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SPIRULINA: A SPOTLIGHT ON ITS NUTRACEUTICAL PROPERTIES AND FOOD PROCESSING APPLICATIONS

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ABSTRACT

Spirulina, blue-green algae is now worldwide used as a dietary supplement owing to its richness in protein (50-60%), antioxidants, essential fatty acids, etc. The amino acid composition of Spirulina protein ranks among the best in the plant world, more than that of soya beans. This review article serves as an overview of why Spirulina is considered as a 'superfood'. Its methodologies of cultivation, richness in nutrients and bioactive components, therapeutic properties in the health food circuits, and versatile utilization in various food supplements are elaborately introduced. All possible fortification of Spirulina platensis emphasizing the elevation of nutrient levels in bakery products, beverages, dairy products, extruded snacks, energy bars, infant foods, and confectionaries is briefed. Beyond, these algae convert carbon dioxide into organic substances and produce oxygen during their growth in alkaline and saline water thereby not wasting fresh water and allowing the production in barren areas. Such an organism with a plethora of health profits to humans, animals and the environment is limitless and has probably more to offer in the future.

Keywords: Spirulina, Antioxidants, Protein, Superfood, Cookies, Energy bar

INTRODUCTION

Blue-green algae, i.e cyanobacteria are one of the first living organisms found on earth. Blue-green algae are of two types, they are edible and toxic species. Some blue-green algae are also edible, the edible species are nostac, spirulina, and aphanizonenon which are used as food for thousands of years **(Vonshak, 1997).** The two species which are most widely used are Spirulina platensis and Spirulina maxima. Blue-green algae used in the field of biotechnology has been employed it as food and feed additives in agriculture, the food industry, the making of perfumes, science, and pharmaceutical medicines **(Saranraj and Sivasakthi, 2014)**. A botanist by the name, Leonard found cakes of blue-green algae in fort Lamy's local market (now called Ndjemena) in Chad, Mexico. A group of people collected the wet algae in pots that were wet made of clay, used a cloth to drain the water and they spread the algae in the sand for it to get dried by the sun (sundry method). These algae cakes were called Dihé, which were sold as small squares. Dihé was mixed with tomato and pepper sauce and poured over millet, bean, fish, or meat. It is eaten by the Kanembu people and accounts for 70% of their meals **(Henrikson, 2010)**. The first country to produce chlorella-based food was Japan **(Sanchez *et al.*, 2003)**. According to Credence Research Market Analysis, the global market for algae products, especially used for nutraceuticals, pharmaceuticals as well as food and feed supplements, will register an annual growth rate of 5.8% in the period 2017-2026, expected to reach more than USD 53 BN **(Grosshagauer *et al*., 2020).** By 2025, the global spirulina market is projected to reach $629.6 million, with a compounded annual growth rate (CAGR) of 9.4% from 2019. The spirulina market is anticipated to increase in volume at a CAGR of 13.6% from 2019 to 2025, reaching 68,025.2 tonnes **(Sharma *et al*., 2014)**.
Current unhealthy lifestyles, stress, and pollution factor demand the need for a more nutritious and healthy diet. Superfoods were introduced to overcome these hurdles of a healthy lifestyle. Superfoods are the ones that prevent disease and thereby promote health more than their designated nutritional value. The clinically proven health benefits of spirulina are hypocholestrolemic, immunological, antiviral, and antiglutagenic B **(McCarty MF, 2007)**. Spirulina is one such superfood that is high in nutrients and prebiotic compounds that promote our health condition. Spirulina is high in protein content **(Srilakshmi, 2001)**.

Microalgae have different applications in food. They are used as:

1. As a consumable food: People from a small group in certain countries have been eating algae that were found in the lakes that were harvested naturally.
2. As a supplementary food: The protein malnutrition in the 1960's addressed by the FAO led to the identification of spirulina as a supplement for combating malnutrition.
3. As a space food: It was proposed by NASA and European space energy as potential space food that can be consumed during long stays in space **(Campanella *et al*., 1999)**.

CULTIVATION OF SPIRULINA

Natural method of cultivation

Spirulina grows naturally in the lakes of the warm regions in alkaline water lakes. It is also a dietary supplement for people living near the lakes of alkaline water etc **(Campanella *et al.*, 1999)**. Spirulina can be cultivated commercially from natural sources. Spirulina maxima is harvested from a lake at 2200m above sea level where the environment is semi-tropical and the temperature on average annually is 18ºC. The algal biomass is filtered, homogenized, pasteurization, and spray dried. In the summer, during the blooming season, spirulina forms thick mats on the lake. It is collected by people in boats of dense concentration in buckets and it is harvested in filters inclined parallel, washed with fresh water, water is removed and pressed again. This paste is extruded into noodle-like filaments which are sun-dried on sheets that are transparent made of plastic. These sun-dried chips are taken to a pharmaceutical factory and pressed into tablets **(Ahsan *et al*., 2008)**.

Laboratory method of cultivation

The factors to be considered while cultivating the spirulina in a laboratory are:

Luminosity (main photoperiod) has to be maintained at 12/12, 4 luxes. The required temperature is 30 °C. Uniformity in the inoculation size and has to be checked. Stirring speed of about 58 cm/s and dissolved solids in the range of 10-60g/L is also maintained. The water quality is tested, and the pH is maintained between 8.5 -10.5. The presence of macro and micro minerals like C, N, P, K, S, Mg, Na, Cl, Ca, and Fe, Zn, Cu, Ni, Co, Se respectively is also important for the cultivation **(Ahsan *et al*., 2008)**.

Figure 1 Kanembu women collecting spirulina from lakes and selling in the local market. Source: **FAO (2008).**

The collected spirulina are processed in the following ways

The spirulina that is collected is screened and checked for pond debris, and microscopic algae that give thickened spirulina finally, the water obtained is recycled to the pond. The spirulina droplets are spray-dried and vacuumed into a hopper in the packaging room for collection, which preserves nutrients, pigments, and enzymes that are sensitive to heat (phycocyanin content). Spirulina powder is compressed directly into tablets and sealed in both glass and plastic bottles **(Henrikson, 2010)**.

Commercial method for the production of spirulina

Commercial production of spirulina involves 4 stages. They are culturing, harvesting, drying, and packaging. All these steps have an impact on the final yield and/or product quality. Therefore, careful, and regular monitoring of these processes is crucial for the profitable production of high-quality spirulina that complies with the very stringent quality and safety standards of the food and supplement industries. A closed-loop system is used in the production process, with constant material recycling and evaporation as the only material loss. Throughout the entire production season, the growing medium is recycled. Each pond is collected to the extent that it has grown over the past 24 hours in a semi-continuous culture method. To maximise growth and provide traceability of the manufacturing lot in the event of a problem, the medium is recycled back into the original pond. A regular supply of make-up nutrients is given to restore the harvested algae's absorption. To ensure consistency and ideal circumstances, laboratory scientists do daily tests and monitor and modify nutrients. Every day, ponds are harvested. A stainless-steel screen is used to clean and concentrate the biomass after the culture is delivered with a pump through PVC pipes into a special processing building. The biomass slurry is then moved to a vacuum belt, where it undergoes a final cleaning process after being further dehydrated as a paste. The moisture from the A. platensis paste is subsequently removed using a spray dryer, producing the fine free-flowing powder that is generally referred to as spirulina. It takes less than 15 minutes to complete the process from pond to powder. In order to conduct microbiological tests and other quality-control inspections, samples of the powder are collected in sterile bags, tagged, and brought to the quality control laboratory. All data is logged by the lab personnel onto written sheets and entered a database on the computer network. Once the study demonstrates that the product complies with the standards and regulations, the Quality Control Department releases the goods for packaging and inventory **(Belay, 2007)**.

The advantages of spirulina cultivation are:

- The culture of spirulina does not require fertile soil and can benefit from saline conditions.
- Spirulina is used as a dietary feed for fish, shrimp, and poultry and also as protein and vitamin supplements for aqua feed and human consumption.

The most important naturally occurring minerals contribute to 7% of the weight of spirulina **(Ahsan *et al*., 2008)**.

- Fresh dried spirulina can be preserved in special oxygen barrier containers for five years or more, maintaining almost the maximum potency of beta carotene **(Henrikson, 2010)**.

NUTRITIVE COMPOSITION OF SPIRULINA

Spirulina is considered as a Superfood owing to its rich nutrient composition including protein, essential amino acids, essential fatty acids, minerals, vitamins, phytochemicals, and sulphated polysaccharides. The nutritional composition of spirulina varies greatly according to the culture conditions and analysis methods. The nutrition profile of spirulina powder per 100g from various companies is listed in
Table 1.

Table 1 Nutritional composition of Spirulina powder (per 100g) reported by various companies

Components	Earthrise Nutritionals, USA (Gershwin and Belay, 2007)	DIC LIFETEC Co. Ltd. Japan	Parry Nutraceuticals. India	Nutrex Hawaii, USA Moorhead *et al.* (2011)	Foundation Antenna Technologies, (FAT) Switzerland Falquet (1997)	Liestianty *et al.* (2019)
Calories (kcal)	373.00	386.00	410.00	333.00	-	-
Moisture (g)	<7.00	<4.10	2.50-4.50	3.00-6.00	3.00-7.00	-
Ash (g)	<9.00	5.60	6.00-9.00	8.00-13.00	7.00-13.00	-
Total carbohydrate (g)	17.80	12.70	15.00-25.00	16.00	15.00-25.00	-
Sugars (g)	1.30	4.40	-	9.00	-	-
Lactose (g)	<0.10	-	-	-	-	-
Dietary fiber (g)	7.70	8.30	-	7.00	4.00-7.00	-
Protein (g)	63.00	69.40	56.00-69.00	67.00	55.00-70.00	64.24
Essential amino acids (g)						
Histidine (His)	1.00	1.17	0.50-1.50	1.50	1.00	1.00
Isoleucine (Ile)	3.50	3.48	3.00-4.00	3.26	3.50	3.60
Leucine (Leu)	5.38	5.61	3.00-5.00	4.89	5.40	5.50
Lysine (Lys)	2.96	3.08	3.00-6.00	2.62	2.90	3.00
Methionine (Met)	1.17	1.59	1.00-6.00	1.33	1.40	1.40
Phenylalanine (Phe)	2.75	2.87	2.50-3.50	2.61	2.80	2.80
Threonine (Thr)	2.86	3.30	1.50-3.00	2.81	3.20	3.30
Tryptophan (Trp)	1.09	1.10	1.00-2.00	8.50	9.00	1.00
Valine (Val)	3.94	3.90	0.10-3.00	3.74	4.00	4.50
Non- essential amino acids (g)						
Alanine (Ala)	4.59	4.91	4.00-5.00	4.66	4.70	4.70
Arginine (Arg)	4.31	4.19	3.00-5.00	4.76	4.30	4.40
Aspartic acid (Asp)	5.99	6.18	1.50-3.00	7.28	6.10	6.00
Cystine (Cys)	5.90	7.00	0.50-0.75	5.60	6.00	7.00
Glutamic acid (Gln)	9.13	9.29	6.00-9.00	8.44	9.10	9.20
Glycine (Gly)	3.13	3.21	2.00-4.00	3.19	3.20	3.20
Proline (Pro)	2.38	2.40	2.00-3.00	2.47	2.70	2.70
Serine (Ser)	2.76	3.21	3.00-4.50	2.65	3.20	3.30
Tyrosine (Tyr)	2.50	2.74	1.00-2.00	2.38	3.00	3.00
Total fat (g)	5.60	8.20	5.00-6.00	5.00	4.00-7.00	8.10
Myristic (C 14:0) (g)	0.01	0.02	0.01-0.03	0.04	-	0.02
Palmitic (C 16:0) (g)	2.44	3.63	2.00-2.50	6.10	1.03-1.81	2.30
Palmitoleic (C 16:1) (g)	0.33	0.24	-	0.59	0.15-0.27	0.60
Heptadecanoic (C 17:0) (g)	0.02	0.02	-	-	-	0.04

Stearic (C 18:0) (g)	0.08	0.09	0.01-0.05	0.25	0.07-0.12	0.07
Oleic (C 18: 1) (g)	0.12	0.25	0.10-0.20	0.05	0.66-1.16	0.14
Linoleic (C 18: 2) (g)	0.97	2.00	0.75-1.20	3.30	0.48-0.84	0.95
Gamma- linolenic (C18: 3) (g)	1.35	1.24	1.00-1.50	3.20	1.60-2.81	1.60
Others (C 20) (g)	0.14	0.72	-	-	-	-
Vitamins						
Vitamin A (IU)	352000	-	-	11250	-	-
Vitamin K (mg)	1.09	2.22	0.90- 1.05	2.5	2.24	-
Vitamin C (mg)	0	0	-	0	Traces	-
Vitamin E (mg)	6.71	10.60	-	9.50	10.00	41.00
Vitamin B1 (Thiamine HCl) (mg)	0.50	4.82	0.15-0.30	0.12	3.50	4.80
Vitamin B2 (Riboflavin) (mg)	4.53	3.93	4.00-7.00	4.67	4.00	5.50
Vitamin B3 (Niacin) (mg)	14.90	39.30	10.00- 25.00	13.33	14.00	15.00
Vitamin B6 (Pyridox. HCl) (mg)	0.96	0.91	0.50- 1.50	1.00	0.80	0.80
Vitamin B12 (Cyanocobalamin)(mg)	0. 16	0.24	0.10- 0.30	0.30	0.32	0. 36
Biotin (µg)	5.00	32.30	-	17.00	0.01	55.00
Pantothenic acid (mg)	0.10	1.23	-	0.15	0.10	0. 20
Folic acid (mg)	Traces	0.07	0.05-0.30	0.21	0.01	0.07
Inositol (mg)	64.00	103.00	70.00-90.00	57.00	64.00	70.00
Minerals						
Calcium (g)	0.47	0.07	0.06-0.11	0.33	1.00	1.50
Iron (g)	0.09	0.08	0.03-0.10	0.22	0.18	0.17
Phosphorus (g)	0.96	0.92	0.70-1.00	1.10	0.80	1.00
Magnesium (g)	0.32	0.28	0.20-0.30	0.50	0.40	0.37
Sodium (g)	0.64	0.21	0.70-1.00	1.00	0.90	-
Potassium (g)	1.66	1.52	1.00-1.50	2.00	1.40	1.60
Iodine (mg)	0.14	-	0.20-0.40	0.50	-	-
Zinc (mg)	1.45	1.04	1.00-3.00	3.00	30.00	0.07
Copper (mg)	0.47	0.26	0.20-0.40	0.67	1.20	-
Manganese (mg)	3.26	3.81	1.00-3.00	13.30	5.00	3.00
Boron(mg)	1.00	-	-	0.73	-	-
Chromium (mg)	<0.40	0.39	0.10- 0.30	1.34	0.28	-
Molybdenum (mg)	1.00	-	-	<0.40	-	-
Selenium (mg)	0.03	-	Traces	0.03	Traces	-
Other minerals (ppm)						
Arsenic	<1.00	-	<0.50	<0.50	0.06-2.00	-
Cadmium	<0.50	-	<0.20	<0.20	0.01-0.10	-
Mercury	<0.05	-	<0.05	<0.03	0.01-0.20	-
Lead	<2.50	-	<0.20	<0.20	0.60-5.10	-
Germanium	<0.6	-	-	-	-	-
Silicon	-	743.00	-	-	-	-
Sulphur	-	6900.00	-	-	-	-
Cobalt	-	0.30	-	-	-	-
Fluorene	-	-	-	-	112.00-630.00	-

From Table 1, it is clear that the average calorific value of spirulina powder lies in the range of 333 kcal-410 kcal and the highest was recorded by **Parry Nutraceuticals, India** which is about 410 kcal. Carbohydrates in spirulina ranged between 12.00-25.00% with the highest value of 25% recorded by Parry Nutraceuticals and FAT (Switzerland) and the total dietary fibre ranged between 4.00-8.30% with the highest value of 8.30% recorded by DIC Lifetec Co Ltd (Japan). In addition, minor amounts of L-arabinose, D-galactose, D-xylose, fructose, rhamnose, mannose, and glucuronic acid were also observed **(Santillan, 1982)**.

Spirulina powder has a high protein concentration in the range of 55-70% with the highest value of 70% reported by FAT. Spirulina protein is highly superior to all other vegetable sources (soyabean) **Leon *et al*. (2005)** and there is no limiting factor for amino acid assimilation **(Henrikson, 2010).** Spirulina can increase the protein value of foods limited in certain amino acids such as rice, wheat, or corn, and is thus seen as a nutritional supplementation **(Becker, 1993).** It is considered a complete protein owing to the presence of all essential amino acids and non-essential amino acids in sufficient quantities. From Table 1, it is found that the highest level of essential amino acid in spirulina is tryptophan (9.00 g/100g) by FAT while the lowest is histidine (0.50 g/100g) by Parry Nutraceuticals**.** Likewise, the highest content of non-essential amino is glutamic acid (9.29 g/100g) by DIC Lifetec Co. Ltd while the lowest is found to be cystine (0.50 g/100g) by Parry Nutraceuticals.

Table 2 Protein quality of Spirulina compared to the reference animal protein

Parameters	**Protein value of Spirulina**	**Reference protein (casein)**
Biological value (BV)	75.00	87.00
Net protein utilization (NPU)	62.00	83.00
Protein digestibility coefficient (DC)	85.00	95.00
Protein efficiency ratio (PER)	1.90	2.50

Source: Gabreila *et al.* (2015)

A typical pregnant woman advised to take a higher protein intake but not a corresponding increase in calories can consume spirulina, a rich protein, and a low-calorie food. Spirulina is suitable in cases where the 'calorie cost' is far lower than dairy, meat, and fish **(Henrikson, 2010).** Spirulina exhibits high BV compared to all other plant-based foods and is proximately close to reference protein casein (animal-based) as shown in Table 2. It has relatively high DC, high NPU, and high PER. Although PER is inferior in spirulina to that of animal protein, it is much higher than most vegetable proteins **(Salmean *et al*., 2015).**

Spirulina has a high amount of polyunsaturated fatty acids (PUFA's- 1.50-2.00%), essential fatty acids (5.00-8.20 %), and saturated fatty acids. The highest total fat percentage in spirulina was given by DIC Lifetec Co. Ltd as 8.20%. In particular, it is rich in gamma- linolenic acid, alpha- linolenic acid, oleic acid, EPA, and DHA **(Henrikson, 1989)**. Ten grams of spirulina has only 36 calories and virtually no cholesterol inferring that it is a low-fat, low-calorie, cholesterol-free source of protein, and is not loaded with fat, grease, calories, and cholesterol of meat and dairy protein **(Henrikson, 2010).** FAT confirmed palmitic acid (6.10 g/100g spirulina) as the highest content of fatty acid in spirulina while myristic acid (0.01 g/ 100g spirulina) is found to be the least in the samples of Earthrise Nutritionals Company. The vitamin content of Spirulina is estimated to be about 0.7% by weight. **Henrikson (1997)** detected traces of vitamins such as β-carotene (precursor of vitamin A), vitamin K, vitamin E, B-complex vitamins, and minerals in Spirulina. It has been identified that an excessive dose of β carotene may be toxic, but when β carotene is ingested from Spirulina or other vegetables, it is usually harmless. Spirulina is a rich source of vitamin B12 that can be used as a supplement in the treatment of pernicious anaemia. Dry spirulina contains 50-190 mg/kg of vitamin E, a level comparable to that of wheat germ.

Mineral content of Spirulina is noticed to be about 7% by weight. It is a rich source of macro and micro minerals namely calcium (0.60-15.00 mg/g), potassium (10.00-20.00 mg/g), phosphorous (7.00-10.00 mg/g), sodium (2.10-10.00 mg/g), iron (0.25-2.17 mg/g), copper (0.20-1.20 mg/100g), manganese (1.00-300.00 mg/100g), zinc (0.07-30.00 mg/100g) and molybdenum, boron, chromium, iodine and selenium in trace amounts **(Moorhead *et al*., 2006)**. Spirulina on the whole is rich in phytonutrients that exhibit potent antioxidant properties **(Estrada *et al.*, 2001)**. It contains high amounts of phycocyanin (140.00 mg/g), chlorophyll (10.00 mg/g), total carotenoids (5.04 mg/g), zeaxanthin (1.01 mg/g), xanthophylls (1.70 mg/g), sulpholipids (1.00 mg/g) and glycolipids (20.00 mg/g) **(Campanella *et al.*, 1999)** Spirulina harvested in its natural environment can contain relatively high levels of arsenic and especially fluorides. On the other hand, when it is grown in an artificial environment has mineral quantity below the relevant norms **IUPAC (1974), Falquet & Humi (1997**) - Arsenic 0.06-2.00 ppm, Selenium 0.01-0.04

ppm, Cadmium 0.01-0.10 ppm, Mercury 0.01-0.20 ppm, Lead 0.60-5.10 ppm, Fluorine 112-630 ppm **(Falquet , 1997)**.
Additionally, Spirulina contains substantial amounts of nutritional phenolic and flavonoid compounds. The amounts of polyphenols present were found to be 0.287 mg GAE/g DW and that of total flavonoids was found to be 0.166 mg QE/g DE. The main phenolic components were salicylic, succinic, catechins, chlorogenic, synaptic caffeic, and trans-cinnamic acids **(Stengel *et al*., 2011)**. Flavonoid compounds such as isoflavones, flavonols, flavanones, and dihydrochalcones were found that have antioxidant and free-radical scavenging properties **(Sehgiri *et al.*, 2019)**. Spirulina has been certified as Generally Recognized as Safe (GRAS) - GRN No.127 by the Food and Drug Administration (FDA) and had its consumption authorized as food or food supplement in 2002 **(European Commission, 2015)**.

MICROBIOLOGICAL QUALITY AND CONTAMINANT SPECIFICATIONS OF SPIRULINA

The author **Sharoba (2014)** has stated that the total viable bacterial count is widely used as an indicator microbiological quality of food. It is evident from the data (Table 3) that the total viable bacterial count and mesophilic spore formers bacteria were absent in baby foods prepared with Spirulina. Furthermore, yeast, and molds were also not detected due to the fact that both are destroyed during drying. Likewise, coliform group, salmonella, and staphylococcus were not detected. On the other hand, spirulina is free from pesticides, rodent hairs, and insect fragments. The level of heavy metals detected is also in line with the specifications of global food.

Table 3 Microbiological examination and contaminant specifications of spirulina

Microbiological quality parameters (cfu/g)	Specifications
TVBC (Total viable bacterial count)	Negative
MSB (Mesophilic spore forming bacteria)	ND
Y & M (Yeasts and Moulds)	ND
Coliform group	ND
Salmonella	ND
Staphylococcus	ND
Contaminant parameters (ppm)	
Arsenic	<1.00
Cadmium	<0.50
Lead	<0.50
Mercury	<0.05
Pesticides	Negative
Rodent hairs and insect fragments	ND

Source: Sharoba (2014)

BIOACTIVE COMPONENTS OF SPIRULINA

Spirulina platensis contains an extremely high number of natural pigments **(Jung *et al*., 2019)**. One of the most important natural pigments is phycocyanin which gives Spirulina a dark blue colour. Spirulina contains phycocyanin which may be called as a precursor of chlorophyll. Chlorophyll is also known as detoxifying and cleansing phytonutrient. Spirulina contains chlorophyll (100.00 mg/100g) which is rich in magnesium and is responsible for giving green colour to Spirulina **(Nieto *et al*., 2010)**. Carotene exists in Spirulina in three forms- alpha, beta, and gamma which are responsible for imparting yellow or orange colour to Spirulina. In general, Spirulina also contains xanthophylls (17.00 mg/100g), myxoxanthophyll (251.20 mg/100g), zeaxanthin (58.80 mg/100g), cryptoxanthin (6.30 mg/100g), echinenone (14.70 mg/100g), and other natural xanthophylls (578.80 mg/100g) which are responsible for the antioxidant property of Spirulina **(Anderson *et al*., 1991)**. The bioavailability of these natural pigments in Spirulina is quite high **(Jung *et al*., 2019)**.
Furthermore, the potential antioxidant property of Spirulina is also due to the presence of two phycobiliproteins which includes phycocyanin and allophycocyanin. The presence of this phycocyanin content gives protection against diabetic neuropathy and also shows free radical scavenging activity **(Pyne *et al*., 2017)**. Spirulina contains highest number of flavonoid compounds (100.78 mg/100g) and relatively low number of phenolic compounds (11.15 mg/100g), alkaloids (2.95 mg/100g), tannins (5.60 mg/100g), ascorbic acid (2.16 mg/100g), terpenoids, saponins, etc. which helps the body to fight against allergy, viruses, and carcinogens **(Adikwu and Deo, 2013). Sehgiri *et al*. (2019)** identified around 20 phenolic and flavonoid compounds in Spirulina (Table 4). Phenolic and flavonoid compounds are rich in secondary metabolites like succinic acid (112.28 mg/100g), quinic acid (84.41 mg/100g), 3-4-hydroxybenzoic acid (68.70 mg/100g), catechin (58.45 mg/100g), citric acid (6.40 mg/100g), vanillic acid (1.62 mg/100g), gallic acid (0.21 mg/100g), and 4- hydroxybenzoic acid (0.10 mg/100g).

Table 4 List of bioactive compounds identified in Spirulina.

S.no	Bioactive compounds	Quantity (mg/100g)	Reference
1.	Alkaloids	0.30, 2.95	**Jisika *et al.* (1992), Chaudhuri *et al.* (2014)**
2.	Ascorbic acid	<0.01, 2.16	**Adikwu *et al.* (2013), Chaudhuri *et al.* (2014)**
3.	Carotenoids	5.00, 75.20	**Pyne *et al.* (2017), Seshadri *et al.* (1993)**
4.	Catechin	2.92 - 58.42	**Seghiri *et al.* (2019)**
5.	Chlorogenic acid	<0.01 - 0.08	**Morais *et al.* (2015)**
6.	Chlorophyll	1.00, 1000.00	**Jung *et al.* (2019), Koru *et al.* (2012)**
7.	Cryptoxanthin	6.30, 10.00	**Henrikson (2009)**
8.	Citric acid	0.32 - 6.42	**Seghiri *et al.* (2019)**
9.	Echinenone	10.00, 14.70	**Henrikson (2009), Jisika *et al.* (1992)**
10.	Ferulic acid	<0.01 - 0.04	**Ahmed *et al.* (2014)**
11.	Flavonoids	2.10, 100.78, 130.00	**Seghiri *et al.* (2019) Chaudhuri *et al.* (2014) Hanaa *et al.* (2009)**
12.	Gallic acid	0.01 - 0.21	**Seghiri *et al.* (2019)**
13.	Myxoxanthophyll	70.00, 251.20	**Henrikson (2009), Donald *et al.* (1990)**
14.	Other xanthophylls	20.00, 578.80	**Henrikson (2009), Donald *et al.* (1990)**
15.	Phenols	0.01, 11.15	**[Chaudhuri *et al.* (2014), Seghiri *et al.* (2019)**
16.	Phycocyanin	12.00, 100.00, 1400.00	**Koru *et al.* (2012) Patel *et al.* (2006), Henrikson (2009),**
17.	Pyrogallol	<0.01 - 0.04	**Seghiri *et al.* (2019)**
18.	Quercetin	<0.01 (in traces)	**Jerez-Martel *et al.* (2017)**
19.	Quercitrin	<0.01 (in traces)	**Pognussatt *et al.* (2014)**
20.	Quinic acid	4.22 - 84.41	**Seghiri *et al.* (2019)**
21.	Resveratrol	<0.01 (in traces)	**Hajimahmoodi *et al.* (2010)**
22.	Rosmarinic acid	<0.01 (in traces)	**Seghiri *et al.* (2019)**
23.	Rutin	<0.01- 0.09	**Pognussatt *et al.* (2014)**
24.	Salicylic acid	<0.01 (in traces)	**Seghiri *et al.* (2019)**
25.	Succinic acid	5.61 - 112.28	**Seghiri *et al.* (2019)**
26.	Tannins	<0.01, 5.60	**Balch and Balch (2000) Chaudhuri *et al.* (2014)**
27.	Tocopherol	18.00, 100.00	**Simpore *et al.* (2005), Miranda *et al.* (2008)**
28.	Vanillic acid	0.08 - 1.62	**Seghiri *et al.* (2019)**
29.	Xanthophylls	20.00, 170.00	**Henrikson (2009), Donald *et al.* (1990)**
30.	Zeaxanthin	58.80, 60.00	**Henrikson (2009), Donald *et al.* (1990)**
31.	3,4-Hydroxybenzoic acid	3.43 - 68.70	**Connan *et al.* (2011)**
32.	4-Hydroxybenzoic acid	<0.01- 0.10	**Safafar *et al.* (2015)**
33.	2-Hydroxycinnamic acid	<0.01- 0.03	**Kepekçi *et al.* (2012)**
34.	4-Hydroxycinnamic acid	<0.01 (in traces)	**Pagnussatt *et al.* (2016)**

NUTRACEUTICAL PROPERTIES OF SPIRULINA

Spirulina is gaining more attention as a nutraceutical and a functional food owing to its perks of several therapeutic effects in the health food circuit. It is described by the United Nations as the ‘most ideal food for mankind’ and was declared as the ‘best food for the future’ in the World Food Conference, in 1974. In the late 1980s and early 90s, both the National Aeronautics and Space Administration (NASA) and the European Space Agency (ESA) proposed Spirulina as one of the principal foods that can be cultivated in long-term space missions and FDA validated it as ‘one of the best protein sources. Spirulina is reported as a powerhouse of various nutrients like carbohydrates, protein, β- carotene, γ-linolenic acid (GLA), vitamins, minerals, phytonutrients, sulpholipids, glycolipids and superoxide dismutase, etc that are missing in most people’s diet. Spirulina incorporated into food products produces functional foods that have enriched nutritional value and numerous health benefits.
The application of Spirulina so far in industries has been in the form of dry powder and not fresh form for value addition and food formulations. Nevertheless, recently **Bchir *et al*. (2019)** incorporated fresh Spirulina in the yogurt formulation and demonstrated superior nutritional and health benefits. The study has successfully eliminated the drying process of Spirulina which presented negligible effect on the product quality. Furthermore, the methanolic and aqueous extract of Spirulina biomass possesses potential nutraceutical effects as represented in Figure 2.

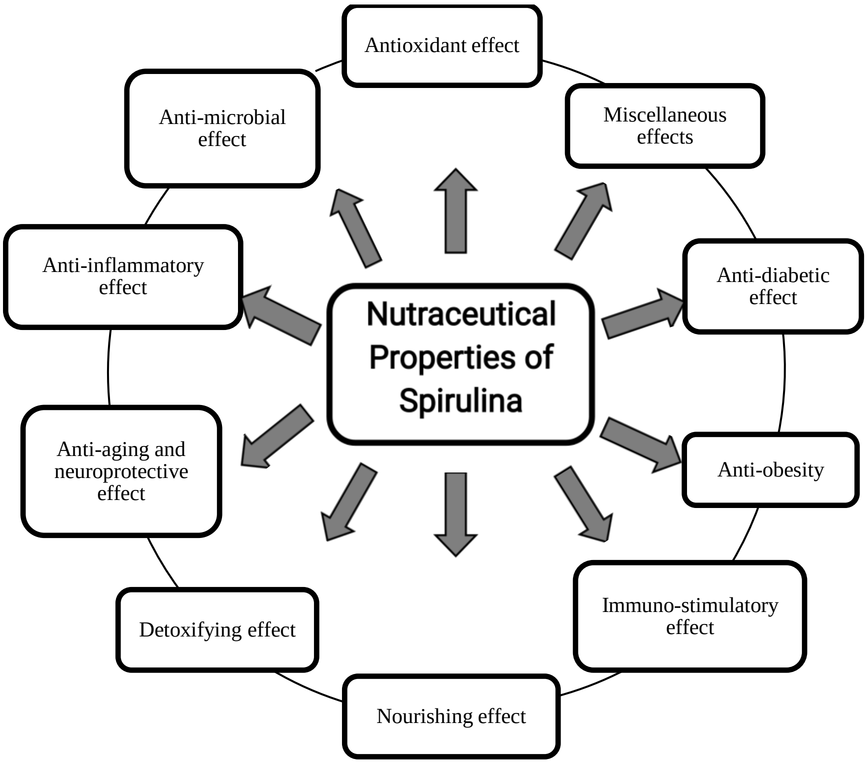

Figure 2 Diverging radial representations corresponding to the health benefits of Spirulina

Antioxidant effect

The C-phycocyanin in Spirulina is an effective potential anti-cancer agent. **Manoj *et al.* (1996)** reported that the alcohol extract of Spirulina inhibited lipid peroxidation more effectively (65%) than the well-known potential antioxidants like tocopherol (35%), butylated hydroxy anisol (45%) and β-carotene (48%). The water extract of Spirulina is also shown to have a more antioxidant effect (76%) than gallic acid (54%) and chlorogenic acid (56%). **Privalov *et al*. (2002)** discovered a new chlorine photosensitizer called Radachlorin derived from S. platensis whose intravenous injection significantly caused full tumor regression. **Bermejo *et al*. (2008)** studied that phycocyanin increases the cellular antioxidant enzymes- glutathione peroxidase and glutathione peroxidase. **Sharma *et al*. (2007)** postulated that increased levels of these enzymes declined lipid peroxidation that in turn reduced the transaminase activity in serum. Moreover, supplementation of Spirulina for 40 days resulted in decreased lactoperoxidase levels. Some researchers reported that the unique polysaccharides in Spirulina enhance cell nucleus enzyme activity and repair the damaged DNA by its endonucleases action thereby preventing tumor growth in hamster cheek pouch mucosa **(Grawish, 2008)**. Additionally, **Chen *et al*. (2009)** have reported that selenium-enriched Spirulina inhibited the growth of MCF-7 human breast cancer cells. **Gad *et al*. (2011)** put forth the chelating activity of Spirulina that inhibits ferrozine- Fe2+ complex formation due to its antioxidant compounds as electron donors. Spirulina plays a hepatoprotective role by inhibiting oxygen stress thereby protecting against diabetic nephropathy **(Zheng *et al*., 2013)**. The total antioxidant capacity (TAC) of Spirulina arises from its whole spectrum of natural antioxidant and phenolic compounds that inhibit the oxidation process **(Hetta *et al*., 2014)**. One such compound is β-carotene (natural antioxidant) that scavenges hydroxyl and peroxyl free radicals in humans thus preventing the prevalence of degenerative diseases like cancer and also lipid peroxidation. While the deep pigmentation of blueberries produces a high ORAC (Oxygen Radical Absorbance Capacity) value of 2600, Spirulina exhibits a fivefold higher ORAC value of 13000. Thus, Spirulina by its antioxidant activity functions as an anti-carcinogen, anti-tumor and a chemopreventive tool.

Anti-microbial effect

Spirulina acts as a functional food, feeding the beneficial intestinal flora, Lactobacillus, and Bifidus. S. platensis biomass was used to maintain the counts of starter organisms in acidophilus-bifidus-thermophilus (ABT) type culture in milk at satisfactory levels during the whole duration of storage. This is a novel opportunity for the production and maintenance of functional dairy foods **(Varga *et al*., 2002)**. **Mendiola *et al*. (2007)** studied the antimicrobial activities of Spirulina extract against Staphylococcus aureus (gram positive bacterium), Escherichia coli (gram negative bacterium), Candida albicans (yeast), and Aspergillus niger (fungus). The results showed that C. albicans was the most sensitive microorganism to all Spirulina fractions extracted by supercritical fluid and this activity is related to the synergistic effect of its functional lipids. Similarly, **Mala *et al*. (2009)** studied the antibacterial activities of organic and aqueous extracts of S. platensis against different species of human pathogenic bacteria by agar-solid diffusion method. It was observed that maximum antimicrobial activity was against Klebsiella pneumoniae and minimum against Proteus vulgaris. Acetone extract also showed the highest biological activity against Klebsiella pneumonia. Spirulina is known to increase Lactobacillus (probiotic efficiency) in humans and also the absorption of B1 and other dietary vitamins. In another study, **Hetta *et al*. (2014)** confirmed that Spirulina extract possesses antimicrobial potential against many pathogenic bacteria and this property is attributed to the presence of functional lipids mainly α- linolenic acid and an antibiotically active fatty acid **(Xue *et al*., 2002)**. It is well known that lipids penetrate the bacterial membrane and cause disintegration by disrupting the extensive meshwork of peptidoglycans in the cell wall. It has antifungal effect against Aspergillus and Candida species and antibiotic effect against Bacillus species and E. coli **(Usharani *et al*., 2015)**. Antiviral and immunostimulatory properties of S. platensis preparations were elicited through increased mobilization of macrophages, cytokine production, antibodies generation, accumulation of NK cells, and mobilization of B and T cells **(Khan *et al*., 2005)** due to the potent glycolipids and sulpholipids present in Spirulina. A recent study by **Balzarini (2007)** on the antiviral activity involved the isolation of Cyanovirin-N (CV-N), a novel cyanobacterial carbohydrate-binding protein that inhibits HIV-I and other enveloped viral particles. The sulfated polysaccharide extracted from Spirulina called Calcium Spirulan (Ca-Sp) is made up of rhamnose, ribose, mannose, fructose, galactose, xylose, glucose, glucuronic acid, galacturonic acid, and calcium sulfate showed anti-viral activity against HIV, Herpes Simplex Virus, Human Cytomegalovirus, Influenza A Virus, Mumps Virus and Measles Virus **(Saranraj and Sivasakthi, 2014)**.

Anti- inflammatory, anti-aging, and neuroprotective effect

The anti-inflammatory effect of Spirulina is due to the presence of phycocyanin which prevented inflammatory stomach and intestinal diseases providing favourable condition for the complete absorption of nutrients **(Kodentsova *et al*., 2001)**. Studies with aged rats showed diets with Spirulina down-regulated markers of inflammation and oxidative stress and improved receptor function in aged rat brains **(Gemma *et al*., 2002)**. An earlier human feeding study conducted in S. platensis, it proved its neuroprotective ability. Hence, it is suggested that chronic treatment with Spirulina can reduce ischemic brain damage **(Wang *et al*., 2005).** Spirulina is a potent food that has many actions in the central nervous system to counteract oxidative stress and inflammation that occur as a consequence of aging and to aid regeneration of the brain following injury or neurodegenerative disease **(Vila and Gemma, 2017).** Spirulina is the only green plant food rich in γ-linolenic acid (GLA), an essential fatty acid which acts as an anti-inflammatory, and sometimes alleviates symptoms of arthritic conditions. Spirulina reduces hepatic damage due to drug abuse and heavy metal exposure, inflammatory response, cell degeneration, and anaphylactic reaction **(Usharani *et al*., 2012)**. Spirulina can be considered a therapeutic intervention for the aging brain. Spirulina aids in reducing stress prevents depression, and improves memory and mental clarity **(Soni *et al*., 2017).**

Detoxifying effect

Spirulina has a unique quality to detoxify (neutralize) or to chelate toxic minerals, a characteristic that is not yet confirmed in any other microalgae **(Okamura and Aoyama, 1994)**. At Beijing University, bioactive molecules from Spirulina have been extracted which could neutralize toxic, and poisonous effects of heavy metals, and showed anti-tumor activity. Therefore, Spirulina could also be used to detoxify the poisonous effect of heavy metals (minerals such as arsenic) from water, food, and the environment. An earlier study successfully showed that Spirulina counteracted heavy metals in toxic kidneys assisting the detoxification process **(Fukino *et al*., 1990).**

Nourishing effect

Spirulina is a very rich source of vitamin B12 (cyanocobalamin) that is used as a supplement in the treatment of pernicious anemia which is caused by vitamin B12 deficiency **(Richmond, 1995)**. A study by **Fox (1984)** proved that Spirulina combated vitamin deficiency in 10 weeks – 2 months with an increase in plasma vitamin B12 and hemoglobin after 12 weeks**. Henrikson (1989)** stressed the iron content of Spirulina (1.18 mg/g) and the importance of Spirulina for treating iron deficiency (anaemia), particularly in pregnant women and children. As a comparison, iron supplements given in form of ferrous sulfate possess a toxicity problem and often cause diarrhoea. Cereals which contain phytic acids and phosphatic polymers sharply limit the bioavailability of the iron they contain. Spirulina contains iron twice as absorbable as that of vegetables. Elderly people who are on restricted diets and are suffering from intestinal malabsorption find Spirulina's protein easy to digest since it is composed of easily digestible soft mucopolysaccharides **(Henrikson, 2010).**
The research conducted by **Madhu *et al*. (2001)** has revealed that the potential use of Spirulina as a food supplement and food additive for combating Protein Energy Malnutrition (PEM) and Protein Energy Wasting (PEW). Researchers conclude that Spirulina is a genuine health food for children as it was studied to curb bad appetite, night sweats, diarrhoea, constipation, and zinc deficiency. It is recommended to take < 4 grams of Spirulina per day for a healthy adult due to the presence of excess vitamin A and not more than 10 grams as it exceeds the RDA values of heavy metals. Fortifying food products with Spirulina effectively decrease diseases linked to nutritional deficiencies, creates nutritional awareness, and increases the acceptance level in developing countries.

Immuno-stimulatory effect

Many beneficial outcomes of Spirulina could be linked to the activation of the innate immune system, first line of defense in our bodies. Phycocyanin prevents degenerative organ diseases by boosting immunity and strengthening the body's resistance through the lymph system. A study by **Blinkova *et al*. (2001)** recommends a phycocyanin dosage in a range of 0.25 to 2.5 grams per day. **Balacharan *et al.* (2006)** reported that Spirulina supplementation increases active macrophages that effectively combat harmful germs. Also, it is observed that food supplementation with phycocyanin stimulated Thymphocytes **(Pugh *et al.*, 2001)** and also natural killer cells **(Batshan *et al*., 2001**). **Kulshreshtha *et al*. (2008)** described Spirulina as a powerful tonic for the immune system.

Anti-obesity

Studies have shown that β- carotene in Spirulina reduces serum cholesterol levels in human beings and significantly reduces body weight. The hypercholesterolemic effect of Spirulina was due to functional lipids like γ-linolenic acid and linoleic acid which accounts for approx. 30% of total lipids **(Henrikson, 2010)**. A study by **Moradi *et al.* (2019)** revealed that Spirulina causes significant weight change in obese subjects by changing the gut microbiota composition and growth of beneficial bacteria **(Nagaoka *et al*., 2005)**. The phenylalanine content in Spirulina is responsible for the release of cholecystokinin that affects the appetite center in the brain **(Fujimoto *et al*., 2012)** and also improves leptin secretion from visceral fat, the impairment of which disturbs the homeostasis leading to obesity **(Vázquez *et al*., 2015)**. Spirulina has also been shown to raise adiponectin levels which are considered as a therapeutic target to treat obesity **(Achari and Jain, 2017)**. Thus, the study by **Moradi *et al*. (2019)** suggested that Spirulina supplementation reduced body weight, body fat percentage, and waist circumference but had no effect on BMI and waist-to-hip ratio.

Anti-diabetic effect

Anitha and Chandralekha (2010) reported supplementation of Spirulina on fasting blood glucose and lipid profile. Spirulina was supplemented for 90 days with 2 capsules (500 mg each) per day. There was a significant reduction from pre- to post-levels of fasting blood glucose, glycosylated hemoglobin, and lipid profile levels of the diabetics upon supplementation with Spirulina. **Takai *et al*. (1991)** found that a water-soluble fraction of spirulina extract was effective in lowering serum glucose levels at fasting while the water-insoluble fraction suppressed glucose levels at glucose loading thereby controlling diabetes mellitus.

Miscellaneous effects

Spirulina contains vitamin A, important in preventing eye diseases; iron and vitamin B12, useful in treating hypo ferric anemia and pernicious anemia, respectively **(Usharani *et al*., 2012)**. Also, the metalloprotective role of Spirulina may be attributed to the presence of beta-carotene, vitamins C, E, superoxide dismutase, selenium, and brilliant blue polypeptide pigment phycocyanin **(Marzieh *et al*., 2013)**. Prostaglandin in humans regulates blood pressure, synthesizes cholesterol, and proliferates cells and inflammation. These are formed from its precursor gamma- linolenic acid (GLA), an essential fatty acid whose deficiency can cause degenerative diseases and chronic health problems. Spirulina, a dietary source of GLA may help overcome heart disease, premenstrual stress, arthritis, manic-depression, and schizophrenia. Thus, Spirulina is highly suitable for children and their growth owing to its high protein, calcium, and iron content for bodybuilding. While Spirulina is often used for human consumption in form of powder, tablets, capsules, and extracts, the functional features of S. platensis led to be used in processing usual foods.
Such a profusion of therapeutic applications - genuine or supposed - is bound to leave Spirulina with the image of a miracle potion. The fact remains that a simple natural food supplement, endowed with the riches of this product, could well improve a good number of pathological conditions.

SPIRULINA-BASED FOOD SUPPLEMENTS

Spirulina fortified bakery products

Bread

Spirulina when added to dough showed a significant increase in essential nutrients. **Minh (2014)** reported a significant increase in the nutritional quality of dough when supplemented with Spirulina (1%, 2%, and 3%). There was an increase in protein (9.6% to 11.0%), lipid (5.0% to 5.7%), total mineral (1.3% to 3.1%), and energy (307.4 kcal to 312.1 kcal). Results showed that bread with 1% of Spirulina is ideal because of its high nutrients and sensory characteristics (Table 5). Similarly, **Burcu *et al*. (2016)** indicated that the bread formulated with 10% Spirulina has a more significant micronutrient content than the control group. The amount of iron found was higher in Spirulina fortified bread. **Rozylo *et al*. (2017)** demonstrated that the fortification of bread with 4% Spirulina considerably increased its loaf volume. Furthermore, rheology and gas retention properties were increased noticeably in the fortified dough. The addition of 6.0% and higher spirulina significantly affected the total phenolics in gluten-free bread and results showed 4% of spirulina fortification is tolerable. The addition of 2.6% of Spirulina in dough increased the protein by 22.6 % **(Lucas *et al*., 2017)**. **Niccolai *et al*. (2019)** developed 2% Spirulina incorporated "crostini", a leavened bakery product that is largely consumed in Europe. Despite a lower volume increase compared to the control, the A. platensis "crostini" dough reached a technologically appropriate volume after fermentation. As A. platensis "crostini" showed higher protein content compared to the control, the newly developed "Spirulina crostini" could be regarded as a very interesting protein-fortified bakery product, with a protein value as high as 14-17% and significantly higher antioxidant capacity. Considering the European Commission Regulation on nutritional claims, "crostini" incorporated with 6% and 10% biomass was claimed to be a "source of protein". The combination of Spirulina biomass addition and sourdough technology led to the development of a novel microalgae-based bakery product with nutritional and functional features. **Wandurraga *et al*. (2019)** enriched breadsticks with 1.5% Arthrospira platensis and classified it as a high in iron and selenium food" with more a stable colour, and texture.

Biscuit

Sharma and Dunkwal (2012) reported that the incorporation of 1% Spirulina in biscuits notably improved the nutritional quality of biscuits. Clearly, there was an increase in energy (506 kcal), protein (19.6%), fiber (2.08%), iron (17.62 mg/100g), and potassium (292 mg/100g) (Table 5). Likewise, **Sahin (2019)** indicated that an addition of 2% of Spirulina presented a significant increase in protein, and essential amino acids in Spirulina-based biscuits compared to the control sample. There is about a 15%-18% increase in essential amino acids and considerably low lipid content seen in Spirulina-based biscuits. According to **Ghaly *et al*. (2015)**, the odor of the Spirulina fortified chocolate chip oatmeal cookies of concentrations 0, 3%, 6%, and 9% were described as pleasant, sweet-yeast smell, musty sea water, and fishy seawater respectively. The cookies that received Spirulina had a grainy texture and dry-chewy mouth feel. Spirulina has enhanced the nutritional value of the cookies by increasing the protein, vitamin, and mineral contents and omega 3 and omega 6 fatty acids. A similar study was conducted by **Batista *et al*. (2017)** and that the biscuits added with 2 and 6% Spirulina showed higher content of polyphenols and an improved antioxidant capacity besides obtaining an attractive and innovative appearance. Spirulina contains all essential amino acids which are not synthesized by the human body but must be supplied in the diet. The daily intake of essential amino acids is 4 mg/day stated by (FAO/WHO, 1991). Fortification of Spirulina (2%) in biscuit as a natural ingredient will fulfill all essential amino acid and protein requirements as suggested by (**Sahin, 2019**).

Cake

Ali et al. (2019) standardized the protocol of the Spirulina soufflé cake since cakes are less in protein. Soufflé is a light airy cake of international reputation. Five treatments T0, T1, T2, T3, and T4 were used in ratios 0%, 4%, 6%, 8%, and 10% of Spirulina incorporated in making soufflé cake. The highest protein % of the Spirulina Soufflé was obtained for the treatment T4 (19.43). The highest % overall acceptability of the Spirulina Soufflé was obtained for the treatment T3 (8.06). However, it can be concluded that the Spirulina Soufflé prepared by incorporating the 8% Spirulina powder extract i.e, treatment T3 outlaid the better organoleptic properties and was found to be rich in protein. Reports of **Golmakani *et al*. (2015)** revealed that the incorporation of Spirulina in Iranian Yazdi cupcakes showed an anti-staling effect over storage. Also, increasing Spirulina concentration significantly ($p < 0.05$) reduced the color parameters in the crust. **Danesi *et al*. (2004)** found that cake samples enriched with 2% Spirulina and 4% cassava bran had higher protein content (4.3%) in comparison with the samples containing 1% microalgae and 2% bran (3.9%). **Branger *et al*. (2003)** identified that the formulation of cakes enriched with Spirulina had higher ash content due to the presence of Fe, Ca, K, P, Cu, Zn, Mg, and Se contained in the microalgae. **Rabelo *et al*. (2013)** found that the addition of Spirulina platensis (5.4%) in cassava doughnuts increased by 66.7% protein content relative to the standard formulation.

Spirulina-fortified dairy products

Cheese

In a study conducted by **Darwish (2017),** the fortification of Spirulina at a concentration of 1.5 % improved the protein and iron content of cheese. Kareish cheese developed with 1% Spirulina was higher in phenolic compound (18.437 mg GAE/100g) and flavonoid compounds (6.391 mg CE/100g) than the control sample. Also, kareish cheese presented a higher β-carotene, DPPH radical scavenging activity. However, 1% Spirulina-fortified cheese was observed to be more acceptable than 0.5% and 1.5% **(Ismaiel *et al*., 2016)**. On the other hand, **Mohamed *et al*. (2020)** indicated that the spreadable processed cheese to sample with 3% Spirulina had higher chemical components like protein (13.66% to 14.67%), and fiber (0.0% to 0.27%). The spreadable processed cheese with 1% of Spirulina was considered to be more acceptable (Table 5). **Agustinia *et al*. (2016)** reported there was an increase in protein, and β-carotene (0.57% to 8.08%) in Spirulina (1.5%) fortified cheese sample. The author says addition of 1.5% was considered the best concentration for cheese preparation.

Yogurt

Malik *et al.* (2013) reported that fortification of yogurt with Spirulina (0.1%, 0.2%, 0.3%, and 0.5%) considerably increased macro-nutrients like protein (3.80% to 4.17%), and iron (0.04 mg to 0.32 mg) and there was a higher curd strength observed with increasing supplements of Spirulina. As suggested by the author, 0.3% of Spirulina concentration was ideal and acceptable in yogurt. **Dubey and Kumari (2011)** indicated that yogurt formulated with 8% Spirulina showed a significant increase in protein (10.5% to 36.94%), and β-carotene (662.5 μg/100g to 2712.75 μg/100g) than the control sample. The incorporation of 6% of Spirulina was considered to be highly acceptable. In another study, **Agustini *et al*. (2017)** compared two samples of yogurt with different concentrations of Spirulina incorporated in it. Yogurt enriched with 1% Spirulina was comfortably accepted. Also, in a study conducted by **Patel *et al*. (2019)** probiotic control yogurt was compared to probiotic Spirulina yogurt (6-10% Spirulina) and results showed that there was an increase in total carotenoid content (0.01 mg/100g to 0.32 mg/100g), and chlorophyll content (0.04 mg/100g to 0.63 mg/100g). The RDA of β-carotene for an adult Indian man and woman is 4800 μg/day and for children (4–6 years) is 3200 μg/day **(ICMR 2010)**. As suggested by the author 7% of Spirulina enriched probiotic yogurt was more nutritious and acceptable.

Ice-cream

Spirulina can be used to increase the nutritional quality of the ice cream as it contains high amount of protein, fat, and moisture. Spirulina powder (0.075%, 0.15%, 0.23%, and 0.3%) was used to replace the stabilizer in ice cream preparation. **Walstra and Jenness (1984)** demonstrated that there was an increase in acidity in Spirulina fortified ice-cream due to buffering action of the additional proteins, phosphates, citrates, lactates, and other miscellaneous milk constituents. Also, **Malik *et al*. (2013)** indicated that the addition of Spirulina by removal of stabilizer showed a significant increase in protein (4.26% to 4.45%), and iron (0.03 mg/100ml to 0.20 mg/100ml). Ice-cream prepared by replacing 50% of stabilizer with 0.15% of spirulina was observed to be more acceptable. **Bulgaru (2019)** demonstrated that replacing gelatin with Spirulina as a stabilizer provides a high-quality index and increased biological value of ice-cream. The addition of Spirulina as a stabilizer increased the iron content from 2.68% to 3.98% and thus the chemical composition of the ice cream was improved. Replacing gelatin with 100% of Spirulina, as a stabilizer was preferable for the preparation of ice cream. **Szmejda *et al.* (2018)** conducted a study in which different flavour of ice-cream was compared to their version supplemented ice cream with Spirulina. There was a greater antioxidant potential, total soluble phenolic content, and β-carotene found in ice cream enriched with Spirulina and was more acceptable.

Spirulina Incorporated Extruded Products

Food extrusion has been widely used to produce ready-to-eat cereals, and snack foods. Physical characteristics of an extruded snack product such as expansion, hardness, and density are important parameters in terms of the consumer acceptability as well as the functional properties of the final product. Successful incorporation of Spirulina into cereal-based extruded products could deliver physiologically active components and represents a major opportunity for food processors who are engaged in providing the consumer with a healthy maize-based product. In connection with this, various studies have been demonstrated to evaluate the applicability of Spirulina in extruded products.

Lucas *et al.* (2018) confirmed that the incorporation of 2.6% Spirulina in the extruded snacks enriched the protein content to11.3% along with enhanced physical and structural properties. In another study, **Vijayarani *et al*. (2012)** developed value-added extruded product with 5% Spirulina that showed a higher amount of nutrients especially protein (62.4 g), energy value (347.96 Kcal), and macro and micronutrient content. On the other hand, **Panesar *et al.* (2012)** noticed that Spirulina powder (7.5%) in the maize flour blend increased the protein, zinc, and carotenoid content of extrudate upto 14.63%, 6.66 mg/kg and 138.83 mg/kg respectively. The in vitro starch digestibility of the product increased 0.98 % and protein digestibility improved to about 85.88% due to thermal denaturation.

Marco *et al*. (2014) observed an increase in protein content, phenolic compounds, and antioxidant activity after incorporating Spirulina biomass in pasta. The potential application of Spirulina in gluten-free pasta was studied by **Fradinho *et al.* (2020).** The products presented an increased content of phenolic compounds, chlorophylls, and carotenoids. Spirulina at a concentration of 2% was preferred by the panellists. **Hameed *et al*. (2018)** prepared spaghetti enriched with 0.5, 1.0, and 2.0 Spirulina platesis which led to the occurrence of γ-Linolenic acid (GLA) in spaghetti and enhanced level of fatty acids (Palmitic acid, Margaric acid, Stearic acid Oleic acid and γ-Linolenic acid). **Fatima and Srivastava (2017)** added 5% Spirulina as a dietary supplement in noodles that showed an increase in protein content (10.28%), fiber content (59.00 %), calcium (144.4 mg/ 100 g), phosphorus (64.8 mg/ 100 g) and iron (1.98 mg/ 100 g).

Spirulina Incorporated Energy Bar

Kumar *et al.* (2018) observed a 167% increase in protein content with the incorporation of Spirulina biomass into nutrition bars. Spirulina contributes small amounts of abundantly nutritious lipids like alpha- linolenic acid, linoleic acid, EPA, DHA, and arachidonic acid. The total phenolic content was found to elevate a maximum of 7.9 mg GAE/g in the 7utria-bar. It was also noticed that bars with higher content of Spirulina (6g/ 80 g nutrition bar) had a stronger appeal.

Spirulina Incorporated Beverages

Martelli *et al*. (2020) noticed Arthrospira platensis (Spirulina) as a useful tool for novel functional fermented dairy food by enhancing the technological performances of starter cultures such as S. thermophilus and Lactobacillus casei. Spirulina at a concentration of 0.25% showed the best boosting effect on the growth of strains and mixed culture of LAB. Mahmoud et al. (2015) demonstrated a significant increase in phagocytosis of macrophages for pomegranate beverage formulated with Spirulina suggesting it as a health-promoting beverage. The incorporation of freeze- dried Spirulina biomass in broccoli soup resulted in increased content of bioaccessible polyphenols (ranging between 32.9 and 45.6 mg/ 100 mL) and a higher antioxidant capacity (356.23 by FRAP assay and 304.66 by DPPH assay) **(Lafarga *et al*., 2019)**. According to **Aleksandrova *et al*. (2019)**, the use of 250 g of a spirulina-enriched smoothie in the athlete's diet will meet the need for protein by 32.5%, carbohydrates by 12.25%, vitamin B4 by 32.5%, vitamin C by 28.8 %, potassium by 26.8% and magnesium by 20%. The maximum effect of Spirulina extract on the auto-oxidation of adrenaline was detected at an exposure times of 3 and 5 minutes: 73.0% and 64.2%, respectively. The study concluded that the use of a drink with Spirulina in the diet of athletes is one of the most important factors in increasing their endurance and performance. A meal replacement shake and a high-calorie food supplemented with Spirulina biomass were developed by Freitas et al. (2019) with Spirulina sp. LEB 18 (750 mg/100 g). The protein content for both developed foods was approximately 20% (w/w). The shelf life of the products with microalgal biomass was estimated at 26 months for the high-calorie food supplement and 17 months for the meal replacement shake.

Spirulina-based Baby Foods

Spirulina is loved by children, and it is safe and highly nutritious for them and can easily assimilate its nutrients. Spirulina can create tissue growth, enhance vision, improves the immune system, healing capacity, and the ability to focus on children. Thus, spirulina formula can be used for babies who are unable to swallow the capsules. It is also possible to blend powdered spirulina formulas with fruit juice, milk, salads, and convenient soups. Sixteen nutritional formulas were prepared for babies (1-3 years of age) as supplementary nutrition using Spirulina at 0, 2.5, 5, and 7.5 percent for the manufacture of sixteen forms of baby foods. Based on the results of sensory evaluation and chemical composition, four formulas containing 5% of spirulina was found to be suitable as a baby food for children aged between 1-3 years. The significance of spirulina is that regular use can regulate the alkaline levels in the body and aids in weight loss, it also nourishes and renews our body and health respectively **(Sharoba, 2014).**

Spirulina based Confectionaries

In any generation, biscuits and chocolates are the most consumed snack foods, considering their low protein content and high presence of unhealthy ingredients. In recent years, there has been an increasing interest in fortifying and/or enriching the nutritional value of foods, especially for children's diets **(Polter *et al*., 2013)**. A study by **Rathod and Annapure (2016),** confectionary samples were prepared by 2g of Spirulina biomass. Appropriate protein content and rich amino acid profile were found in homemade biscuits and chocolates designed with 2 % (w / w) of Spirulina as a natural ingredient, with a substantial increase in the daily intake of essential amino acids, histidine, and arginine, for infants and children Amino acids like phenylalanine and tyrosine (aromatic) and methionine (sulphur) in Spirulina enriched chocolates almost fulfilled the daily intake (RDI) as 25 and 15 mg/kg respectively **(Sahin, 2019)**.

Spirulina Millet Mix Flour (SMMF)

Saggu and Sundaravalli (2016) developed Spirulina millet mix flour (SMMF) that contained a rich nutrient profile of 378.84 Kcal energy, 16.07 g protein, 1.24 g fiber, 299.6 g calcium, 20.16 g of iron per 100g and 4.6 meq peroxide content per 1000 g. Of the traditional recipes developed, 50% SMMF chapathi, and burfi, 75% SMMF dhoklas and onion pakoras, and 100% SMMF muffins were found most acceptable. Thus, by the efficient combination of Spirulina and millets, SMMF can reach households and help tackle public health issues like iron deficiency anaemia.

Table 5 List of Spirulina fortified food products

S.no	Category of products	Principle raw materials	Level of spirulina incorporated (%)	Optimum level of spirulina (%)	Changes in the formulated product	Reference
1.				**Bakery products**		
	Bread	Whole wheat flour, water, and yeast	1, 2, 3, 4, and 5	1	Increase in protein, mineral (iron), micro-nutrient and energy.	**Minh (2014)**
	Crostini	Wheat flour, water, *L. farciminis, and S. cerevisiae*	2, 6, and 10	2	Enhanced green colour, Increase in protein, phycocyanin and total phenolic content.	**Niccolai *et al*. (2019)**
	Breadsticks	Wheat flour, salt, fresh yeast, water, and sunflower oil	1.5	1.5	High in micro-minerals selenium and iron, product is more stable in colour and texture.	**Uribe-Wandurraga *et al.* (2019)**
	Biscuits	Whole wheat flour, semolina, oat flour, butter, yoghurt, and water	1, 2, and 3	2	Increase in protein, fibre, iron potassium, essential amino acids and energy.	**Sahina (2019)**
	Chocolate chip oatmeal cookies	Soft butter, brown sugar, wheat flour, cooking oats, chocolate chips, eggs, baking soda, salt, and vanilla	3, 6, and 9	3	Adding spirulina to cookies affected their smell, color, appearance, texture and taste. The addition of spirulina affected the easiness with which breaking a cookie was made, the fragmentation and the appearance of the break line.	**Ghaly *et al.* (2015)**
	Souffle cake	Eggs, corn flour, dry fruits, and sugar	4, 6, 8, and 10	8	Better organoleptic properties viz, Colour and Appearance, Body and Texture, Flavour and Taste and Overall Acceptability. The microbial count was found to be within the limit.	**Ali *et al.* (2019)**
	Yazdi cupcake	Flour, sugar, eggs, liquid oil, yoghurt, rose water, sodium bicarbonate, sugar syrup, cinnamon, and water	0.5, 1.5, and 2.5	2.5	High nutritional value, desirable texture, and flavor. Highest content of ash, protein, Mn, Mg, and Se. The lowest hardness, gumminess, and chewiness.	**Golmakani *et al.* (2015)**
	Cassava doughnuts	Cassava, water, sucrose, whole egg powder, salt, instant dry baking powder, and inverted sugar	2.59-5.41	5.41	Increase in protein, mineral, fibre and lipid composition.	**Rabelo *et al.* (2013)**
2.				**Dairy products**		
	Kareish cheese	Pasteurized buffalo skim milk, salt, yoghurt culture *(S. thermophiles* and *L. delbrueckii)*	0.5, 1.0, and 1.5	1.0	Significant effect of protein, β-carotene and antioxidant activity. Higher phenolic content and flavonoid compounds.	**Darwish *et al.* (2017)**
	Cheese	Pasteurized buffalo skim milk, salt, and yoghurt	0.5, 1.0 and 1.5	1.5	Increase in protein, fibre, phenolic compounds, β-carotene and flavonoid compounds.	**Agustinia *et al.* (2016)**
	Yoghurt	Toned milk, skimmed milk powder, and sucrose	0.1, 0.2, 0.3, 0.4, and 0.5	0.3	Increase in protein, macronutrients iron, total carotenoids and chlorophyll.	**Malik *et al.* (2013)**
	Icecream	Skimmed milk powder, pasteurized milk, butter, cream, crystal sugar, eggs, gelatin, and water	0.75, 0.15, 0.23, and 0.30	0.15	Increase in protein, iron, antioxidant potential and β-carotene.	**Dubey *et al.* (2011)**

3.			**Extruded products**			
	Pasta	Semolina flour, gluten, and water	0.00, 0.25, 0.50, 0.75, and 1.00	0.25	Increase in protein content, phenolic compounds and antioxidant activity.	**De Marco *et al.* (2014)**
	Noodles	Wheat Flour and water	5	5	Developed food products were nutritious and their nutritional value is much greater, higher acceptability on organoleptic parameters.	**Fatima *et al.* (2017)**
	Snack foods	Organic corn flour, and organic rice flour	0.4, 1.8, and 3.2	2.6	Increase protein concentration, expansion index, ΔE and to reduce the bulk density and hardness of the snacks.	**Lucas *et al.* (2018)**
	Spaghetti	Durum semolina flour, and water	0.5, 1.0, and 2.0	1.0	Increase of fatty acids, polyunsaturated fatty acids (PUFA). Appealing colour, increasing colour intensity.	**El-Hameed *et al.* (2018)**
4.	**Energy bars**	Bengal gram, groundnut, puffed rice, coconut flakes, cornflakes, jaggery, ghee and liquid glucose	3, 4, 5, and 6	6	Increased in the carotenoid content, protein content and zinc content, showed desirable hardness and did not lack cohesion.	**Kumar *et al.* (2018)**
5.			**Beverages**			
	Fermented drink	LAB strains, probiotic and commercial mix culture, substrates, and yeast extract	0.25 and 0.50	0.25	Boosting effect to enhance the technological performances of starter LAB strains and mix culture.	**Martelli *et al.* (2020)**
	Pomegranate beverage	Pomegranate fruit, *Echinacea purpurea* leaves, and stevioside	4	4	Could activate innate immune response and may be considered as a supplementary therapy. High antioxidant activity, High total phenols.	**Mahmoud *et al.* (2015)**
	Sports nutrition drink	Banana, wild berries, and natural yoghurt	1.5, 2.0, and 2.5	2.0	Increased protein and a vitamin-mineral complex on the improvement of competitive activity noted.	**Aleksandrovna (2019)**
	Broccoli soup	Boiled broccoli, boiling water, olive oil, and salt.	0.5, 1.0, 1.5, and 2.0	0.5	Higher phenolic content and antioxidant activity, The amount of bio accessible polyphenols as well as the antioxidant capacity of the digestive enzymatic extracts was also higher.	**Lafargaa *et al.* (2019)**
6.	**Baby foods**	Fruits, sugar, wheat flour, rice, barley, lentil, chickpea, spinach, cauliflower, and peas	0, 2.5, 5 and 7.5	5	Regulating the alkaline levels in the body and aids in weight loss; also nourishes and renews body.	**Sharoba *et al.* (2014)**
7.	**Chocolates**	Coconut butter, cacao, and grape molasses	2	2	Substantial increase of amino acids like phenylalanine, tyrosine and methionine.	**Rathod *et al.* (2015)**
8.	**Millet mix flour**	Sprouted Finger Millet, Foxtail Millet, and Kodo Millet.	6.6, 8.3, and 10.0	10.0	Increased protein, iron and calcium content,	**Saggu and Sundaralli (2016)**

DOSAGE AND RECOMMENDATION

Spirulina when consumed anywhere between 1.0 g– 8.0 g per day is found to offer various health benefits. The maximum intake of spirulina for an elderly person is 2.0-3.0g/day, and for children, it is 1.0-2.0g/day (94). Nevertheless, the exact dosage of Spirulina depends on the disorder for which it is prescribed **(Patel, 2020**).

- For cholesterol, a dosage between 1.0g to 8.0g per day has some effect.
- For muscle efficiency, doses of 2.0g to 7.5g per day shows some effect.
- For blood glucose control, dosage of 2.0g per day showed a mild effect.
- For blood pressure, dosage of 3.5 to 4.5 shows some effect.

As per **Patel (2020)**, the suggested dosage of Spirulina for a person based on weight is as follows:

- 150 lb person-11.0g of spirulina
- 200 lb person- 14.5g of spirulina
- 250 lb person- 18.2g of spirulina

According to the experience of long-time consumers and scientific evidence, consumption of 3.0 to 10.0 grams of spirulina per day provides a significant health benefit. It is evaluated that daily use is most beneficial. Spirulina consumed in the form of tablets, between, before, or with meals is convenient. Many consumers mix spirulina powder in fruit smoothies as instant breakfast and vegetable smoothies as an instant food for the afternoon **(Seema and Sonia, 2015)**. The SCF (The Scientific Committee on Food) and EFSA (European Food Safety Association) also recommend 10g as the maximum dosage per day. Spirulina as a daily supplement has no records of toxicity **(FDA, 2013**). Though spirulina has been termed as a non-toxic supplement and proved safe for consumption, researchers continue to check its safety **(Shiomi and Waisundara, 2017)**. It is still a debate if spirulina can be consumed only once a day in the recommended dosage or multiple times a day in small dosages. Even when consumed less or more than the recommended dosage, there were no significant effects **(Patel, 2020).**

CONCLUSION

Exploring microalgae as a source of human and animal feed is not new and is been followed for centuries. They offer a wide range of food and non-food applications and can a stand extreme conditions, also they are non-seasonal. Spirulina particularly is extremely nutritious and even reported to be non-toxic consequently it can be safely consumed and recommended as a human food. We can conclude that the benefits of spirulina consumption are innumerable i.e., it has significantly higher essential nutrients, bioactive compounds, physiological health benefits, and non-toxic to name a few. Hence, spirulina can be considered to be a promising alternative to feed the future world and fight against malnutrition as well as other nutritional disorders.

FUTURE PERSPECTIVES

Spirulina being titled the 'Food for future' has also been entitled as the 'Biofuel of the future'. From a long history, algae are an essential part of Earth's self-regulating life support system. Diverse applications of it have led to an innovative dream of utilizing algae to re-green the deserts, re-fertilize depleted soils, clean the oceans, etc moving to a more sustainable and realistic business model. Better discovery of Spirulina with superior properties will pave way for futuristic developments such as biofuel and bio-packaging that will represent the return of the origin of life for individuals and planetary health and restoration. Multitalented Spirulina alga will reduce the production costs of many products by replacing fossil fuel chemical products with biopolymers and bioplastics thus envisioning a bright future with it.

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