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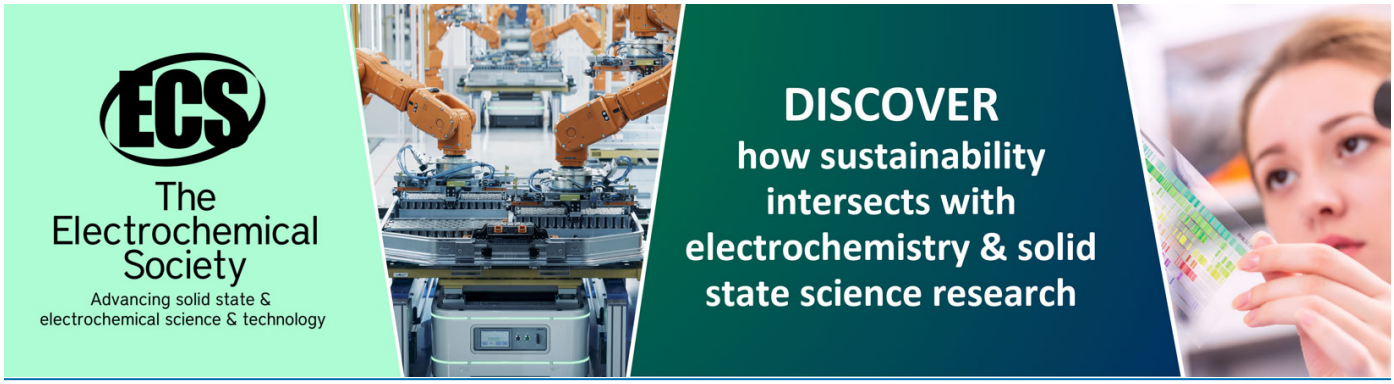

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Nutritional analysis of *spirulina sp* to promote as superfood candidate

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Abstract. An analysis of the nutrition content of *Spirulina sp* is reported. The main objective of this study is to evaluate the nutritional value of *Spirulina* sp that produced by the large culture at ambient temperature. The analysis method was used high-performance liquid chromatography, gas chromatography, spectrophotometers UV-Vis and inductively Coupled Plasma-OES. The analysis has found vitamins, minerals, amino acids, pigments, and essential fatty acids. It is concluded that *Spirulina sp* which is produced by large-scale culture is a good source of nutrition and potent an alternative superfood.
Keywords: Spirulina sp, Superfood, Supplement food, Nutrition.

1. Introduction

The Biomass production of microalgae such as phytoplankton received special attention recently, as it could be a good source of alternative protein in foods. Production and commercialization of microalgae rich in pro-vitamin A carotenoids are mostly performed on *Spirulina, Dunaliella*, and *Chlorella* [1, 2]. These microalgae are widely produced commercially because of the high provitamin A content, and also provide a variety of other nutrients such as proteins, minerals, and vitamins. therefore, *Spirulina* has been used as supplements foods, drugs, functional and additives, foods [3]. Some microalgae have been studied, but the *Spirulina* blue-green algae are considered more promising because of their high protein content (DM 65 to 70%). In addition, the amino acid content is high and a large number of vitamins and minerals [4-6].

The *Spirulina* is known a rich foods source of micro and macronutrients such as protein, vitamins, gamma-linolenic acid, phycocyanin and sulfated polysaccharides [7, 8]. *Spirulina* has a cell wall consisting of proteins, carbohydrates, and fats that are easily digested so that it has more nutritional content than other vegetable food sources [9]. Therefore, *Spirulina* is potentially used as a functional

food and supplements that are safe to consume in the right amount [10]. The term functional refers to foods that have been shown to help certain body functions, resulting in promoting health and /or reducing the risk of disease beyond nutritional function [11]. Several studies such as antioxidant actions, hypercholesterolemia, anti-inflammatory, immuno-stimulatory, anticancer, antivirals have been conducted to verify the benefits of *Spirulina* [12, 13]. In other hands, *Spirulina* is also shown that good acceptance as an important food prospect or nutritional supplement) and the *Spirulina* has not shown chronic or acute toxicity, it is safely consumed as humans foods [14, 15].

Nutritional foods composition are important to both health professionals and consumer. Several analytical techniques were used to analysis food composition, such as high-performance liquid chromatography (HPLC) to determine the carotenoid, lipid and amino acids composition. Spectrophotometric was used to determine pigment and total carotenoid. Inductively coupled plasma optical emission spectrometry (ICP-OES) was used to determine the micronutrients and heavy metal contents. Some of the research shows that concentration of organic and inorganic compounds such as carbohydrate, carboxylic acids, metals and anions in vegetable samples were determined by high-performance liquid chromatography (HPLC)–ICP OES hyphenation technique [16-19].

The nutritional composition of *Spirulina* varies greatly according to the culture conditions and analysis methods. In this study, we report for the first time the nutritional status of *Spirulina* cultured at ambient temperature. The objectives of the study were as follows: (i) to investigate the nutritional value of *Spirulina* sp, and (ii) to assess the impact of large-scale culture at ambient temperature on the nutrition status of *Spirulina* sp.

2. Methods

The biomass of *Spirulina* is produced batch culture by large-scale culture (1000 L) cultivated experiment was conducted in the laboratory. This study was conducted at the wet laboratory, Marine and Fisheries Faculty, Universitas Khairun. The *Spirulina* was large-scale culture (volume 1000 L) photo-bioreactors with central aeration, utilizing media containing Urea, triple superphosphate (TSP), nitrogen phosphate potassium (NPK), Biotin, Vitamin $B_{12.}$ Culture pH is maintained at 6.5–7.5 and grown at a mean temperature of 28-30°C. The biomass of Spirulina was harvested by drying method at exponential growth phase to obtain protein and highly unsaturated fatty acids contents. after harvested, it was lyophilized and stored in airtight containers in darkness about -10°C.

The protein contains *Spirulina* is determined by the *Kjeldahl method*. The amino acids composition of *Spirulina* was analysed by high-performance liquid chromatography (HPLC) using o-phthaldialdehyde-mercaptopropionic acid (OPA-MPA) as reagent and amino acid standards. The total carotenoids, chlorophyll a and chlorophyll b were determined by spectrophotometric methods. one gram of *Spirulina* biomass is crushed (slurry), then extracted with 80% acetone, stirred until chlorophyll and carotenoid are dissolved, filtered, and filtrate is measured by spectrophotometer at a wavelength of 480 nm, 646 nm, and 663 nm.

Th**e** fatty acid (FA) content was analysed by a gas chromatographic (GC) instrumentation. one gram of *Spirulina* biomass is extracted with 2,2-dimethoxy propane solution and then concentrated. It was diluted with n-hexane solution and then injected into the gas chromatography instrumentation. The concentrations of minerals (K, Ca, P, Mn, Mg, Na, Fe, and Zn) were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) following microwave-assisted acid digestion. One gram of Spirulina biomass is added with 10 mL of nitric acid and then deconstructed using a microwave digestion. It was added with sterile water (distilled water) to 50 mL, filtered, diluted and measured using ICP-OES.

The vitamins Assay for vitamin C by the titrimetric method, where ascorbic acid is oxidized by dichlorophenol-indophenol into de-hydro ascorbic compounds. The end of the titration was characterized by the formation of a red colour from the excess of dichlorophenol-indophenol. The cyanocobalamin, beta-carotene, Thiamine, niacin, riboflavin, pyridoxine, folic acid, vitamin K, vitamin E, inositol, biotin, and pantothenate were determined by the high-performance liquid chromatography system method. Determination of multivitamin content in *Spirulina* biomass was carried out by HPLC

using Column Zorbax Eclipse plus C18 Agilent 4.6 x 150 mm 5-micron, Mobile phase (the mixture of methanol: air = 35: 65), Flow rate (1.5 ml/minute), UV detector 550 nm, and injection volume 20 ml. one gram of *Spirulina* biomass is extracted with 100 mL of water and then put into the injection column to obtain a retention time. then It was adjusted to the retention time of each vitamin standards.

3. Results

The nutritional and chemical composition of dried powder *Spirulina* is summarized in Fig. 1 and 2 and Table 1. The *Spirulina's* protein content is determined by *the Kjeldahl* method; it is about 64.24 % of its dry weight (DW). This is an important information because that majority of plant-based of foods which known as good protein sources contain only about 35% of their dry weight.

3.1. Amino acids composition

Spirulina contains both essential and non-essential amino acids in moderately high amounts (Fig. 1). The essential amino acids contents of *Spirulina* such as leucine (55 mg g^{-1}), tryptophane (10 mg g^{-1}), methionine (14 mg g^{-1}), phenylalanine (28 mg g^{-1}), lysine (30 mg g^{-1}), thionine (33 mg g^{-1}), isoleucine (36 mg g^{-1}) and valine (45 mg g^{-1}). The essential amino acids are organic compounds which needed in biological processes. They are the main component of all tissues in the body. Because of the essential amino acids are not produced naturally by the body, therefore they are included in the daily diet. Based on the essential amino acids content, the use of Spirulina as a supplement food can provide the daily needs of essential amino acids.

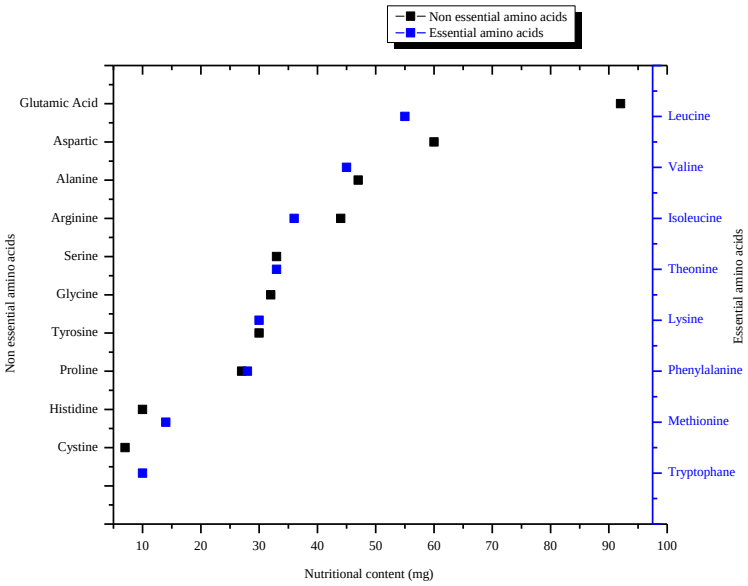

Figure 1. The both of the essential and non-essential amino acids of *Spirulina*.

Spirulina also contains non-essential amino acids such as cysteine (7 mg g^{-1}), histidine (10 mg g^{-1}), proline (27 mg g^{-1}), tyrosine (30 mg g^{-1}), glycine (32 mg g^{-1}), serine (33 mg g^{-1}), arginine (44 mg g^{-1}), alanine (47 mg g^{-1}), aspartic (60 mg g^{-1}), glutamate acid (92 mg g^{-1}). Non-essential amino acids play an important role in the human body. In the human body, it can incorporate with essential amino acids to produce a new protein needed by body cells. In addition, it can be converted into glucose as a source of fuel.

3.2. Fatty acids content

The fatty acids contents of *Spirulina* showed in Fig. 2. Several fatty acids component have been analyzed such as myristic, heptadecanoic, stearic, oleic, palmitoleic, omega-3, omega-6, linoleic acid (LA), gamma linoleic acid (GLA), and palmitic. The myristic and palmitic are the lowest and the highest content of fatty acids.

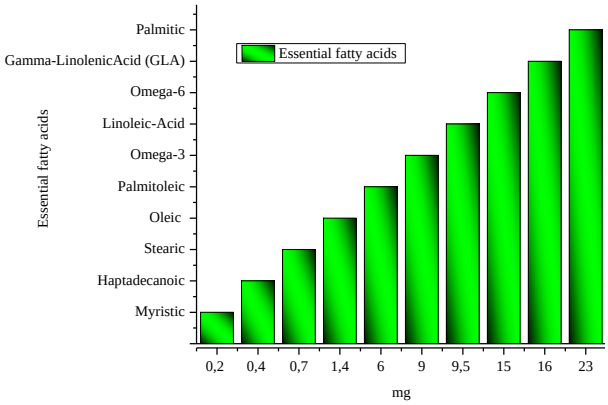

Figure 2. The essential fatty acids of Spirulina.

The total fatty acid content of *Spirulina* is 81.2 (mg g^{-1} dry weight). This result greater than the total fatty acid found by Kent et al, only about 48,73 mg g^{-1} DW [20]. Fig. 1, showing the gamma linoleic acids (GLA) contents is 16 mg. Compare with the previous study, gamma linoleic acid (GLA) content of Spirulina about 9.42 mg g^{-1} DW. It is a source of breast milk which contains gamma linoleic acid (GLA), essential amino acids and essential fatty acids which helps regulate the whole hormone system.

3.3. Minerals, vitamins, and pigments

The minerals content of *Spirulina* showed in Table 1. The most relevance of inorganic micronutrients in *Spirulina* is Potassium, magnesium, calcium, zinc, phosphorous and iron. They are important for nutrition human body. Iron can help to improve anaemia and hypertensive disorders. Phosphorus and calcium are comparable to those in milk. The proportion (Ca:P) of this micronutrient is compatible with the bone health to reduce decalcification. The iron, calcium and phosphorous content of *Spirulina* are 1.7, 15, and 10 mg, respectively.

Table 1. Vitamins, Minerals and pigments composition of *Spirulina* (per 1 g sample)

Vitamins composition		Minerals composition	
Biotin	0.55 μg	Potassium (K)	16 mg
Folic Acid	0.71 μg	Calcium (Ca)	15 mg
Pantothenic Acid	2 μg	Phosphorus (P)	10 mg
Cyanocobalamin (Vitamin B12)	3.6 μg	Manganese (Mn)	3 mg
Pyridoxine (Vitamin B6)	8 μg	Zinc (Zn)	70 μg
Thiamine (Vitamin B1)	48 μg	Magnesium (Mg)	3.7 mg
riboflavin (Vitamin B2)	55 μg	Sodium (Na)	2.5 mg
Niacin (Vitamin B3)	0.15 mg	Iron (Fe)	1.7 mg
Tocopherol (Vitamin E)	0.41 mg	Pigments	
Inositol Acid	0.7 mg	Phycocyanin	180 mg
Beta-carotene (pro-vitamin A)	5.8 mg	Chlorophyll	11 mg
Bioflavonoids	10 mg	Total Carotenoids	6 mg

The dried powder *Spirulina* contains some of the vitamins like Thiamine, riboflavin, Niacin, Pyridoxine, Cyanocobalamin, and Tocopherol. One of the important and complex vitamins is cobalamin (vitamin B12). It is very important because vitamin B12 is only contained in animal origin foods. according to Table 1, the cyanocobalamin (B12) content of *Spirulina* about 3.6 μg. The Spirulina is also known as a good beta-carotene source because it is containing about 5.8 mg g-1. beta-carotene will be absorbed to bio-transformed into vitamin A. Human body requirement of vitamin an about 1 mg/day, therefore 1-2 g of *Spirulina* will be sufficient for this need.

The phycocyanin, chlorophyll, and carotenoid are an important group of pigment found in *Spirulina*. Carotenoid is played as lipophilic antioxidants and responsible as an anticancer agent. The pigment component of *Spirulina* showed in Table 1. The contains both of the phycocyanin, chlorophyll, and carotenoid were 180.11 and 6 mg, respectively.

4. Conclusions

The *Spirulina* can be categorized as a superfood, because of its high nutrient content. *Spirulina* contains nutrients like protein, vitamins, essential fatty acids, amino acids, minerals, and phytonutrients. The high phytonutrient content of *Spirulina* can be considered an alternative food for vitamin supplements.

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