

FORMULATION OF A NEWLY DEVELOPED DIETARY SUPPLEMENT FROM  
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## ABSTRACT

Supplementation of foods is of current interest because of increasing nutritional awareness among consumers. A dietary supplement is a manufactured product intended to supplement the diet when taken by mouth as a pill, capsule, tablet, or liquid. Spirulina is selected to this study for its high protein content and its varieties health benefits. The objective of this study is to produce dietary capsule from marine sources and evaluate its efficacy on health and nutrition. Proximate composition of Spirulina plus capsule was analyzed. Protein content of Spirulina plus capsule was (71.19±1.11) % and it removes protein energy malnutrition. It contains a considerable amounts of ash, fiber which has beneficial health effects. The final product Spirulina plus capsule contained (9.16±0.004) % fat which can help for brain development. Spirulina plus capsule had (12.09±0.02) % fiber which can remove constipation. Microbial test of final product represents that the product was safe. It was concluded that Spirulina plus capsule is enriched with the mixture of some other ingredients and it becomes a good dietary supplement to the consumers.

**KEYWORDS:** Spirulina; Dietary supplement; Marine sources.

## INTRODUCTION

A supplement can provide nutrients either extracted from food sources or synthetic, individually or in combination, in order to increase the quantity of their consumption. Spirulina is selected to this study for its high protein content and its varieties health benefits.

Many marine algae produce antibiotic substances capable of inhibiting bacteria, viruses, fungi. It appears that the antibiotic characteristic is dependent on many factors, including the particular alga, the microorganisms, the season, and the growth conditions.<sup>[1]</sup> Algae contained 51% crude protein. It appears to have potential as a livestock feed because of the high content of protein, plus significant amounts of carotene, phosphorus, calcium and trace minerals.<sup>[2]</sup> The marine environment, which contains a vast array of organisms with unique biological properties, is one of the most underutilized biological resources. Algae and microalgae are referenced in the literature as sources of bioactive compounds for use as functional food ingredients.<sup>[3]</sup> Algae comprise a complex and heterogeneous group of organisms characterized by their photosynthetic nature and their simple reproductive structures. According to their size, algae can be roughly divided into unicellular organisms, known as microalgae and multicellular

organisms referred to as microalgae. Algae frequently live in extreme environments of light, salinity, and temperature.<sup>[4]</sup> Marine algae comprising a few thousands of species represent a considerable part of the littoral biomass. Many algal species have long been used as human food, animal fodder, and source of valuable substances.<sup>[5]</sup> Marine algae are rich in polyunsaturated fatty acids (PUFAs).<sup>[6]</sup>

## 1.1. Spirulina

Spirulina is a "Superfood." It is the most nutritious, concentrated whole food known to humankind. It has a rich, vibrant history, and occupies an intriguing biological and ecological niche in the plant kingdom. A food that can help regulate blood sugar, blood pressure and cholesterol; alleviate pain from inflammation and deliver antioxidant activity to ward off life-threatening diseases like cancer, Alzheimer's, heart disease and stroke; protects the liver and kidneys and removes radiation from the body; improves the immune system, alleviates allergies; helpful for our eyes and brain; help us lose weight and improve digestion. Spirulina is about sixty percent complete, highly digestible protein. Spirulina contains every essential amino acid.

## 1.2. Species

Various spirulina species are *Spirulina maxima*; *Spirulina platensis*, *Spirulina pacifica* (also known as *Arthrospira platensis*, *Arthrospira maxima*). *Arthrospira platensis* is the predominant species and commercially cultivated worldwide though *Arthrospira maxima* is produced in the South and Central American regions.<sup>[7]</sup> Nutrient Value of Spirulina is one of the natural sources containing the highest amount of protein — five times that of meat. Spirulina provides the majority of essential and nonessential amino acids. It has a fairly well-balanced amino acid pattern and contains the highest amount of beta-carotene, a precursor of vitamin A. It is the only vegetable source of vitamin B12 having two and half times the amount in liver. It is also the source of the essential fatty acid  $\gamma$ -linolenic acid which is the precursor of hormones involved in the regulation of body functions. The constituents of spirulina include protein (50-70%) including all essential amino acids, essential fatty acids, polysaccharides, B vitamins particularly vitamin B12, beta-carotene and minerals particularly iron.<sup>[8]</sup>

## 1.3. The main pigments found in Spirulina

The most visible pigment in Spirulina is chlorophyll. Chlorophyll is known as the cleansing and detoxifying phytonutrients increases peristaltic action and thus relieves constipation. It also normalizes the secretion of digestive acids.

Spirulina is the richest food source of beta-carotene which is a Vitamin A precursor. It has 21 times more beta carotene than raw carrots and with a spectrum of 10 mixed carotenoids, about half are orange carotene. These are alpha, beta, and gamma. These components used to enhance healthy eyes and vision and antioxidant protection.

Phycocyanin is a brilliant blue polypeptide which is a source of biliverdin (a green pigment excreted in bile) which is the most potent intra-cellular antioxidants and related to human pigment bilirubin and stem cells. Its components are important to healthy liver function and digestion of amino acids.

Porphyrin is a red compound that forms the active nucleus of hemoglobin. It is essential for the formation of red blood cells. It is used as a chelator for heavy metal toxicity and circulation problems.

Spirulina contains a number of enzymes. One of the most significant enzymes is superoxide dismutase (SOD), which is important in quenching free radicals and in retarding aging. This essential enzyme is crucial to the body's ability to assimilate amino acids. In fact, Spirulina is so high in enzyme activity that even after being dried (at 160°C) it will often start growing again if placed in the right medium, temperature and sunlight. Spirulina has been scientifically demonstrated to increase

the reproduction of lactobacilli (bacteria that digests our food). It contains over 2000 different enzymes.<sup>[9,10]</sup>

## 1.4. Nutritional food value

The use of Spirulina as complementary feed in various sector of aquaculture resulting in fast growth factors, enhancing the pigmentation and immunity systems. It is considered as a portion of excellent food, lacking toxicity and having corrective properties against the pathogenic microorganisms. It lacks cellulose cell walls and therefore does not requires chemical or processing in order to become digestible. The digestibility is 83 – 84%. Spirulina is regarded as a rich source of protein, vitamins, an essential mineral, amino acids, EFFA like gamma LNA and antioxidant pigments like carotenoids.

## 1.5. Biochemical composition of Spirulina

### 1.5.1. Protein & Amino acids

Spirulina contains 60-70% protein along with phenolic acids, tocopherols, carotenes and linolenic acids for which represents an important staple in diets. The essential amino acids are present around 47% of total protein weight. The spectrum of amino acid represents that the biological value of proteins in Spirulina is very high.

### 1.5.2. Carbohydrates

Spirulina contains about 15 -21% carbohydrates in the form of Glucose, fructose, sucrose, rhamnose, mannose, xylose and galactose. It provides appropriate and important foodstuff for aquatic culture animals with problems of poor intestinal absorption. A high molecular weight polysaccharide is believed to have an effect on DNA repair mechanisms, immune stimulatory and immune-regulatory properties.

### 1.5.3. Nucleic acids

Spirulina contains 2.2% - 3.5% of RNA and 0.6%-1% and DNA, which represents less than 5% of these acids, based on the dry weight.

### 1.5.4. Essential fatty acids

Spirulina has a high amount of polyunsaturated fatty acids (PUFAs) and 1.5–2.0 percent of total lipid. Spirulina is rich in  $\gamma$ -linolenic acid (ALA), linoleic acid (LA), stearidonic acid (SDA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid (AA).

### 1.5.5. $\beta$ -carotene and vitamins

Spirulina contains vitamin B1 (thiamine), B2 (riboflavin), B3 (nicotinamide), B6 (pyridoxine), B9 (folic acid), B12 (cyanocobalamin), vitamin C, vitamin D and vitamin E. The  $\beta$ -carotene, B-group vitamin, vitamin E, iron, potassium and chlorophyll available in the Spirulina can promote the metabolism of carbohydrate, fats, protein, alcohol, and the reproduction of skin, muscle and mucosa. Spirulina contains large amounts of natural  $\beta$ -carotene and this  $\beta$ -carotene is converted into vitamin A.

### 1.5.6. Minerals

Spirulina is a rich source of potassium, and also contains calcium, chromium, copper, iron, magnesium, manganese, phosphorus, selenium, sodium, zinc, molybdenum, chloride, germanium and boron.

### 1.5.7. Photosynthetic pigments

Spirulina contains many pigments including chlorophyll a, xanthophyll, betacarotene, echinenone, myxoxanthophyll, zeaxanthin, canthaxanthin, diatoxanthin, 3-hydroxyechinone, beta cryptoxanthin.

### 1.6. Health Benefits of Spirulina

In addition to antioxidant and anti-inflammatory effects other potential health applications are: protection of the liver and kidneys; improvement of blood quality and prevention of anaemia; benefits for diabetes; reduction in blood Pressure; removal of heavy metals from the body; radioprotection; prevention of liver and renal toxicity; antioxidant action; immune protection and relief in allergic reactions.<sup>[11]</sup> These antioxidants can become pro-oxidants and protect the body from oxidative stress.<sup>[12]</sup>

Study showed that the total tumor regression was found in 30% of the animals receiving the Spirulina and Dunaliella extracts, while partial tumor regression was found in all of the remaining 70% of the animals; meanwhile, in the control group, no tumor regression was found.<sup>[13]</sup> Chemoprevention of cancer & reduced incidence of liver tumors has also been documented.<sup>[14]</sup> The chemo preventive capacity to reverse precancerous lesions of spirulina is attributed to the antioxidant property with a high amount of beta carotene and superoxide dismutase.<sup>[15,16]</sup> It may produce high NADP<sup>+</sup>, which results in down regulation of lipogenesis and lower risk of the tissues for oxidation stress and high resistance for diabetes.<sup>[17]</sup> It was also shown that spirulina reduced systolic and diastolic blood pressure when given by oral route (4.5 g/day, for 6 weeks).<sup>[18]</sup> This lipid lowering property has been attributed to the C-phycoyanin molecule in spirulina.<sup>[19]</sup> Oral administration of spirulina is also associated with a reduction in systolic and diastolic blood pressure.<sup>[18]</sup> Levels of anaemia also decreased in children when their diet was supplemented with spirulina.<sup>[20]</sup> Spirulina extracts have also been shown capable of inhibiting carcinogenesis.<sup>[21]</sup> The anaemic condition induced by irradiation was also reduced.<sup>[22]</sup> Spirulina is rich in iron, magnesium and trace minerals, and is easier to absorb than iron supplements. Spirulina is the highest source of B-12, essential for healthy nerves and tissue, especially for vegetarians.<sup>[23]</sup>

### 1.7. Socio-economic Importance

St. Martin's Island is not only significant for its biodiversity value but also important for Bangladesh in defining its Exclusive Economic Zone. They grow vastly in winter and spring. Spirulina plus capsule will play an important role in preventing cardiovascular disease, osteoarthritis, diabetes and also presenting antiviral, anti-

inflammatory, antitumor, antimicrobial and antioxidant activities. Iodine rich functional food will be a cheap, reliable and available effective food for goiter prevention which will reduce mortality and morbidity. Algae as potential sources of bioactive compounds for nutraceutical, pharmaceutical, food or cosmetic industries. It will also be beneficial for our national economy if we will export this functional food to a foreign country after fulfilling our demand.

## 2. MATERIALS AND METHODS

### 2.1. Study place

The study was conducted in the laboratories of BCSIR (Bangladesh Council of Scientific and Industrial Research) and laboratory of Food Technology and Nutritional Science department of Mawlana Bhashani Science and Technology University.

### 2.2. Manufacturing process of Spirulina plus capsule

Marine algae (spirulina species) collection from Saint Martin Marine algae were collected from Saint Martin in February two times. Processed and dried Spirulina species was washed very carefully and dried by sun drying. Firstly, Spirulina was dried and dried parts were transferred into a machine. Finally, Spirulina powder was ready. Add carrot powder. After formation of Spirulina powder carrot powder was added into it. Add ginger powder and formation of mixture of Spirulina plus Ginger powder was added into Spirulina plus mixture. Spirulina plus mixture was entered into capsuling machine machine and Spirulina plus capsule was formed. Spirulina plus capsule was tested in a laboratory by several tests such as moisture, ash, fat, protein, microbial test etc. Filling Spirulina plus capsule was filled into a food grade plastic bottle. Sealing and labeling Information of ingredients, net quantity, shelf life of product was labelled in the outer place. Certified by a registered doctor in Dhaka and the result was positive.



Fig. 1: Spirulina plus capsule filling.

### 2.3. Nutrient composition of spirulina plus capsule

Proximate composition represents the gross content of important chemical constituents –moisture, ash, fat, vitamin and minerals. These gives positive indication as to the nutritive quality of the under investigation but this parameter cannot be used to generalize the nutritive value of Spirulina plus capsule. The study of the proximate composition serves as an important base to study the nutritive quality of Spirulina plus capsule.

#### 2.3.1. Determination of moisture content

Moisture is always present in food stuffs. Estimation of moisture is done by (AOAC, 1984). (%) Percentage of moisture content =  $\frac{\text{Initial weight}-\text{Final weight}}{\text{Sample weight}} \times 100$

#### 2.3.2. Determination of ash content

Ash content was done (AOCC, 1984) method. (%) of moisture content =  $\frac{\text{Initial Weight}-\text{Final weight}}{\text{Sample weight}} \times 100$

#### 2.3.3. Determination of fat content

##### Principle

Fat is estimated by dissolving the food sample into organic solvents (petroleum ether) separating die filtrate by filtration, placing the filtrate into separating funnel and then separated mixture is then dried to measure the extract and finally % of fat is estimated.

Weight of the extract (%) of Fat content =  $\frac{\text{Weight of extract}}{\text{Sample weight}} \times 100$

Procedure 10 gm sample was taken. Then 3 pieces of filter papers and pins were also weighted. Sample was carefully packed into filter paper and pinned up. Then sample was placed into thimbles of Soxhlet apparatus and sufficient amount of petroleum ether poured into flat bottom flask through thimble. After setting condenser, condensed water was supplied. Then, Soxhlet apparatus was started for heating at (60-70) °C. After separating fat about 4 times it was stopped. Condenser and thimble are separated from apparatus. Flat bottom flask containing fat and petroleum ether was again heated for removing ether. Then the residue was poured into weighted beaker and kept into desiccators. After 5 minutes beaker with fat was weighted and then calculated.

### 3. Experimental results and discussions

#### 3.1. Spirulina powder, carrot powder and ginger powder characterization

Table 1: Spirulina powder, carrot powder and ginger powder characterization.

Characteristics	Spirulina powder	Carrot powder	Ginger powder
Powder color	Greenish	Orange	Yellowish
Texture	Amorphous	Bulky	Coarse

Table 1 shows that the color and texture of Spirulina powder, carrot powder and ginger powder. It represents that the color of Spirulina powder is greenish, carrot powder is orange and ginger powder is yellowish. The texture of Spirulina powder is amorphous, carrot powder is bulky and ginger powder is coarse.

#### 2.3.4. Estimation of protein content

##### Principle

Protein content can be measured by estimating the nitrogen content of the material and then multiplying the nitrogen value by 6.25. This is referred to as crude protein content, since the non-protein nitrogen (NPN) present in the material was taking into consideration in the present investigation.

The estimation of nitrogen was made by modified kjeldahl method (AOAC, 1990).

% of Protein =  $\frac{B \times 29 \times \text{Moisture factor}}{\text{Weight sample}}$  Where,

#### 2.4. Microbiological analysis

Total Plate count (TPC), total coliform count (TCC), total fungal count (TFC) microbiological tests were conducted for the samples.

##### 2.4.1. Determination of total plate count

For total plate count of the sample, standard plate count was done according to the method described in standard methods for the examination of dairy products (Robertson, 1952).

##### 2.4.2. Determination of Total Coliform Count

Coliform count of sample was done according to the method as described in the standard methods for examination of dairy products (Robertson, 1952).

##### 2.4.2. Determination of Total Fungal Count

Yeast and mold count of sample was done according to the method as described in the standard methods for examination of dairy products (Robertson, 1952).

##### 2.4.3. Water Retention

The volume of water separated after centrifugation was measured and water retention was calculated using following formula as per method given by (McConnell, 1974).

Water retained (ml) Water retention (ml/g) =  $\frac{\text{Water retained (ml)}}{\text{Weight of sample (g)}}$   
 =  $\frac{\text{Water retained (ml)}}{\text{Volume of water added 20 ml} - \text{Volume of water obtained after centrifugation}}$

#### 3.2. Spirulina plus mixture characterization

Table 2: Spirulina plus mixture characterization.

Characteristics	Spirulina plus capsule
Color	Brownish-green
Texture	Amorphous

Table 2 represents that color of Spirulina plus mixture is brownish-green and texture is amorphous.

**3.3. Physical properties of Spirulina powder**

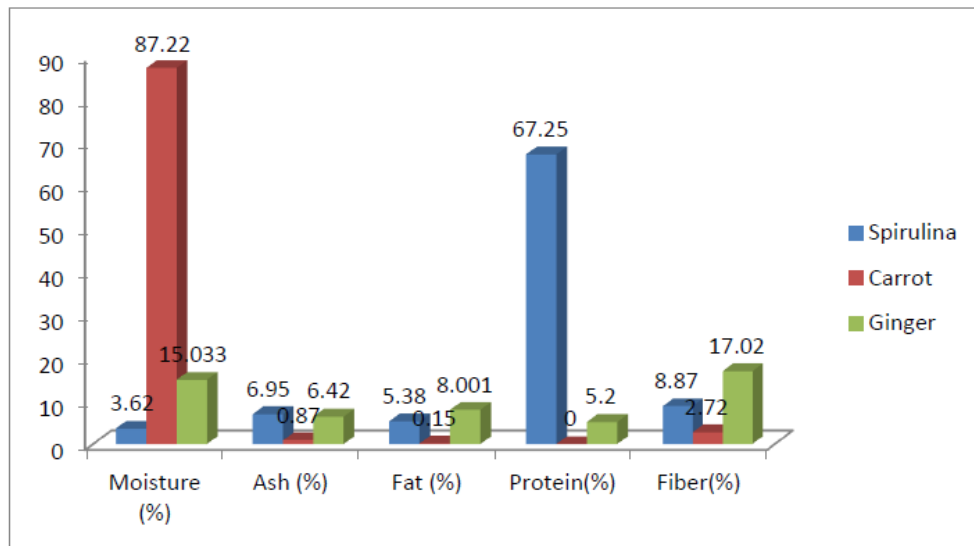
**Table 3: Physical properties of Spirulina powder.**

Physical properties	Values
p <sup>H</sup>	6.93±0.09
Particle size	100% of mesh
Color	Blue green to green
Appearance	Fine, uniform powder
Odor and taste	Mild like sea weed
Consistency	Powder

Table 3 depicts the physical properties of Spirulina powder. It indicates that PH of Spirulina powder is 6.93±0.09, color is blue green to green, odor and taste is mild like sea weed and consistency is powder.

**3.4. Composition of raw Spirulina, carrot and ginger**

Fig 2 shows that the highest moisture content present in raw carrot is 87.22% and lowest in fresh Spirulina is 3.62. The highest protein content present in raw Spirulina is 67.25%. The lowest fat content present in raw carrot is 0.15%. Fiber content is high in raw ginger that is 17.02%.



**Figure 2: Composition of raw Spirulina, carrot and ginger.**

**3.5. Composition of Spirulina plus capsule**

**Table 4: Composition of Spirulina plus capsule.**

Sample	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Fiber (%)	
Spirulina plus capsule	16.12±0.01	12.09±0.02	15.43±0.003	9.16±0.004	71.19±1.11	12.09±0.02

Table 4 explains that the final product composition and it indicates that protein content is very high and fat content is almost poor.

These findings are almost same to the recording findings by researchers.

**3.6. Moisture content (%) of stored Spirulina powder, carrot powder and ginger powder mixture which was used for supplement**

**Table 5: Moisture content (%) of stored Spirulina powder, carrot powder and ginger powder mixture which was used for supplement.**

Storage	Moisture (%)						
	Storage period (Months)						
Stored in food grade plastic box at condition (24-26°C)	0	1	2	3	4	5	6
	5.37	5.39	5.43	5.57	5.61	5.78	5.85

Moisture content is one of the important parameters which interfere in the quality of the powder during the storage. In the Table 5, it is clearly depicted that, there was an increase in the moisture content from the initial value (5.37) to 5.39, 5.43, 5.57, 5.61, 5.78 and 5.85 per cent in ambient temperature with the intervals of a

month. Peak increase in the moisture level was observed at 1st (5.39%) and 4th (5.85%) months.

**4. CONCLUSIONS**

Spirulina plus capsule has emerged as the wonder food supplement. Several leading organizations are utilizing

this beneficial action. The highly diverse nutritive nature of spirulina plus capsule together with its antioxidant and protective health benefits have been utilized in various health related problems. The effectiveness of spirulina plus capsule is summarize: Spirulina plus capsule contain high amount of protein which can remove protein energy malnutrition. After microbial test, it was noticed that there were very few colonies in sample media. So, this supplement is free from microorganisms. Spirulina plus capsule were suggested to take their supplement of diabetic patients. Blood sugar level was decreased after consuming this supplement. So, it has antidiabetic property. Spirulina plus capsule also contains 9.16% fat that is poly unsaturated fatty acids which can help our brain development. This dietary supplement mixed with ginger which has antioxidant property.

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