anaphylaxis caused by the *Spirulina* pigment phycocyanin was also reported. Upon an oral challenge with increasing *Spirulina* doses, corresponding to four *Spirulina* tablets over a 3-h period, a 14-old year adolescent experienced diarrhea and erythema.[55] People with phenylketonuria should avoid the consumption of *Spirulina*.[51] It has been suggested that *Spirulina* when present in the diet exerted a neuroprotective effect in a mouse model of amyotrophic lateral sclerosis (ALS), by retarding or stopping motor neuron degeneration.[56] Nevertheless, as emphasized by the ALSUntangled Group,[57] at this time there is no evidence that *Spirulina* is effective for ALS and there appears to be real and theoretical toxicities that patients with ALS may encounter with it. Until better efficacy and safety studies are published, the ALSUntangled Group does not support the use of *Spirulina* in patients with ALS.[57]

It must be emphasized that the consumption of *Spirulina* of unknown origin or originating from countries that do not guarantee the quality and safety of the product should be avoided. The major areas of concern for the safe consumption of *Spirulina* are microbiological load, heavy metal content, pesticides, extraneous matter and cyanobacterial toxins.[13,50]

4. CONCLUDING REMARKS

Spirulina, a microscopic and filamentous cyanobacterium with widespread usage throughout the world as a dietary supplement and a potential solution for combating malnutrition, seems to offer significant health advantages to the consumer. The positive effects of *Spirulina* in relation to its immunomodulation and antiviral properties are based on clinical evidence but larger trials are required. The microalga chromophore phycocyanobilin, as a potent inhibitor of NADPH oxidase, may have versatile potential in the prevention and therapy of various diseases mediated by overactivity of this enzyme. *Spirulina* is accepted as a safe food supplement and the total number of side effects reported in the literature is relatively small. The iodine and nucleic acid content of *Spirulina* do not pose health risks assuming a consumption of *Spirulina* of up to 30 g per day.

ИЗВОД

ЗДРАВСТВЕНИ АСПЕКТИ МИКРОАЛГЕ *Spirulina* (*Arthrospira*) КАО ХРАНЉИВОГ СУПЛЕМЕНТА

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Спирулина (*Spirulina*), позната и као Артроспира (*Arthrospira*), је микроскопска филаментозна цијанобактерија која је дуго у употреби у исхрани. Комерцијално се производи у великим отвореним базенима, у контролисаним условима. Циљ овог прегледног рада је да изнесе нове податке о клиничком потенцијалу и примени спирулине, као и о резултатима који се односе на безбедност и споредне ефекте. Потенцијалне здравствене благодети спирулине потичу од њеног хемијског састава, укључујући протеине (највећи садржај протеина од све природне хране, 55–70 %), угљене хидрате, есенцијане аминокиселине, минерале (посебно гвожђе), есенцијалне масне киселине, витамине и пигменте. Три најважније компоненте спирулине које имају значајну улогу у побољшавању телесних функција код људи су протеин фикоцијанин, сулфати полисахарида и γ-линоленска киселина. Нови експериментални докази упућују на закључак да спирулина има имуномодулаторне и антивирусне ефекте. Према подацима америчког комитета ("*Dietary Supplements Information Expert Committee of United States Pharmacopeial Convention*"), досадашње клиничке студије не указују на здравствени ризик услед употребе спирулине, мада је пријављено неколико случајева са озбиљним пратећим појавама.

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