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Nutritional and toxicological aspects of *Spirulina (Arthrospira)*

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Abstract

Undernutrition constitutes a public health problem particularly in developing countries. The utilization of algae, particularly *Spirulina*, as a functional food was suggested decades ago due to the fact that it is not only a protein-dense food source, but because its amino acid profile is considered as of high biologic-value protein content. *Spirulina* provides essential fats (e.g., gamma-linolenic oleic acids), concomitant to low content nucleic acids. It also has an exceptionally high content of vitamin B_{12}, is a good source of beta-carotene, iron, calcium and phosphorous. Moreover, *Spirulina* has also proven to have good acceptance as of its organoleptic properties (thus making it a possible prospect for food or a nutrition supplement) and it has not exhibited neither acute nor chronic toxicities, making it safe for human consumption.

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Key words: *Arthrospira. Functional foods. Nutritional value. Spirulina. Toxicological profile.*

ASPECTOS NUTRICIONALES Y TOXICOLÓGICOS DE *SPIRULINA (ARTHROSPIRA)*

Resumen

La desnutrición constituye un problema de salud pública, fundamentalmente en los países en desarrollo. El uso de algas, particularmente *Spirulina*, como alimento funcional para combatir dicha patología se propuso desde hace algunas décadas debido a que estos alimentos no solo son fuentes alimentarias de alta densidad proteica, sino que también ofrecen un perfil de aminoácidos de alto valor biológico. Además, *Spirulina* provee ácidos grasos esenciales (p. ej., el gamma linolénico), concomitante con un bajo aporte de ácidos nucleicos. También tiene un contenido excepcionalmente alto de vitamina B_{12}, es considerada una buena fuente de betacaroteno, hierro, calcio y fósforo. Más aún, se ha demostrado que *Spirulina* tiene una buena aceptación organoléptica, lo que le confiere un gran potencial para considerarse como suplemento nutricional y, finalmente, no se han reportado toxicidades crónicas ni agudas, haciéndola segura para el consumo humano.

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Palabras clave: *Alimentos funcionales. Arthrospira. Perfil*

Abbreviations

BV: biological value.
DC: digestibility coefficient.
g: gram.
GLA: gamma linolenic acid.
kg: kilogram.
mg: miligram.
NPU: net protein utilization.
PER: protein efficiency ratio.
SOD: superoxide dismutase.

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Introduction

Undernutrition is the outcome of insufficient food intake, resulting in a decadent nutritional status characterized by lower weight and/or height that those expected for one's age[1]. Such condition, being frequently related to protein deficiency, constitutes a public health problem all over the world, but particularly in developing countries[2].

In the interest of developing novel more effective protein sources for preventing/reversing malnutrition, increasing attention has been turned to microalgae. Single cell protein, i.e., crude or refined sources of protein that originates from microorganisms such as bacteria, yeasts, fungi or algae[3], represent an attractive offer to many industries (e.g., fuel, cosmetic, therapeutic), including the feed, food and nutritional ones[4,5].

The possible utilization of algae as a nonconventional protein source was suggested some decades ago[6]. Since then, several types of algae have been tested for this purpose and, although toxicity problems have been reported for some species, promising results have been demonstrated for others. Among the latter, we have *Spirulina*.

Spirulina (*Arthrospira*) is a microscopic blue-green algae –or cyanobacteria– from the Oscillateriaceae family. It naturally grows in alkaline and warm media; in the sea and fresh water of Asia, Africa, Europe, South and North America[7]. In Mexico, the Texcoco lake used to be abundant in the subclass *S. maxima* and there is evidence that it was used as food –called *tecuitlatl*– during prehispanic times and it was, later, during the conquest, harvested from the lake, dried and sold for human consumption[8,9]. Unfortunately, this practice was later lost with time.

In terms of nutrition, *Spirulina* is a rich food source of macro- and micronutrients including high quality protein, iron, gama-linolenic acid, vitamins, minerals, sulfated polysaccharides and phycocanin[10,11]. (El-Baky, 2008; Chu, 2010). Hence *Spirulina* is of great interest as it offers the possibility of being used as a *functional food*[12]. This term refers to those foods that have proven to aid specific body functions, yielding health-promoting properties and/or reduce the risk of disease beyond its nutritional functions[13]. Moreover, *Spirulina* has also proven to have good acceptance as of its organoleptic properties (thus making it a possible prospect for food or a nutrition supplement) and it has not exhibited neither acute nor chronic toxicities, making it safe for human consumption[14,15].

The objective of the present paper was to systematically review the nutritional and toxicological properties of *Spirulina*, since scarce information has been reported, although some pharmacological activities –mainly those related to the algae's antioxidant and enzyme inhibitor capacity– were reviewed several years ago[16,17].

Nutritional composition

Spirulina composition may vary according to the culturing conditions, and the methods of analysis. Table I shows the results obtained by a third party laboratory and by Earthrise Nutritionals LLC (CA, U.S.A) in terms of macronutrients, vitamins, minerals and phytonutrients.

Table I
Nutritional profile of Spirulina *Powder (composition by 100 g)*

Macronutrients		***Vitamins***	
Calories	373	Vitamin A (as β-carotene)[b]	352.000 IU
Total fat (g)	4.3	Vitamin K	1090 mcg
Saturated fat	1.95	Thiamine HCL (Vitamin B1)	0.5 mg
Polyunsaturated fat	1.93	Rivoflavin (Vitamin B2)	4.53 mg
Monounsaturated fat	0.26	Niacin (Vitamine B3)	14.9 mg
Cholesterol	< 0.1	Vitamin B6 (Pyridox. HCL)	0.96 mg
Total carbohydrate (g)	17.8	Vitamin B12	162 mcg
Dietary fiber	7.7		
Sugars	1.3	***Minerals***	
Lactose	< 0.1	Calcium	468 mg
Protein B	63	Iron	87.4 mg
Essential amino acids (mg)		Phosphorus	961 mg
Histidine	1000	Iodine	142 mcg
Isoleucine	3500	Magnesium	319 mg
Leucine	5380	Zinc	1.45 mg
Lysine	2960	Selenium	25.5 mcg
Methionine	1170	Cooper	0.47 mg
Phenylalanine	2750	Manganese	3.26 mg
Threonine	2860	Chromium	<400 mcg
Tryptophan	1090	Potassium	1,660 mg
Valine	3940	Sodium	641 mg
Non-essential amino acids (mg)			
Alanine	4590	***Phytonutrients***	
Arginine	4310	Phycocyanin (mean)[b]	17.2%
Aspartic acid	5990	Chlorophyll (mean)[b]	1.2%
Cystine	590	Superoxide dismutase (SOD)	531,000 IU
Glutamic acid	9130	Gamma linolenic acid (GLA)	1080 mg
Glycine	3130	Total carotenoids (mean)[b]	504 mg
Proline	2380	β-carotene (mean)[b]	211 mg
Serine	2760	Zeaxanthin	101 mg
Tyrosine	2500		

[a] Most data are based on recent analysis by third-party laboratory.
[b] In Earthrise Nutritional LLC.

Protein and Amino Acids

Spirulina's protein content ranges between 60 to 70% of its dry weight. This is an exceptional proportion since the vast majority of plant-based foods (even the ones that are known to be "good protein sources") contain only about 35%[18]. In fact, C-phycocyanin, a molecule which contains phycocyanobilin, an homolog of biliverdin[19], is one of the major proteins present in *Spirulina*, accounting for about 20% of algae's dry weight[20].

In addition to the quantity, it is also important to assess the protein's quality, which is determined by the contents, proportion, and availability of the protein's amino acids[21]. *Spirulina* provides a *complete protein*, as it contains all of the essential amino acids and –in fact– these represent almost half of the protein[6].

It is possible that some authors state that *Spirulina* "is somewhat low in these amino acids" (methionine and cystein). This is true; however, it must be considered that such a conclusion is reached by comparing versus a standard or reference protein (usually, egg albumin or milk casein). To this respect, most researchers agree that, despite being mildly inferior to the standard protein (reaching an 80% of the limiting amino acids), *Spirulina* protein is highly superior to that of almost any other vegetable source (including legumes, e.g., soybean)[8,22] and there is no limiting factor for amino acid assimilation[23].

Spirulina protein exhibits other characteristics that increase its nutritional value. For example, its biologic value (BV) –i.e., a measure of the nitrogen retained within the body in relation to the nitrogen absorbed–, its net protein utilization (NPU), which represents the percentage of nitrogen ingested that remains within the organism. Moreover, *Spirulina* has a relatively high digestibility coefficient (DC), which is the proportion of nitrogen contained in foods that is actually absorbed[24]. The algae also exhibits a good protein efficiency ratio (PER), the simplest and most common method used to evaluate proteins by animal feeding tests[25]. For the blue-green algae, although inferior to that of animal protein, the PER is much higher than most vegetable protein.

What does all of the above mean? That *Spirulina* is not only a good source of protein –due to its high content– but that such protein is used in a better and more efficient way, thus the cyanobacteria has been considered as a possible protein supplement for both humans and animals[26]. Some results of the previous characteristics are presented in table II.

Moreover, as the blue-green algae would be used not as a sole food, but rather as a nutritional supplement, it could be seen as a complementation to common cereals such as rice, wheat or corn. Since these foods are limited in certain amino acids, *Spirulina* is able to increase its protein value[27].

Table II
Protein values of Spirulina *as compared to the gold standard*

Characteristic	Spirulina	*Reference protein (casein)*	*% of the reference*
Biologic value	75	87	86.20
Net protein utilization	62	83	74.69
Digestibility	85	95	89.47
Protein efficiency	1.9	2.5	76.00

Lipids

Spirulina presents a lipid fraction of approximately 5-10% of its dry weight. The important thing to this respect is that fats that make up such fraction are –mainly– essential lipids to human. Hence, *Spirulina* is considered a good source of gamma-linolenic, linoleic and oleic acids. The first one has received much attention since there are not many food sources that contain a significant amount; in fact, *Spirulina* is considered the vegetable source with the highest quantity (representing approximately a 20% of its total fatty acid content)[28,29]. The importance of gamma-linolenic acid relies on the fact that it is the precursor of prostaglandins, leukotrienes and thromboxanes; thus, as they are mediators in inflammation and immune processes, they participate in the course of conditions such as arthritis, diabetes, cardiovascular disease, and cellular aging[30]. Finally, gamma-linolenic acid is being investigated as a potentially antineoplasic agent as is has been shown that it can suppress tumor growth[31-33].

Nucleic acids

This fraction refers to the DNA and RNA content in *Spirulina*. Nucleic acids' catabolism yields uric acid, since purines –adenine and guanine– are being degraded. High levels of uric acid are correlated to the development of gout, kidney stones and, more recently, cardiovascular disease[34-37].

Spirulina's content of nucleic acids is about 4-6% of its dry weight; this values are –for much– lower than that of other single-cell protein sources (e.g., yeast contains about 20% of its dry matter) and other microalgae like Chlorella. The World Health Organization recommends that the daily total nucleic acid consumption should not exceed 4 g; to get such quantity from the blue-green algae, one would have to consume up to 80 g.

Vitamins

Of all the vitamins, vitamin B_{12} is the largest and most complex; it represents all of the biologically active cobalamins[38]. The fact that *Spirulina* has an exceptionally high content of vitamin B_{12} –as other sea weed do– is of great importance because such vitamin is –usually– contained only in animal origin foods. Thus this alga might be considered as a good source for vegans, since they do not consume any animal-origin foods[20].

Spirulina is also a good source of beta-carotene, containing about 700-1700 mg/kg, which –once absorbed– will be biotransformed into vitamin A. Human requirements of vitamin A are of approximately 1 mg/day; hence 1-2 g of algae will be enough to assure this need. Moreover, opposite to the use of commercial supplements, an overdose would be non probable because beta-carotene (vs. retinol) is not cumulatively-toxic and its bioavailability has been verified in preclinical and clinical studies[39-42].

Carotenoids are the second most important group of pigments found in algae. They play a role as lipophylic antioxidants and they are thought to be responsible for the therapeutic property of carotene as an anticancer agent.

Minerals

The inorganic nutrients of most relevance in *Spirulina* are iron, calcium and phosphorous. Populations that consume little animal foods –because of their own beliefs, preferences, or accessibility– are at a higher risk of developing iron deficiency; this disease is clinically manifested as microcytic and hypochromic anemia since hemoglobin is not present in sufficient quantities in erythrocytes[43,44]. Additionally, those same people tend to consume a great amount of fiber which contains phytates and oxalates which, in turn, lower the bioavailability of iron in vegetable sources. Finally, plant foods contain only non-heme iron, which is more prone to be affected by absorption inhibitors (i.e., phytates).

Spirulina could be able to counteract these two aspects: a) its iron content is substantially high: comparatively, cereals –which are usually considered good sources of iron– contain between 150-250 mg/kg; blue-green algae contains about 580-1800 mg/kg; b) algae does not have pericardium (as cereals do), hence it does not present phytates/oxalates that could chelate iron and lower its absorption (this is what happens, for example, with spinach)[45,46].

For its side, calcium and phosphorous contents are comparable to those of the milk. The relative proportion (Ca:P) of these micronutrients is compatible with the preservation of bone health since it reduces decalcification risk. Moreover, as it was previously stated, the cyanobacteria of interest is an oxalate-free plant food, thus –as with iron- it provides calcium with high availability, thus it improves its absorption[47].

Human Studies on the Nutritional Potential of *Spirulina*

In humans, studies are even scarcer than in preclinical subjects. However, *Spirulina* has been implemented as a reversion agent for protein undernutrition, giving positive results in terms of weight gain and improvement of the general nutritional status[48].

With regards to protein, reports of favorable results in the case of Mexican children or infants suffering of severe malnutrition have been cited[49,50]. In a more detailed study, diets in which up 50% of the protein came from *Spirulina* were fed through a plastic tube to five undernourished adults for periods varying from 4 to 5 days. A significant weight gain and a positive nitrogen balance were observed and no side effects were reported[51]. Another study carried out in Africa, supplemented children with protein or energy-protein undernutrition with *Spirulina* for eight weeks. As a result, nutritional status showed a significant improvement and the number of children diagnosed with undernutrition was reduced; in average, the *Spirulina*-supplemented group gained 25 g/day of weight, while the control group gained 15 g/day[2,52]. These results do not concord with those published by Branger[53], who found no apparent benefit from the algae supplementation in children and, in fact, recommend a “traditional” renourishement program.

Finally, another study concludes that a large-sized, randomized, double-blinded and placebo-controlled trial is needed in order to reach conclusions with more confiability and improve the current knowledge on the potential impact of the microalgae on nutritional rehabilitation[51].

Now, as of vitamin supplementation, a large trial including 5000 pre-school children previously diagnosed with vitamin A deficiency –evidenced by Bitot’s spots– showed that the intake of 1 g/day of *Spirulina* for almost 4 months significantly decreased the prevalence of the deficiency in 70% (80 to 10%)[54].

For its side, although some investigations have stated that vitamin B_{12} contained in cyanobacteria is not a reliable source of its active form, in fact, the supplementation of *Spirulina* to children with macrocytic anemia has shown that despite significant increases in the vitamin’s intake and its plasma levels –finding a correlation with Spearman’s $\rho=0.9$); hematological values (hemoglobin and medium corpuscular volume) did not show any improvement[55]. Such findings suggest that vitamin B_{12} contained in *Spirulina* is well absorbed but it is not “used” by the body. Some authors report that cyanobacteria certainly produce vitamin B_{12}, however, it is a non-cobalamin analogue, which makes it unavailable to humans and may even block the vitamin’s metabolism[56,57].

Finally, a study was conducted in Germany with the aims of evaluating the effect of *Spirulina* tablets as a supplement to a weight-loss diet. Tablets were administered for four weeks, resulting in a significant weight reduction of 1.4 kg compared to subjects in the placebo group[25].

Toxicological Studies

Because organisms can be a source of toxins, anti-nutrients or other potentially harmful compounds, this issue needs to be addressed in the safety assessment (Howlett, 2003). Herein, we present a summary table III that shows the effects found on various toxicological tests performed on laboratory animals for the assurance of human health (modified from Chamorro-Cevallos)[58]. It is worth mentioning that phycocyanin, the blue colorant from in *Spirulina*, fed to both-sexes rats for 14 weeks in a concentration of up to 5% in the diet, did not show and symptoms of toxicity[59].

Perspectives/discussion

Nutritional supplements may be consumed for different reasons, e.g. compensating an insufficient energy, macronutrient (carbohydrates, lipids, proteins) or micronutrient (vitamins and minerals) intake with the objective of preventing or reversing an illness[60]. The main reason for the interest put on microalgae consumption is its potential to be used as a treatment for protein deficiency. The latter affects is a serious problem with an estimated prevalence of more than 300 million people around the world. *Spirulina* might represent a possible vehicle of protein due to its high contents and remarkable quality: bioavailable iron and complete protein, as it has been stated before.

The use of microorganisms for production of human or animal feed, especially regarding protein, has been explored since World War II. It offers several advantages over plants and animals food sources: microorganism are able to synthesize protein from inorganic nitrogen, they are not season-dependent and can double their mass within hours[61]. Additionally, since the cyanobacteria is able to grow in extreme conditions (i.e., salinity and pH), it is likely to be a highly hygienic food, because not many other microorganisms are able to survive such conditions.

Furthermore, *Spirulina* has already shown no toxicities –neither acute nor chronic– hence, it might be safely used as a human food. The levels, of this alga, tested during the toxicological analysis were higher than any anticipated human consumption. Therefore, it would appear that no toxicological hazard is related to the present use of this cyanobacterium as a source of single cell protein.[15]

For all the above, we can conclude that the advantages of *Spirulina* are multiple: its high nutritional value, the availability of its nutrients, its simple production method due to its moderate requirements for growth, its excellent conservation after recollection, and its security in relation to consumption (no toxicities), to name a few[51].

Although other microalgae (e.g., *Chlorella, Dunaliella, and Scenedesmus*) have been also used as food supplements[62], *Spirulina* seems to be the most promising strain in the attempt of using unconventional sources to fight nutritional deficiencies[63,64].

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Table III
Toxicity of Spirulina

Toxicity Test	*General Results*
Acute	The oral and single treatment with as much 800 mg/kg of *Spirulina* to rats produced no mortality, nor alterations in body weights, tissues and organs. Also, there was no allergic skin reaction with an application of up to 2000 mg/kg.
Subchronic	The feeding of *Spirulina* in different experiments to rats or mice at a dietary level until 30% for 13 weeks, produced no toxic effects on body and organ weights, hematology, serum, urine and histopathology values. Only one study, in mice, with a diet containing 60% of the algae induced an increase in the kidney, heart and lung weights and a nephrocalcinosis syndrome.
Chronic	*Spirulina* given in as much as 48% in the experimental diet for 86 weeks, produced no adverse effects on hematology, urine, serum biochemistry, nor in macroscopic or histopathological findings.
Reproductive	Fertility, teratogenic, peri- and postnatal development and multigenerational studies in different species of rodents, showed no deleterious effects by *Spirulina* treatment at 10, 20 and 30% levels in the diet.
Genotoxic	Short and long-term studies with *Spirulina* at 10, 20 and 30% levels included in the diet, failed to reveal germinal mutations of the dominant-lethal type in rats and mice. Negative results were also reported using the *Salmonella typhymurium* test.

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