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Earth Food *Spirulina* (*Arthrospira*): Production and Quality Standarts

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1. Introduction

According to researchers, in the first photosynthetic life forms known on earth 3.6 billion years ago, was created by the God. Blue-green algae, cyanobacteria, is the evolutionary bridge between bacteria and green plants. It contained within it everything life needed to evolve. This immortal plant has renewed itself for billions of years, and has presented itself to us in the last 40 years. *Spirulina* has 3.6 billion years of evolutionary wisdom coded in its DNA. *Spirulina*, or what was most likely *Arthrospira*, is a photosynthetic, filamentous, spiral-shaped, multicellular and blue-green microalga that has a long history of use as food. For this microorganism contain chlorophyll *a*, like higher plants, botanists classify it as a microalga belonging to *Cyanophyceae* class; but according to bacteriologists it is a bacterium due to its prokaryotic structure (Fig. 1). *Spirulina*, essentially an exceptional simple extract of blue-green algae, has been extensively studied and is now in widespread usage throughout the world as a food product and as a dietary supplement (Fox, 1996; Paleaz, 2006).

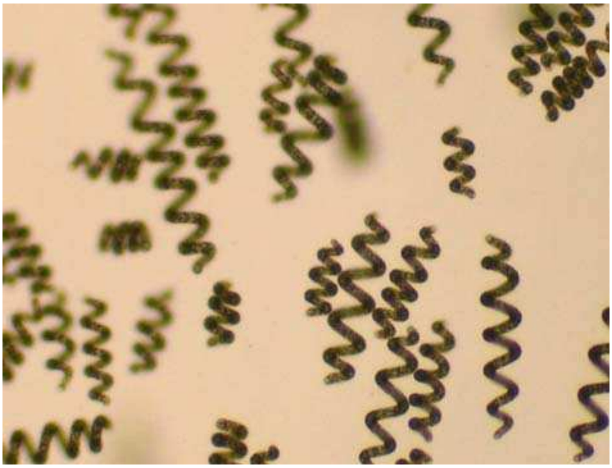

Fig. 1. Microscopic view of microalgae *Spirulina* (Cyanobacteria), Photo by E. Koru

The goals of this paper are: to review the present literature on the historical evolution and potential nutritional use of *Spirulina* and its products; to provide insight into the potential implications of the studies reviewed in the context of possible nutritional and therapeutic applications in health management, and; to identify areas of interest for future research.

2. Historical background on the use of *Spirulina*

2.1 Evolutionary history

The cyanobacteria are believed to have evolved 3.5 billion years ago. Fossils discovered in the 3.5-Ga-old Apex chert in northwestern Western Australia crude filamentous cyanobacteria with strikingly similar morphologies to present-day filamentous cyanobacteria (*Oscillatoriacea*). The occurrence of aerobic respiration and oxygenic photosynthesis, photosynthetic carbon dioxide fixation like that of extant cyanobacteria, cell division more similar to the extant cyanobacterial and recent rRNA analyses showing that the *Oscillatoriacea* are among the earliest evolved also lend further evidence to the fossil record. *Arthrospira* belongs to the Class *Oscillatoriacea* and therefore has a very old lineage. Despite their old lineage, the fossil cyanobacteria are morphologically very similar to their extant forms, suggesting a slow evolutionary process. *Spirulina* (*Arthrospira*) is a ubiquitous organism. After the first isolation by Turpin in 1827 from a freshwater stream, species of *Spirulina* have been found in a variety of environments: soil, sand, marshes, brackish water, seawater, and freshwater. Species of *Spirulina* have been isolated, for instance, from tropical waters to the North Sea, thermal springs, salt pans, warm waters from power plants, fish ponds, etc. Thus, the organism appears to be capable of adaptation to very different habitats and colonizes certain environments in which life for other microorganisms is, if not impossible, very difficult (Ciferri, 1983; Tomaselli, 2003).

2.2 Use of *Spirulina* as human food and animal feed

It is not known with accuracy when human began to use microalgae. The current use of these resources has three precedents: tradition, scientific and technological development, and the so-called, “green tendency”. There are reports that it was used as food in Mexico during the Aztec civilization approximate 400 years ago. Bernal Díaz del Castillo, a member of Hernán Cortez´s troops, reported in 1521, that *S. maxima* was harvested from the Lake Texcoco, dried and sold for human consumption in a Tenochtitlán (today Mexico City) market (Sánchez, et al., 2003). In 1940, a French phycologist Dangeard published a report on the consumption of dihé by the Kanembu people near Lake Chad. Scientist Dangeard, also noted these same algae populated a number of lakes in the Rift Valley of East Africa, and was the main food for the flamingos living around those lakes. Twenty-five years later during 1964-65, a botanist on a Belgian Trans-Saharan expedition, Jean Léonard, reported finding a curious greenish, edible cakes being sold in native markets of Fort-Lamy (now N’Djamena) in Chad. When locals said these cakes came from areas near Lake Chad, Léonard recognized the connection between the algal blooms and dried cakes sold in the market (Habib et al., 2008). It is still being used as food by the Kanembu tribe in the Lake Chad area of the Republic of Chad where it is sold as dried bread called “*dihe*” (Fox, 1996; Belay, 2002). In 1967 *Spirulina* was established as a “wonderful future food source” in the International Association of Applied Microbiology (Sasson, 1997). This traditional food

(Fig. 2), by a European scientific mission, and is now widely cultured throughout the world. In its commercial use, the common name, *Spirulina*, refers to the dried biomass of the cyanobacterium, *Arthrospira platensis*, and is a whole product of biological origin. *A. platensis* and *A. maxima*, that are commonly used as food, dietary supplement, and feed supplement (Wikfors & Ohno, 2001). The re-introduction of *Spirulina* as a health food for human consumption in the late 1970s and the beginning of the 1980s was associated with many controversial claims which attribute to *Spirulina* a role of a 'magic agent' that could do almost everything, from curing specific cancer to antibiotic and antiviral activity. Since most claims were never backed up by detailed scientific and medical research, they will not be discussed in this chapter. Nevertheless, one cannot ignore the fact that more than 70 per cent of the current *Spirulina* market is for human consumption, mainly as health food. Primarily concern in *Spirulina* focused mainly on its rich content of protein, essential amino acids, minerals, vitamins, and essential fatty acids. *Spirulina* is 60-70% protein by weight and contains a rich source of vitamins, for example vitamin B12 and provitamin A (β-carotene), and minerals, especially iron. One of the few sources of dietary γ-linolenic acid (GLA), it also contains a host of other phytochemicals that have potential health benefits. This first data was enough to launch many research projects for industrial purposes in the 1970s, because micro-organisms (*Chlorella*, *Spirulina*, yeast, some bacteria and moulds) seemed at that time to be the most direct route to inexpensive proteins – the iconic "single cell proteins". In 1970's, a request was received from a company named Sosa-Texcoco Ltd by the "Institut français du pétrole" to study a bloom of algae occurring in the evaporation ponds of their sodium bicarbonate production facility in a lake near Mexico City. As a result, the first systematic and detailed study of the growth requirements and physiology of *Spirulina* was performed. This study, which was a part of Ph.D. thesis by Zarrouk (1966), was the basis for establishing the first large-scale production plant of *Spirulina* (Sasson, 1997). *Arthrospira* has been produced commercially for the last 30 years for food and specialty feeds. Commercial algae like *Spirulina*, are normally produced in large outdoor ponds under controlled conditions (Fig. 3). Some companies also produce directly from lakes. Current production of *Spirulina* worldwide is estimated to be about 3,000 metric tons.

The incidence of toxic episodes in freshwaters due to the presence of toxic cyanobacteria has considerably increased over the last few years. There is also increasing evidence that low levels of exposure may have chronic effects in humans and therefore strict and reliable control of these toxins should be carried out in order to prevent serious public health problems. It is known that cyanobacteria have been implicated in a number of episodes of human illnesses worldwide (Martinez, 2007; Šejnohová, 2008). For this reason toxicological tests obligation done so the *Spirulina* culture and biomass. There is no report of cyanobacterial toxins in *Arthrospira* species to date. Although inadvertent harvest of these toxic species is a risk when harvesting algae from natural bodies of water with mixed phytoplankton populations, it is very unlikely to be a problem in properly controlled and properly managed monoculture of *Arthrospira* (Belay, 2008). So, sold widely in health food stores and mass-market outlets throughout the world, *Spirulina*'s safety as food has been established through centuries of human use and through numerous and rigorous toxicological studies (Belay, 1997; Cevallos, et al., 2008).

The nutritive value of *Arthrospira* is amplified in that it has a relatively low percentage of nucleic acids (~4%) as comperad with the high content of nucleic acids in bacteria. Also, it is

Fig. 2. Harvesting, traditionally drying *Spirulina* dihé in a sand filter, and selling local market (www.AlgaeIndustryMagazine.com, Algae in Historical Legends March 10, 2011, by Robert Henrikson)

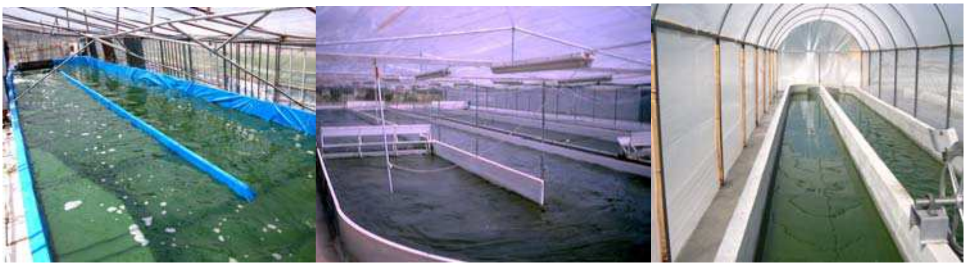

Fig. 3. *Spirulina* production ponds under controlled conditions (İzmir-Aydın/Turkey), Photo by Edis Koru

extremely high in vitamin B_{12}, the mucoprotein cell walls are easy to digest, unlike the cellulose cell wall found in many other nutritional algae, it is completely non-toxic, and its lipids are made up of unsaturated fatty acids that do not form chlesterol. This makes *Spirulina* a potential food item for persons suffering from coronary illness and obesity (Richmond, 1992). In addition to being exceptionally high in protein, *Arthrospira* appears to have the highest vitamin B_{12} content of any unprocessed plant or animal food, representing a boon to vegetarian diets. Two or three heaped tablespoons of *Spirulina* (~20-25 g), provide all the daily body requirements of vitamin B_{12}, as well as significant quantities of other B-complex vitamins, including 70% of the recommended daily allowance for vitamin B_1 (thiamine), 50% for B_2 (riboflavin), and 12% for B_3 (niacin). Also, 10 g. of *Spirulina* contains about 25000 international units of vitamin A, representing over 500% of the recommended daily allowance. Other nutritional attributes of *Spirulina* include essential unsaturated fatty acids, the most important of which is γ-linolenic acid (6,9,12-octadecatrienoic-acid) of which it has a high content (Becker, 1992, Richmond, 1992). Also, researchers have reported the therapeutic effects of *Spirulina* as a growth promoter, probiotic, and booster of the immune system in animals including fishes (Venkataraman, 1993; James et al., 2006).

Some of the best worldwide known *Spirulina* producing companies are: Earthrise Farms (USA), Cyanotech (USA), Hainan DIC Microalgae Co., Ltd (China), Marugappa Chettir Research Center (India), Genix (Cuba) and Solarium Biotechnology (Chile) (Belay, 1997).

Nowadays, *Spirulina* has been marketed and consumed as a human food and has been approved as a food for human consumption by many governments, health agencies and associations of these countries: Argentina, Australia, Austria, Bahrain, Bahamas, Bangladesh, Belarus, Belgium, Brazil, Bulgaria, Canada, Chad, Chile, China, Colombia, Costa Rica, Croatia, Czech Republic, Denmark, Ecuador, Egypt, Ethiopia, Finland, France, Germany, Greece, Guam, Gulf States Haiti, Hungary, India, Iceland, Indonesia, Ireland, Israel, Italy, Jamaica, Japan, Kenya, Korea, Kuwait, Liechtenstein, Luxembourg, Macedonia, Malaysia, Mexico, Myanmar, Monaco, Netherlands, New Zealand, Nigeria, Norway, Peru, Philippines, Poland, Portugal, Romania, Russia, Saudi Arabia, Serbia, Singapore, Slovenia, South Africa, Spain, Sweden, Switzerland, Taiwan, Thailand, Togo, Turkey, Ukraine, United Kingdom, United States, Venezuela, Vietnam, Zaire, Zimbabwe. (Becker & Venkataraman, 1984; Vonshak, 2002; Koru, 2009, Henrikson, 2010).

3. Biological properties of *Spirulina*

3.1 Morphology and taxonomy

Spirulina (*Arthrospira*) is symbiotic, multicellular and filamentous blue-green microalgae with symbiotic bacteria that fix nitrogen from air. *Spirulina* can be rod- or disk-shaped. Their

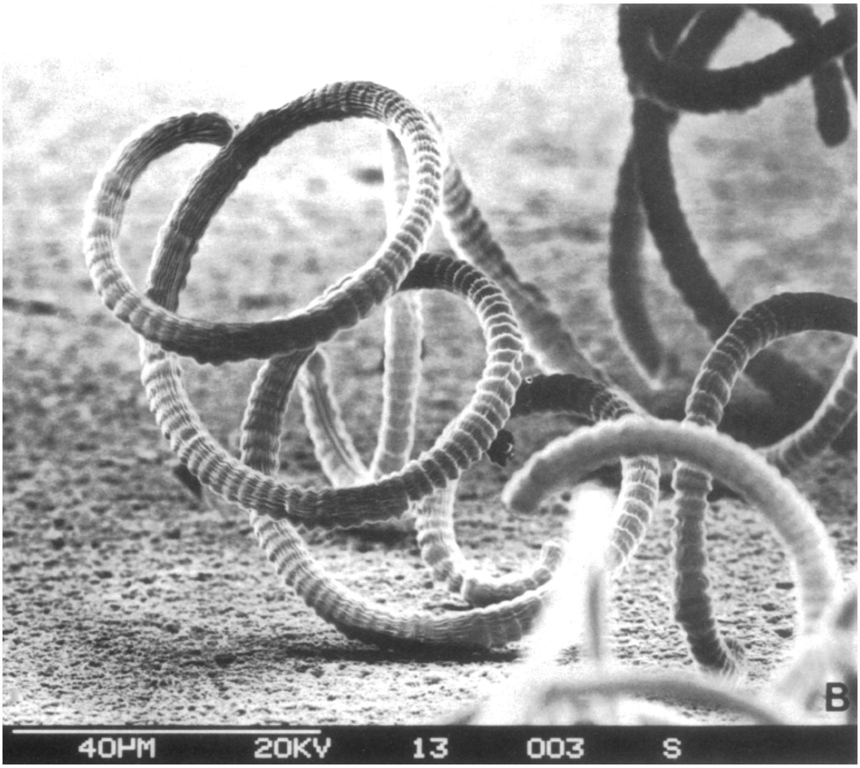

Fig. 4. Morphology of *Spirulina (Arthrospira)*, Scanning electron micrograph of a trichome of axenic *S. platensis*. (Ciferri, 1983), Photo by R. Locci

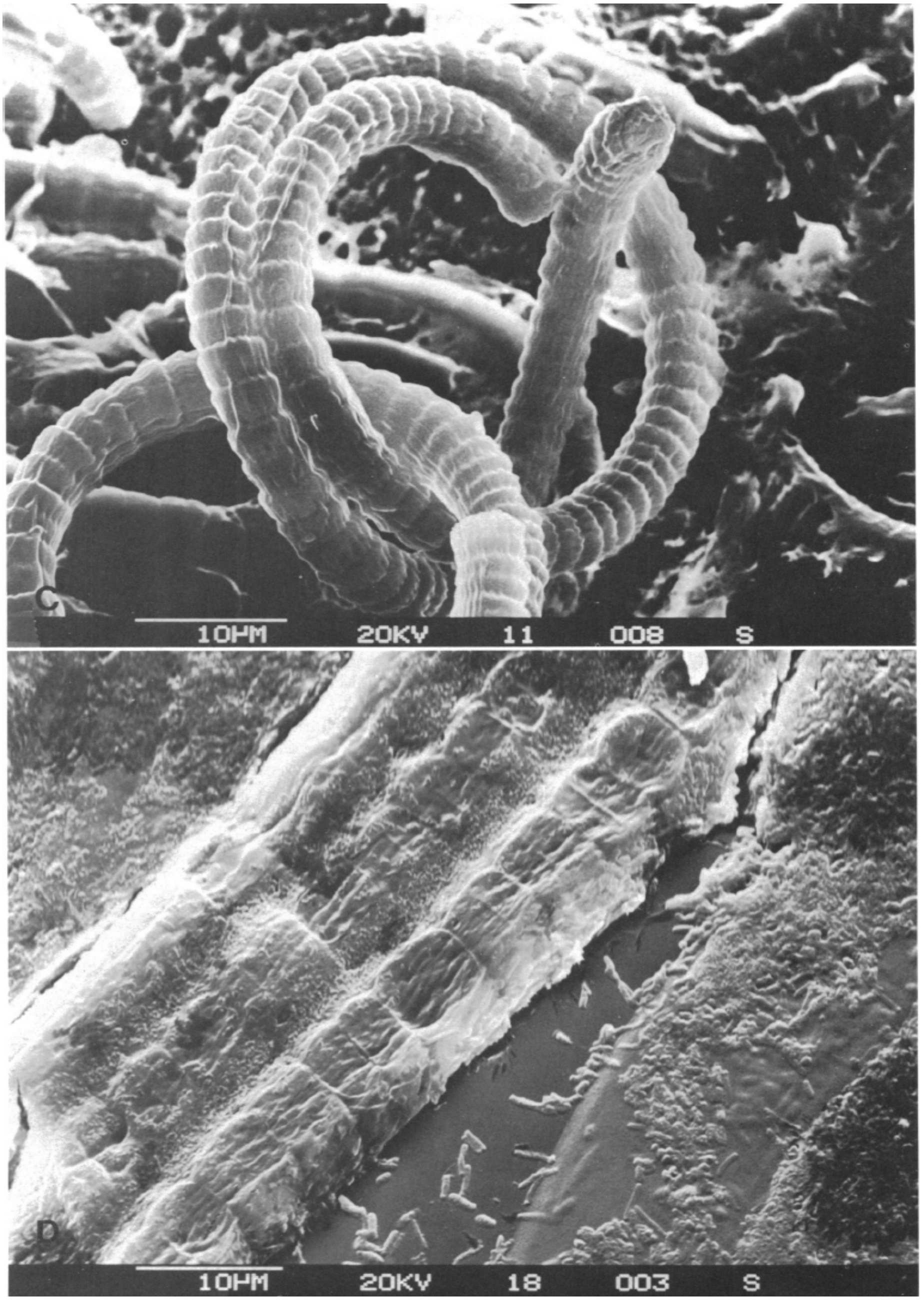

Fig. 5. Morphology of *Spirulina(Arthrospira)*, (C). Scanning electron micrograph of a portion of a trichome of axenic *S. platensis*; (D). Scanning electron micrograph of non axenic trichomes of *S. maxima*. (Ciferri, 1983), Photo by R. Locci

main photosynthetic pigment is phycocyanin, which is blue in colour. These bacteria also contain chlorophyll *a* and carotenoids. Some contain the pigment phycoythrin, giving the bacteria a red or pink colour. *Spirulina* are photosynthetic and therefore autotrophic. *Spirulina* reproduce by binary fission. *Arthrospira* (*Spirulina*) species show great plasticity in morphology. This is attributed to environmental factors like temperature and other physical and chemical factors and possibly also due to genetic change. Transmission Electron Microscope observations show for *Spirulina* prokaryotic organization, capsule, pluri-stratified cell wall, photosynthetic or thylakoid lamella system, ribosomes and fibrils of DNA region and numerous inclusions (Fig. 4,5). The capsule has fibrillar structure and covers each filament protecting it. The irregular presence of capsule around the filaments in *S. platensis* is a differentiating morphological characteristic to compare with *S. maxima* (Ciferri, 1983; Fox, 1996; Belay, 1997; Tomaselli, 2002).

3.2 Spirulina biochemical composition

Microalgae could be utilized for the production of several chemicals which are either unique to the algae or found at relatively high concentrations and command a high market value. In this respect, *Spirulina* is one of the more promising microalgae. It is especially rich, relative to other sources, in the polyunsaturated fatty acid γ- linolenic acid (GLA), and in pigments such as phycocyanin, myxoxanthophyl and zeaxanthin. The biochemical composition of *Spirulina* has been analyzed since 1970, showing high protein concentration, 60–70% of its dry weight, whose nutritive value is related to the quality of amino acid. *Spirulina* contains essential amino acids, including leucine, isoleucine and valine. It also contains a relative high concentration of provitamin A, vitamin B_{12} and β-carotene. *Spirulina* have 4–7% lipids, essential fatty acids as linolenic and γ-linolenic acid, and ω-3 and ω-6 polyunsaturated fatty acids (Wu, L.C. et al., 2005) (Table 1,2,3). *Cyanobacteria* and algae possess a wide range of colored compounds, including carotenoids, chlorophyll, and phycobiliproteins. C-phycocyanin is the principal phycobiliprotein. A selenium-containing phycocyanin has been isolated from *S. platensis* (Shekharam et al., 1987). *A. platensis* contains about 13.5% carbohydrates, the sugar composition is mainly composed of glucose, along with rhamnose, mannose, xylose, galactose, and two unusual sugars: 2-*O*-mehtyl-l-rhamnose and 3-*O*-methyl-l-rhamnose. Nowadays the antiviral activity of *Spirulina* has been attributed to three groups of substances: sulfated polysaccharides, sulfoglycolipids, and a protein-bound pigment, the allophycocianin (Barrón et al., 2008). *Spirulina* contains 2.2%-3.5% of RNA and 0.6 %-1% of DNA, which represents less than 5% of these acids, based on dry weight.

Food Type	**Crude Protein (%)**
Spirulina powder	65
Chicken Egg	47
Beer Yeast	45
Chicken meat	24
Skimmed Powdered milk	37
Cheese	36
Beef meat	22
Fish	22

Table 1. Quantity of *Spirulina* proteins and other foods (Henrikson, 1994)

Vitamins	mg 100/g
Provitamin A	$2.330x10^3$IU/kg
(β-carotene)	140
Vitamin E	100 a-tocopherol equiv.
Thiamin B_1	3.5
Riboflavin B_2	4.0
Niacin B_3	14.0
Vitamin B_6	0.8
Vitamin B_{12}	0.32
Biotin	0.005
Folic acid	0.01
Phantothenic acid	0.1
Vitamin K	2.2

Table 2. Vitamins in *Spirulina* powder (Belay, 1997)

Composition	Quantity (per 100 g dry wt)
General composition	
Moisture	3.5 g.
Protein	63.5 g.
Fat (Lipids)	9.5 g.
Fibre	3.00 g.
Ash	6.70 g.
N-free extract	15 g.
Colorants	
Phycocyanin	15.6 g.
Carotenoids	456.00 mg
Chlorophyll-*a*	1.30 g
Vitamins	
Provitamin A	213.00 mg
Thiamin (V.B_1)	1.92 mg
Riboflavin (V.B_2)	3.44 mg
Vitamin B_6	0.49 mg
Vitamin B_{12}	0.12 mg
Vitamin E	10.40 mg
Niacin	11.30 mg
Folic acid	40 µg
Panthothenic acid	0.94 mg
Inositol	76.00 mg
Minerals	
Phosphorus	916.00 mg
Iron	53.60 mg
Calcium	168 mg
Potassium	1.83 g
Sodium	1.09 g
Magnesium	250 mg

Table 3. A typical analysis of *Spirulina* product. Based on sample of dried *Spirulina* powder of Turkish Algae Company analyzed by Republic of Turkey Ministry of Agriculture and Rural Affairs Izmir Province Control Laboratory and Ege University (Koru et al., 2008)

Composition	Fatty acids (%)	
(C14) Myristic acid	0.23[a]	1.6-1.7[b]
(C16) Palmitic acid	46.07[a]	18-38[b]
(C16:1)Δ9 Palmitoleic acid	1.26[a]	5-7[b]
(C18:1)Δ9 Oleic acid	5.26[a]	5-6[b]
(C18:2)Δ9,12 Linoleic acid	17.43[a]	6-15[b]
(C18:3)Δ9,12,15 γ-Linolenic acid	8.87[a]	4-22[b]
Others	20.88[a]	10-68[b]

Table 4. Fatty acid composition of *Spirulina platensis* powder ([a] Othes and Pire, 2001; [b] Diraman et al., 2009)

3.3 General quality and safety assurance for *Spirulina*

In 1970's, *Spirulina* underwent extensive safety studies with animals and fish. Independent feeding tests in France, Mexico and Japan showed no undesirable results and no toxic side effects on humans, rats, pigs, chickens, fish and oysters. Many independent rat feeding trials were conducted in Japan and no negative effects at all were found for acute or chronic toxicity or reproduction. The re-introduction of *Spirulina* as a health food for human consumption in the late 1970s and the beginning of the 1980s was associated with many controversial claims which attribute to *Spirulina* a role of a 'magic agent' that could do almost everything, from curing specific cancer to antibiotic and antiviral activity. Since most

Spirulina food standards: quality requirements of France, Sweden, Japan and Earthrise Farms (USA)				
Standard	France[a]	Sweden[b]	Japan[c]	USA[d]
Protein	%55-65	%55-65	≥%50	%55-65
Total Carotenoids	♦	♦	>100 mg %	300 mg/100g
Chlorophyll-a	>500 mg %	♦	>500 mg %	900 mg/100g
Phycocyanin	♦	♦	>2000 mg %	8,000 mg/100g
Moisture	♦	♦	<7 %	<7 %
Standard Plate Count	<100,000/g	1,000,000/g	<200,000/g	<200,000/g
Mold	♦	<1000/g	♦	<100/g
Saccharomyces sp.	♦	♦	♦	< 40/g
Coliform bacteria	<10/g	<100/g	negative	negative
Staphylococcus aureus	<100/g	<100/g	♦	negative
Salmonella enteritidis	negative	negative	♦	negative
total heavy metals (Lead, Mercury, Cadmium, Arsenic)	♦	♦	< 20.0 ppm	2.1 ppm
Insect fragmen	♦	♦	♦	<30 pcs per 10 g
Rodent hair	♦	♦	♦	< 1.5 pcs per 150 g

[a] Superior Public Hygiene Council of France, 1984, 1986.
[b] Ministry of Health, Sweden.
[c] Japan Health Foods Association, auth. by Ministry of Health and Welfare.
[d] Earthrise Farms, 1995.
definitions: < = less than, ≥ more than, ♦ = no set Standard, /g = per gram, parts per million (ppm)

Table 5. General Quality and Safety Standards for *Spirulina* (Shimamatsu, 2004, Henrikson, 2010)

claims were never backed up by detailed scientific and medical research, they will not be discussed in this chapter. Nevertheless, one cannot ignore the fact that more than 70 per cent of the current *Spirulina* market is for human consumption, mainly as health food. (Vonshak, 2002, Shimamatsu, 2004, Henrikson, 2010). So, there are established national and international quality standards for *Spirulina* products. Tables 5 show the typical analysis of the contents of *Spirulina* product, the quality standard in Europe, Japan and United States Food and Drug Administration's (FDA) requirement for *Spirulina* product respectively. Recently,cyanobacterial toxins have become a major issue in public health due to the increased occurrence of toxic cyanobacterial blooms. These toxic blooms contain algae that produce hepatotoxins called microcystins (Carmichael, 1994). *Spirulina* companies like Earthrise Farms have already developed methods for the determination of these toxins and actually certify each lot of their product to be toxin free. *Spirulina* does not normally contain microcystins but contamination of outdoor culture by other cyanobacteria is a possibility.

4. Conclusion and outlook

A bibliographical review on *Spirulina* (*Arthrospira*) identifies this microorganism as microalgae or bacteria, by bacteriologist and botanists respectively. This study has revealed many research studies done on its properties, some of these are related to human and animal food uses. *Spirulina* is claimed as a non-toxic, nutritious food, with some corrective properties against viral attacks, anemia, tumoral growth and low prostaglandins production in mammals; and as a source of the yellow coloration of egg yolk when consumed by hens, and a growth, sexual maturation and fertility factor, in poultry and bovines. This material contains proteins, carbohydrates, essential fatty acids, vitamins, minerals, carotenes, chlorophyll *a* and phycocyanin. *Arthrospira* may be produced in rather simple pilot plants or industrial installations if good conditions and quality controls are assured. *Spirulina* has been used as food for centuries in human history. Moreover, it has been produced commercially over the past 30 years and consumed by thousands of people without problem. Many research show that it has a good nutritional profile in addition to containing some phytonutrients that have potential health benefits. The technology of *Spirulina* production has also advanced in the past 30 years, resulting in higher-quality of product at relatively lower cost. *Spirulina* or *Arthrospira* has attracted the attention of researchers for many years, as shown by the hundreds of publications in its various aspects. There are more and more information is being made available about its biology, biotechnological and nutrition applications, and health application and even in what seem to be remote applications like biofuel production and as a life support system in space studies. This interest will no doubt continue. Research along these lines will be rewarding both socially and professionally.

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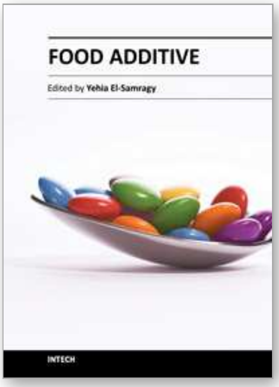

Food Additive

Edited by Prof. Yehia El-Samragy

ISBN 978-953-51-0067-6
Hard cover, 256 pages
Publisher InTech
Published online 22, February, 2012
Published in print edition February, 2012

A food additive is defined as a substance not normally consumed as a food in itself and not normally used as a characteristic ingredient of food whether or not it has nutritive value. Food additives are natural or manufactured substances, which are added to food to restore colors lost during processing. They provide sweetness, prevent deterioration during storage and guard against food poisoning (preservatives). This book provides a review of traditional and non-traditional food preservation approaches and ingredients used as food additives. It also provides detailed knowledge for the evaluation of the agro-industrial wastes based on their great potential for the production of industrially relevant food additives. Furthermore the assessment of potential reproductive and developmental toxicity perspectives of some newly synthesized food additives on market has been covered. Finally, the identification of the areas relevant for future research has been pointed out indicating that there is more and more information needed to explore the possibility of the implementation of some other materials to be used as food additives.

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