SPIRULINA (ARTHROSPIRA): AN EDIBLE MICROORGANISM. A REVIEW.

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ABSTRACT

Spirulina is a photosynthetic, filamentous, spiral-shaped, multicellular and green-blue microalga. The two most important species of which are *Spirulina maxima* and *Spirulina platensis*. For these microorganisms cell division occurs by binary fission. Since this material contains chlorophyll *a*, like higher plants, botanists classify it as a microalga belonging to *Chyanophyceae* class; but according to bacteriologists it is a bacterium due to its prokaryotic structure. Before Columbus, Mexicans (Aztecs) exploited this microorganism as human food; presently, African tribes (Kanembu) use it for the same purpose. Its chemical composition includes proteins (55%-70%), carbohydrates (15%-25%), essential fatty acids (18%) vitamins, minerals and pigments like carotenes, chlorophyll *a* and phycocyanin. The last one is used in food and cosmetic industries. *Spirulina* is considered as an excellent food, lacking toxicity and having corrective properties against viral attacks, anemia, tumor growth and malnutrition. It has been reported in literature that the use of these microalgae as animal food supplement implies enhancement of the yellow coloration of skin and eggs yolk in poultry and flamingos, growth acceleration, sexual maturation and increase of fertility in cattle.

Key words: food, microalgae, nutrition, *Spirulina.*

RESUMEN

Spirulina es una microalga verde-azul, fotosintética, filamentosa, en forma helicoidal, multicelular. Las dos especies más importantes son *Spirulina máxima* y *Spirulina platensis.* La división celular se realiza por fisión binaria. Según los botánicos es una microalga debido a la presencia de clorofila *a* al igual que en plantas superiores. Pertenece a la división Cianofita y a la clase Cianoficea, pero según los bacteriólogos es una bacteria debido a su estructura procarionte. Se conoce desde tiempos precolombinos, que este microorganismo fue utilizado como alimento por tribus mexicanas (Aztecas) y actualmente por tribus africanas (Kanembu). Su composición química incluye proteínas (55%-70%), azúcares (15%-25%), ácidos grasos esenciales (18%), vitaminas, minerales y pigmentos como carotenos, clorofila *a* y ficocianina; éste último utilizado en industrias de alimentos y cosméticas. Se le considera excelente alimento, exento de toxicidad y poseedor de propiedades correctoras de ataques virales, anemia, crecimiento tumoral y malnutrición. La literatura ha reportado, que *Spirulina*, usada como alimento de animales conlleva al realce de la coloración amarilla de piel y yema de huevos, en gallináceos y flamencos; aceleración de crecimiento, maduración sexual y aumento de fertilidad, en bovinos.

Palabras clave: alimento, microalga, nutrición, *Spirulina*.

HISTORY OF *SPIRULINA* IN HUMAN CONSUMPTION

It is not known with accuracy when man began to use microalgae. The current use of these resources has three precedents: tradition, scientific and technological development, and the so-called, "green tendency" (Henrikson, 1994). 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, (Figure 1). This author makes reference to "..small cakes made of a mud-like algae, which has a cheese-like flavor, and that natives took out of the lake to make bread,..." (Ciferri, 1983). Years later, the Franciscan friar Bernardino de Sahagún wrote: "... in certain periods of the year, very soft things are gathered from Mexican lakes. They look like curdles, have a clear blue color, and are used to make bread, that then eaten cooked..." Natives gave this food the name of Tecuitlalt, which in their language literally means "excrements of stones". In 1524, friar Toribio of Benavente related that the Aztecs harvested the Tecuitlalt, using clothes for pressing and the resulting dough was placed on sand and exposed to the sunshine for its drying. Once Spanish Conquest was over, the topic of the Tecuitlalt was not mentioned again, and its elaboration fell into oblivion, possibly due to contagious disease outbreaks, attributed to the new customs adopted by the Indians, new foods, and the deep social, political and religious changes brought by the Europeans (Henrikson, 1994).

In 1940, the French phycologist P. Dangeard mentioned a cake called *dihé*, consumed by the people of the Kanembu tribe, near the African Lake Chad, in the sub-desert area of Kanem. *Dihé* is a hardened cake of blue-green algae, collected at the banks of small ponds surrounding the lake and later on sun-dried. Dangeard studied the *dihé* samples and concluded that it was a purée of a spring form blue algae, main constituent of the phytoplankton in a large number of the African Valley's lakes (Ciferri, 1983).

Between 1964 and 1965, the botanist Jean Leonard (Leonard, 1966), who participated in the Belgian Trans-Saharan Expedition, was impacted when he observed "a curious bluish green substance, similar to cookies…" Leonard confirmed that *dihé* was made up of *Spirulina*, obtained from alkaline lakes in the Kanem desert, northeast of Lake Chad. This investigator and his colleague Cómpere corroborated the previous report by P. Dangeard, from whose observations the chemical analyses of *Spirulina* began. At that time, a group of French investigators studied some samples of *Spirulina* (*S. maxima*) that grew abundantly in Lake Texcoco, near Mexico City (Ciferri, 1983; Richmond, 1992).

From the scientific point of view, the microalgae cultivation began in 1919 with Warburg's investigations. This scientist was well known for his works on dense suspensions of *Chlorella,* as a tool to study photosynthesis. The easy manipulation under controlled conditions and the experimental reproducibility made the microalgae favorite organisms for biochemical, vegetable physiology and photosynthetic studies. In 1950, the United States and Japan began the experimental cultivations of this microorganism to investigate its chemical composition and industrial applications. Japan was the first country to produce *Chlorella* using this microorganism as diet food or a water-soluble extract, denominated *Chlorella* Growth Factor (Devlin, 1975).

From 1970, the nutritional and medicinal studies on *Spirulina* have proliferated (Chamorro, *et al.*, 1996; Fox, 1993; Hayashi, 1996*a*; Richmond, 1992; Saxena, *et al.*, 1983; Schwartz and Shklar, 1987). In 1970, the German Federal Republic supported investigations on human consumption of *Spirulina* in India, Thailand and Peru. In the Asian countries, the production was focused on nutritious support for the undernourished population; in Peru, efforts have been made to industrialize the production of *Scenedesmus*. In 1970, the massive production of microalgae, which could be used in protein production and in water treatment, was projected (Ayala and Vargas, 1987; Cañizares, *et al.,* 1993; Ciferri and Tiboni, 1985; Oxa and Ríos, 1998).

Spirulina is marketed and consumed in: Germany, Brazil (Lacaz and Nascimento, 1990), Chile, Spain, France, Canada, Belgium, Egypt, United States, Ireland, Argentina, Philippines, India, Africa, and other countries, where public administration, sanitary organisms and associations have approved human consumption (Henrikson, 1994). 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) (Ayala, *et al.,* 1988; Jourdan, 1993; Belay, 1997).

SYSTEMATIC

According to the classification in Bergey's Manual of Determinative Bacteriology, *Spirulina* (*Arthrospira)*, (Figure 2) belongs to the oxygenic photosynthetic bacteria that cover the groups *Cyanobacteria* and *Prochlorales* (Castenholz and Waterbury, 1989; Whitton, 1992), which are, by phylogeny, related to the sequence of the rARN (ribosomal ribonucleic acid) sub-unit 16S. As a function of the sequence data of this sub-unit and the rRNA sub-unit 5S, these prokaryotes are classified within the eubacteria group.

In 1827, P. J. Turpin isolated *Spirulina* from a fresh water sample (Ciferri, 1983). In 1844, near the city of Montevideo, Wittrock and Nordstedt reported the presence of a helical, septal and green-blue microalgae named *Spirulina jenneri* f. *platensis*. But it

was not until 1852, that the first taxonomic report written by Stizenberger, appeared. He gave this new genus the name *Arthrospira* based on the septa presence, helical form and multicellular structure.

Gomont confirmed Stizenberger's studies in 1892. This author attributed the aseptate form to the *Spirulina* genus, and the septal form to the *Arthrospira* genus. Geitler in 1932, because of the helical morphology, reunified the members of the two genera under the designation *Spirulina* without considering the septum presence only morphological similarity. In 1989, these microorganisms were classified into two genera, according to a suggestion by Gomont in 1892 (Castenholz and Waterbury, 1989); this classification is currently accepted (Tomaselli, *et al.,* 1996; Vonshak and Tomaselli, 2000).

The systematic position of cyanobacteria has been a matter of discussion, as these photosynthetic organisms were first considered algae. In 1962, a distinction between prokaryotes and eukaryotes was clearly established. The main difference is based upon the presence of cell organelles enveloped by a phospholipidic membrane in eukaryotes. Stanier and Van Neil (1969) incorporated green-blue algae into the prokaryote kingdom and proposed to call these microorganisms cyanobacteria. This designation was accepted and first published in 1974 in the Bergey`s Manual of Determinative Bacteriology (Guglielmi, *et al.*, 1993).

Spirulina and *Arthrospira* must be admitted as different genera. The worldwide investigation on microalgae has been carried out under the name of *Spirulina*; this common designation between scientist and consumers has proved difficult to change. The microalgae exploited as food with excellent health properties belongs to the genus *Arthrospira*, but it will probably be called *Spirulina* for some time.

Spirulina and *Arthrospira* morphologies are differentiated fundamentally by: helix type, distribution of pores in the cell wall, visibility of septos under light microscopy, diameter and fragmentation type of Trichomes (filaments) (Guglielmi, *et al.*, 1993; Vonshak and Tomaselli, 2000). As mentioned, *S. maxima* and *S. platensis* are the most important species in this genus and among these exist taxonomic differences in filaments, vacuoles and external cover or capsule regularity of each filament (Tomaselli, 1997).

The names cyanobacteria and green-blue algae *(Cyanophyceae),* are considered compatible terms. The first one refers to the phylogenetic / taxonomic relationship, while the second represents the ecological/biological correlation (Castenholz and Waterbury, 1989).

ULTRA-STRUCTURE

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. 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* (Balloni, *et al.*, 1980; Belay, 1997). Trichome width varies from 6 to 12 μm, and is composed of cylindrical cells. The helix diameter varies from 30 to 70 μm (Tomaselli, 1997); the trichome length is about 500 μm, although in some cases when stirring of culture is deficient the length of filament reaches approximately 1 mm. It is very important to explain that the helical shape of *Spirulina* in liquid culture is changed to spiral shape in solid media (Figure 3). These changes are due to hydratation or dehydratation of oligopeptides in the peptidoglycan layer (Ciferri, 1983).

Spirulina cell wall is formed by four numbered layers, from the inner most outward as: LI, LII, LIII and LIV. All these layers are very weak, except layer LII made up of peptidoglycan, substance that gives the wall its rigidity (Ciferri, 1983). The LI layer contains β-1,2-glucan, a polysaccharide not very digestible by human beings. However, the low concentration (<1%) of this layer, thickness its (12 nm), and the protein and lipo-polysaccharide nature of the LII layer are favorite reasons for the easy human digestion of *Spirulina* (Balloni, *et al.,* 1980).

In this microorganism chlorophyll *a*, carotenes and phycobilisomes, which contain phycocyanin (blue pigment) are located in the thylakoid system or photosynthetic lamellas. The inter-thylakoid space is limited by the presence of electronically transparent protein gas vesicles, with the cylindrical form that give *Spirulina* its floating capacity (Ciferri, 1983).

Ribosomes and fibrils of DNA region are generally of central localization (Balloni, *et al.,* 1980).

Spirulina contains numerous characteristic peripheral inclusions associated to thylakoids. Those are: cyanophycin granules, polyhedral bodies, polyglucan granules, lipid granules, and polyphosphate granules (Balloni, *et al.,* 1980; Ciferri, 1983). The cyanophycin granules, or reserve granules, are important due to their chemical nature and a series of pigments. The polyhedral bodies or carboxysomes mainly contain the enzyme ribulose 1,5-diphosphate carboxylase that allows the fixation of CO_2 in photosynthetic organisms and probably carry out a reserve function. The polyglucan granules or glycogen granules or α-granules are glucose polymers, small, circular and widely diffused in the interthylacoidal space. The lipid granules, β-granules or

osmophile granules form the reservation deposit, constituted by poly-β-hydroxybutyrate (PHB), found only in prokaryotes. PHB acts as a carbon and energy reserve (Vincenzini, *et al*., 1990).

LIFE CYCLE

A fundamental aspect of *Spirulina* biology is its life cycle (Figure 4) due to the taxonomic, physiologic and cultivation implications (Ciferri, 1983; Richmond, 1984). This period is summarized in three fundamental stages: *trichomes* fragmentation, hormogonia cells enlargement and maturation processes, and trichome elongation. The mature trichomes are divided into several small filaments or hormogonia through previous formation of specialized cells, necridium cells, in which the cell material is reabsorbed allowing fragmentation. The number of cells in the hormogonias is increased by binary fission. For this process, the trichomes grows lengthwise and takes their helical form (Balloni, *et al.*, 1980).

CHEMICAL COMPOSITION

Since 1970, *Spirulina* has been analyzed chemically. It has been shown to be an excellent source of proteins, vitamins and minerals (Switzer, 1980).

Proteins. *Spirulina* has a high protein concentration (60%-70% of its dry weight), (Table 1) (Ciferri, 1983). *Spirulina* is useful in human nutrition, due to the high quality and quantity of its protein. The nutritive value of a protein is related to the quality of amino acids, digestibility coefficient, as well as by its biological value (Dillon and Phan, 1993; Richmond, 1992). *Spirulina* contains essential amino acids; the highest values are leucine (10.9% of total amino acids), valine (7.5%), and isoleucine (6.8%), (Cohen, 1997). Denaturation of *Spirulina* protein is observed when algae are heated above 67 ºC, at neutral aqueous solution. Hydrophobic regions interaction during heating and hydrogen bonds formation during cooling are aggregation and gelation factors of *Spirulina* protein (Chronakis, 2001).

Vitamins. Among food, *Spirulina* has a relative high provitamin A concentration (Belay, 1997), (Table 2). An excessive dose of β-carotene may be toxic, but when the β-carotene is ingested from the *Spirulina* or another vegetable it is usually harmless since the human organism only converts into vitamin A the quantity it needs (Henrikson R., 1994). *Spirulina* is a very rich source in vitamin B_{12}, and that is a reason why these cyanobacteria is of great value for people needing supplements in the treatment of pernicious anemia (Richmond, 1992; Becker, 1984; Belay, 1997).

Lipids. *Spirulina* contains 4-7% lipids. *Spirulina* has essential fatty acids: linoleic acid (LA) $(C_{18:2})\Delta^{9,12}$ and γ-linolenic acid $(C_{18:3})\Delta^{9,12,15}$ (GLA) (Othes and Pire, 2001), (Table 3). The latter is claimed to have medicinal properties and is required for arachidonic acid and prostaglandin synthesis (Dubacq and Pham-Quoc, 1993). GLA lowers low-density lipoprotein, being 170-fold more effective than LA (Cohen, 1997).

Minerals. Iron in some nutritional complements is not appropriately absorbed. Iron in *Spirulina* is 60% better absorbed than ferrous sulfate and other complements. Consequently, it could represent an adequate source of iron in anemic pregnant women (Pyufoulhoux, *et al.,* 2001) (Table 4).

Carbohydrates. *Spirulina platensis* contains about 13.6% carbohydrates; some of these are glucose, rhamnose, mannose, xylose and galactose (Shekharam, *et al.,* 1987). *Spirulina* does not have cellulose in its cell wall, a feature that makes it an appropriate and important foodstuff for people with problems of poor intestinal absorption, and geriatric patients (Richmond, 1992). A new high molecular weight polysaccharide, with immunostimulatory activity has been isolated from *Spirulina* and is called "Immulina". This highly water-soluble polysaccharide represents between 0.5% and 2.0% (w/w) of the dry microalgae (Pugh, *et al.,* 2001).

Nucleic acids content. One of the main concerns about the consumption of microorganisms is their high content of nucleic acids that may cause disease such as gout. *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. These values are smaller than those of other microalgae like *Chlorella* and *Scenedesmus* (Ciferri, 1983).

Pigments. Some natural pigments are found in *Spirulina,* (Table 5). These pigments are responsible for the characteristic colors of certain flamingo species that consume these cyanobacteria in the African Valley. This knowledge has promoted the use of this microorganism as source of pigmentation for fish, eggs (Ciferri, 1983; Saxena, *et al*., 1983; Henrikson, 1994) and chicken. *Spirulina* also increases the yellowness and redness of broiled chicken due to accumulation of zeaxanthin (Toyomizu, *et al.,* 2001).

SOME *Spirulina* CONSUMPTION BENEFITS

Studies have shown that *Spirulina* consumption during 4 weeks reduces serum cholesterol levels in human beings by 4.5% (Henrikson, 1994) and significantly reduces body weight by 1.4 +/- 0.4 Kg after four weeks (Becker, *et al*., 1986). These reports indicated no changes in clinical parameters (blood pressure) or in biochemical variables (hematocrite, hemoglobin, white blood cells, sedimentation rate) and absence of adverse effects. The reduction of cholesterol is partly owed to the γ-linolenic acid cyanobacteria high content (Henrikson, 1994).

The β-carotene is one of the most effective substances to counteract those free radicals that alter cells causing cancer (Fedkovic, *et al*., 1993; Schwartz, *et al*., 1990). Studies at the Harvard University School of Dental Medicine found a reduction in mouth cancer when β-carotene extracts, obtained from *Spirulina*, are consumed. The β-carotene solution applied to oral cancer tumors in hamsters reduced the tumor number and size and in some cases these disappeared (Schwartz and Shklar, 1987; Schwartz, *et al*., 1988). *Spirulina* extract induces the tumor necrosis factor in macrophages, suggesting a possible tumor destruction mechanism (Shklar and Schwartz, 1988).

An extract of sulfated polysaccharides, called Calcium-Spirulan (Ca-SP), made up of rhamnose, ribose, mannose, fructose, galactose, xylose, glucose, glucuronic acid, galacturonic acid, and calcium sulfate, obtained from *Spirulina*, showed activity against HIV, Herpes Simplex Virus, Human Cytomegalovirus, Influenza A Virus, Mumps Virus and Measles Virus (Henrikson R, 1994; Hayashi, 1996*b*). Current investigation in this field is searching for extracts that inhibit the AIDS virus replication (Ayehunie, *et al*., 1998) and allows these patients to improve their health.

Spirulina excretes variable quantities of products from its metabolism such as: organic acids, vitamins, and phytohormones. Cell extract of *S. maxima* has shown antimicrobial activity against *Bacillus subtillis*, *Streptococcus aureus*, *Saccharomyces cerevisiae,* and *Candida albicans*. The presence of high quantities of acrylic acid in *Spirulina* was substantiated at the end of the seventies. This substance shows anti-microbial activity, in a 2 mg/L of biomass concentration. Propionic, benzoic and mandelic organic acids were also found (Balloni W. *et al*., 1980).

Lactobacillus population in human gastrointestinal tract is increased by *Spirulina* consumption. This means: food digestion and absorption improvement, intestinal protection against bacterial infections and immune system stimulation (Henrikson, 1994; Schiffrin, *et al*., 1997). Immune system modulation is due to interference on production and NK cytotoxicity (Hirahashi, *et al.,* 2002).

Spirulina reduces: hepatic damage due to drug abuse and heavy metal exposure, inflammatory response (Richmond, 1984; González, *et al*., 1999), cells degeneration (Bulik, 1993), anaphylactic reaction (Yang, *et al*., 1997), Bitot's spots, and Cesium-137 and Strontium-90 radiation in Chernobyl children (Henrikson, 1994).

Spirulina contains vitamin A, important in preventing eye diseases; iron and vitamin B_{12}, useful in treating hypoferric anemia and pernicious anemia, respectively; γ-linolenic acid, appropriate in treatment of atopic child eczema therapy; to alleviate premenstrual syndrome, and in immune system stimulation (Pascaud, 1993). *Spirulina* also has a positive effect on cardiac disease, Parkinson`s disease, malnutrition, sclerosis (Richmond A, 1992; Fox, 1993, Fox, 1998; Thein, 1993) and wounds cure (Richmond, 1992).

Other benefits are attributed to *Spirulina:* anti-arthritic effect due to the anti-inflammatory and antioxidative properties of phycocyanin (Ramirez, *et al.*, 2002); anti-atherogenic property (Kaji, *et al*., 2002), tumor burden inhibition (Dasgupta, *et al.*, 2001); chemo protective and radio-protective effect (Zhang, *et al*., 2001); and antioxidant activity on lead-induced toxicity in rats (Upasani, *et al*., 2001).

In Mexico, *Spirulina* is used in to enrich candies. In Australia and New Zealand beverages of this substance are marketed. In Japan, India, and Singapore *Spirulina*-enriched appetizers are sold specially to pregnant women, children and elderly. *Spirulina* is not only food, but also a natural coloring in Japanese chewing gums. Countries like Chile, France, Cuba, Germany, Switzerland, Spain, Portugal, Sweden, Holland, Belgium, Denmark, United Kingdom, Australia, and New Zealand market food complements, which include *Spirulina* as the main component. Internationally, skin care products, shampoos, dyes, masks, creams and tonics containing this microorganism are marketed. In Sweden low calorie bread enriched with *Spirulina* is sold, and in France a vegetable pâté, made of *Spirulina*, is sold as bread spread (Henrikson, 1994).

Many agricultural and industrial materials are being prepared from cyanobacteria. These include: biomass (Ciferri, 1983; Richmond and Becker, 1984; Shang-Hao, 1988; Thein, 1993), restriction nucleases (Kawamura, *et al*., 1986), antifungal, antineoplastic (Moore, *et al*., 1984; Clardy, *et al.*, 1990), antimicrobial (Gerwick, *et al.*, 1987), anti-leukemia (Moore, *et al.*, 1977) and herbicidal compounds (Entzeroth, *et al.*, 1985). Some pigments have been produced from cyanobacteria (Jung and Dailey, 1989; Paniagua-Michel and Sasson, 1995). Other products from microalgae are: amino acids (Kerby, *et al.*, 1988), and fertilizers (Boussiba, 1988).

Spirulina has been studied as an animal cell-growth stimulant (Kerby and Rowell, 1992) and in the treatment of residual waters using alginate (Cañizares, *et al.,* 1993; Patnaik, *et al.,* 2001). Phycocyanin shows activity on vegetable cell cultures with production of secondary metabolites as anthocyanin (Ramachandra, *et al.*, 1996). This pigment has the ability to inhibit oxidative damage in DNA and hence it may be used as a therapeutic agent (Bhat, *et al*., 2001).

Spirulina is used in Japan and Taiwan as aquarium fish food, in United States to enhance color, speed the growth and sexual maturation of canaries and exotic birds (Saxena, *et al.*, 1983). Cattle and horse breeders affirm that when adding *Spirulina* to silage, the quantity of sperms in males and the fertility in females are increased (Henrikson, 1994*). Labeo rohita (rohu),* an Indian carp, showed greater growth after being fed with *Spirulina* (Nandeesha, *et al*., 2001). In chickens, *Spirulina* increases the mononuclear phagocyte system function thereby enhancing their disease resistance (Al-Batshan, *et al.,* 2001).

TOXICOLOGY

This microorganism in general terms do not exceed the metal concentration limits recommended by international agencies. But due to the use of fertilizers, possible water and environmental pollution optimal quality control and periodic revisions of this cyanobacteria culture is necessary to detect high metal concentration values (Chamorro, *et al.*, 1996). Studies in Mexico showed that the administration of *S. platensis* to mice does not cause embryonic or fetal damages (Chamorro, *et al.*, 1989; Chamorro and Salazar, 1990).

Absence of phycotoxins in *Spirulina* is an advantage with respect to *Microcystis*, *Anabaena* and *Aphanizomenon*, fresh water cyanobacteria that have caused death in livestock and allergic or gastrointestinal reactions in human beings (Chamorro, *et al.*, 1996).

Chronic and sub-chronic toxicity studies have not revealed toxic effects by *Spirulina*. The lethal dose (LD_{50}) of *Spirulina* has not been determined, since it would be necessary to dispense high quantities in one single dose (Chamorro, *et al.*, 1996; Switzer, 1980).

PRODUCTION

The main commercial large-scale culture of microalgae started in the early 1960s in Japan with the culture of *Chlorella,* followed by *Spirulina* in the early 1970s at Lake Texcoco, Mexico. The third major microalgae industry was established in Australia in 1986. Commercial production of *Dunaliella salina* was cultured as a source of β-carotene (Borowitzka, 1998).

The first plant in USA (*Earthrise Farms)* for the exploitation of *Spirulina*, built in 1981 in California, emerged as the result of a research work on its culture by Dainippon Ink & Chemicals, Inc. of Japan and Proteus Corporation of California (Vonshak, 1997), (Table 6). *Spirulina* grows quickly and produces 20 times more protein by surface unit that soy beams (Henrikson, 1994). When comparing the growth of *Spirulina* and the agricultural crop cycles, the difference in the time of production is noticeable. In agriculture, the harvest is obtained after several months of cultivation, while *Spirulina* is produced continually (Switzer, 1980).

Production process of *Spirulina* requires clonal or unialgal cultures (isolation of a single algal unit or trichome), (Vonshak, 1984; González, *et al.*, 1995; Hoshaw and Rosowski, 1979). The method begins with the determination of physical and chemical parameters of the water sample, which constitutes the main ingredient of the growth medium. The *Spirulina* samples should remain under dim light or in darkness and at 20-25°C; freezing temperature is not recommended because it favors lysis and death (Rippka, 1988). Isolation of the microorganism is carried out under an intensely lighted microscope and with a capillary pipette so that one and only one filament is selected (Ayala, 2000) by its morphological attributes (color, size of trichomes, length and apical filament characteristics). If axenic cultures, specific for physiologic and biochemical studies of algae, are wanted, special treatments such as: centrifugation followed by ultrasonic treatments with antibiotics and potassium tellurite are required (Hoshaw and Rosowski, 1979).

Cyanobacteria are grown in many liquid and solid culture media such as: BG11, ASM-1, Z8, SAG, BBM, AA, KMC, Kn Cg-10, D (Rippka, 1988) and *Spirulina* grows in culture media such as: Zarrouk, SSM (*Sea Saltpeter Medium*) , Vonshak, Spirulina and AO (Ogawa and Aiba, 1977; Ayala and Bravo R, 1982).

Zarrouk medium (Zarrouk, 1966; Borowitzka, 1992) is frequently used during the isolation process and the SSM medium is preferred in the industrial production stage (Ayala, 2000). Eight mayor medium factors influence the productivity of *Spirulina*: luminosity (photo-period 12/12, 4 luxes), temperature (30°C), inoculation size, stirring speed, dissolved solids (10-60 g/L), pH (8.5-10.5), water quality, macro and micronutrient presence, (C, N, P, K, S, Mg, Na, Cl, Ca and Fe, Zn, Cu, Ni, Co, W) (Ciferri, 1983; Ayala, 1998).

Spirulina production may be carried out in closed and open systems. The first one involves laboratory photo bioreactors (Materassi, *et al.*, 1980; Torzillo and Carlozzi, 1996; Watanabe and Hall, 1996). This is not used in industrial production. The

open system, denominated *raceway* (Figure, 5), due to its low production cost, easy handling and high production of biomass, is frequently chosen for industrial production. This method uses a pond with a central islet, a motor operating a paddle wheel which allows continuous displacement of the liquid culture in the peripheral channel. Paddle wheels speed in the order of 20 cm s^{-1} has been recommended. If necessary, plates may be introduced to avoid dead point formation. When this type of reactor is located outdoors the following factors should be considered as modifiers on the cyanobacteria growth: the medium composition (Ciferri, 1983), evaporation speed, culture contamination, and temperature (35°C–38ºC)(Walmsley, *et al.*, 1981).

Productive process has five stages: *Filtration and Cleaning*, a nylon filter at the entrance of the water pond is needed; *Pre-concentration,* to obtain algal biomass which is washed to reduce salts content; *Concentration,* to remove the highest possible amount of interstitial water (located among the filaments); *Neutralization,* to neutralize the biomass with the addition of acid solution; *Disintegration,* to break down trichomes by a grinder; *Dehydration* by spray-drying; this operation has great economic importance since it involves about 20-30% of the production cost (Ayala and Laing, 1990); *Packing* is done in sealed plastic bags to avoid hygroscopic action on the dry *Spirulina*; and *Storage,* in corrugate cardboard boxes, and in fresh, dry, dim, pest-free, and clean storeroom, preventing *Spirulina* pigments from deteriorating (Ayala, 1998). Quality control for *Spirulina* as a food includes microbiological standard tests, chemical composition test, and test for heavy metals, pesticides and extraneous materials (insect fragments, rodent hair and feather fragments) (Belay, 1997).

The above-mentioned facts stress *Spirulina* is a non-noxious microorganism with a very high nutritional and economic potential for animal consumption including man. It may be cultured in laboratory, pilot plant and at industrial scale in a simple way. However, like in all food processing, it is necessary to maintain good production conditions and quality.

CONCLUSIONS

A bibliographical review on *Spirulina* identifies this microorganism as microalgae or bacteria, by botanists and bacteriologist respectively. This study has revealed a rather significant number of 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 bovines. This material contains proteins, carbohydrates, essential fatty acids, vitamins, minerals, carotenes, chlorophyll *a* and phycocyanin. *Spirulina* may be produced in rather simple pilot plants or industrial installations if good conditions and quality controls are assured.

TABLES

Food Type	***Crude Protein %***
Spirulina powder	65
Whole Dried egg	47
Beer Yeast	45
Skimmed powdered milk	37
Whole soybean flour	36
Parmesan Cheese	36
Wheat germ	27
Peanuts	26
Chicken	24
Fish	22
Beef meat	22

Table 1. Quantity of *Spirulina* proteins

and other foods (Henrikson, 1994).

Vitamins	***mg 100 g^{-1}***
Provitamin A	2.330.000 IU kg^{-1}
(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
Folic acid	0.01

Biotin	0.005
Phantothenic acid	0.1
Vitamin K	2.2

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

Fatty acid	***Fatty acids (%)***
(C_{14}) Myristic acid	0.23
(C_{16}) Palmitic acid	46.07
($C_{16:1}$)Δ^9 Palmitoleic acid	1.26
($C_{18:1}$)Δ^9 Oleic acid	5.26
($C_{18:2}$)$\Delta^{9,12}$ Linoleic acid	17.43
($C_{18:3}$)$\Delta^{9,12,15}$ γ-Linolenic acid	8.87
Others	20.88

Table 3. Fatty acid composition of *Spirulina*

platensis powder (Othes and Pire, 2001).

Mineral	***mg 100g^{-1}***
Calcium	700
Chromium	0.28
Copper	1.2
Iron	100
Magnesium	400
Manganese	5.0
Phosphorus	800
Potassium	1400
Sodium	900
Zinc	3.0

Table 4. Minerals in *Spirulina* powder (Belay, 1997).

Pigments	***mg 100g^{-1}***
Carotenoids	370
Chlorophyll *a*	1000
Phycocyanin	14000

Chart 5. Pigments in *Spirulina* powder (Belay, 1997).

Name of Company	***Location***	***Total area***	***Production (ton)***
Earthrise Farms	Calipatria, California, USA	[a] Intensive ponds, total area 150.000m^2	[a] 1995: 360 [a] 1996: 400 [b] 2002: 450
Myanma Microalgae Biotechnology Project	Yangon, Myanmar	[a] Mainly native ponds with a total area 130.000m^2	[a] 1995: 32 [a] 1996: 40
Cyanotech Corporation	Kailua Kona, Hawaii, USA	[a] Intensive ponds, total area 100.000m^2	[a] 1995: 250 [a] 1996: 300
Hainan DIC Microalgae Co., Ltd	China	[b] Total area 100.000m^2	[b] 2002: 330
Ballarpur Industries Ltd	Nanjangud, Mysore District, India	[a] Intensive ponds, total area 54.000m^2	[a] 1994 - 1995 : 25 [a] 1995 - 1996: 85

Nao Pao Resins Chemical Co., Ltd	Tainan, Taiwan, ROC	[a] Intensive ponds, total area 50.000m^2	[a] 1995: 70 [a] 1996: 80 [a] 2000: 150
Neotech Food Co., Ltd	Banpong, Rajburi, Thailand	[a] Intensive ponds, total area 50.000m^2	[a] 1995: 30 [a] 1996: 40
Genix	Cuba	[b] Intensive ponds, total area 45.000m^2	[b] 2001: 100
Siam Algae Co., Ltd.	Thailand	[b] Total area 30.000m^2	[b] 2002: 135
Solarium Biotechnology	La Huayca, Chile	[b] Intensive ponds, total area 24.000m^2	[b] 2000 (Oct-Dec): 4.5 [b] 2001: 28,6 [b] 2002 (Jan - Oct): 13

Table 6: Some commercial producers of *Spirulina*

([a] Vonshak, 1997 ; [b] *Pers. Comm*. October 2002).

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