

CHEMICAL AND TECHNOLOGICAL STUDIES ON MICRO ALGAE EXTRACTS AND ITS UTILIZATION IN SOME FOOD PRODUCTS. 1-CHEMICAL, BIOCHEMICAL CHARACTERISTICS AND NUTRITIONAL VALUE OF SPIRULINA ALGAE.

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ABSTRACT

The objective of the present study was to evaluate the chemical, biochemical properties and nutritional value of Spirulina algae powder. The dried spirulina contained (53.8%) protein, (5.92%) lipid; beside the total carbohydrates, ash, crude fiber was 15.13, 9.67 and 3.32%; respectively.

The dried spirulina had seven essential amino acids; histidine, lysine, methionine, tryptophan, phenylalanine, isoleucine and leucine. The dried spirulina contained a valuable amount of vitamins, B2, folic acid, B12, C and E. Bioactive compounds such as gallic acid (75.27µg/mg), chlorogenic acid (12.12 µg/mg), naringenin (1.18 µg/mg), methyl gallate (0.96 µg/mg) and caffeic acid (0.45 µg/mg) were found in dried spirulina. Heavy metals content in spirulina powder; nickel, cadmium, mercury was determined and were not found. While copper and lead were detected at 9.5 and 3.58 p.p.m; respectively.

***Conclusively,** the chemical, biochemical and nutritional analysis of dried spirulina showed a gross chemical composition (protein, lipids, carbohydrate, ash and fiber) and bioactive compounds (Gallic acid, chlorogenic acid, naringenin.... etc.). For these reasons may be use the spirulina to fortified some food products were suitable for human nutrition.*

Key words: Micro algae extracts, , food products, chemical & biochemical characteristics, nutritional value, spirulina algae.

INTRODUCTION:

Production of blue green microalga *Spirulina platensis*, serves as an alternative approach as feed and food additives due to containing protein, poly

unsaturated fatty acids (linolenic acid), vitamins as well as minerals, pigments and enzymes. Spirulina has several pharmacological activities such as anticancer, antiviral, antibacterial. Potential health effects of *S. platensis* (nutritional and therapeutic applications) identify the fields of interests for future researches. Research results show that using *S. platensis* powder in food products is very practical and functional. Increased demand for healthy food, more global acceptance of fortified foods by *S. platensis*, low production cost and its high nutritional content enhance its application in food industry. (Marzieh *et al.*, 2013).

Spirulina is a nature's gift as super food to mankind. It is a photosynthetic filamentous microalga which has emerged as a potent food supplement because of its rich micro- and macronutrient contents. The body of Spirulina is smooth and with weak cell wall that makes it easily digestible. It is a valuable source of proteins, vitamins, minerals, β - carotene, fatty acids, etc. which makes it perfect as food and fodder. (Mathur, 2018).

Srivastava (2017) focused on adding spirulina as a dietary supplement in cookies, pasta and noodles to fortify the food product with enriching protein, calcium, iron, vitamin B12 phosphorus to enhance the foundation years of a child's life. Spirulina is blue green algae having very good nutritional value. It also has many therapeutic properties, in the same area the *spirulina* produces 20 times more protein than soya, and Spirulina typically contains about 60% protein. It is a good source of vitamin B12, copper and iron.

The researcher added spirulina in specific quantity to food product recipes of cookies, pasta and noodles. For that reasons the aims of this study were to evaluate the chemical, biochemical properties and nutritional value of Spirulina algae powder.

MATERIALS AND METHODS:

Spirulina:

Spirulina platensis was purchased from National Research Centre (NRC), Cairo, Egypt.

Chemical analysis:

The chemical composition of *Spirulina platensis* was determined in the Central Laboratory for Soil, Foods and Feedstuffs (International Accredited Lab, has ISO 17025 since 2012), Faculty of Technology & Development, Zagazig University, Zagazig, Egypt. Chemical analysis was performed according to the International Standard Methods (ISO). Moisture content was determined according to ISO 6496: 1999, crude ash according to ISO 5984:2002, crude

protein according to ISO 5983-1:2002, crude fat and crude fiber were determined according to the methods described in Official Journal of the European Union (EN), 2009, L54/ 37 and 40; respectively Volume 52.

Preparation of sample for amino acid analysis:

One gram of the sample was mixed with 4 mL H₂O and 5 mL of HCl (Note: final concentrate. of HCl is 6 M) and then heated at 100°C for 24 hrs and then filtered. Finally 1 mL of the filtrate was injected to HPLC according to Campanella, *et al.* (2002); Jajić, *et al.* (2013) and Laurens, *et al.* (2012). Analysis was carried out using an Agilent 1260 series.

Determination of vitamins

Ten grams of *Spirulina platensis* powder were homogenized with methanol for extraction of water-soluble vitamins while acetone-chloroform (30:70 v/v) was used for extraction of fat-soluble vitamins. The mixtures were shaken on a vortex mixer for 5 min, centrifuged at 4000 rpm for 5 min and filtered through a Milli pore TM filter (45 µm). The filtrates were evaporated under nitrogen and the residues were re-dissolved in 1 ml water for water soluble vitamins and in 1 ml butanol for fat soluble vitamins that were quantified by HPLC. (Manz and Vuilleumier, 1988) and El-Moataaz *et al.* (2019) analysis was carried out using an Agilent 1260 series. Phenolic compound determined with HPLC according to by (Abd El-Baky *et al.*, (2009) analysis was carried out using an Agilent 1260 series.

Fatty acids composition was determined according to Jubie and Dhanabal, (2012) with GC-MS model 7890B from Agilent Technologies was equipped with MS detector at Central Laboratories Network, National Research Centre, Cairo, Egypt.

Mineral content with exception of copper, nickel, cadmium, mercury and lead. were determined by atomic absorption spectrophotometer (Perkin Elmer 2380, Serial No.13186, USA) using ISO 6869:2000. Phosphorus was determined by spectrophotometer (Manufacturer Labomed, Inc., USA, Model Spetro22, S.N 221101) according to ISO 6491 (1998). Sodium and potassium contents were determined by flame photometer spectroscopy apparatus (CIBA Corning model 410, USA, Serial No., 4887).

RESULTS AND DISCUSSION:

1: Chemical composition of dried Spirulina platensis:

Table (1) shows the chemical composition of spirulina powder (g/100g). From this Table, it could be noticed that moisture content was 12.2; protein

Table (1): Chemical composition of dried *Spirulina platensis* (g/100g)

Components	Values%*
Moisture	12.2
Protein	53.78
Lipid	5.92
Ash content	9.67
Fiber	3.22
Total Carbohydrates**	15.21
Total calories (cal.)	329.24

* Value represents average of three determinations

** Calculated by difference.

content was 53.78%. while the lipid content was 5.92 %. on the other hand, total carbohydrates, ash and fiber content were 15.13,9.67 and 3.32%; respectively. These results are in line with the results obtained by Ortega-caivo *et al.* (1993) and Soni *et al.* (2017).

2: Amino acids profile of dried *Spirulina*:

Data in Table (2) represented the amino acids profile of the dried *Spirulina platensis*. From this Table, it could be observed that the dried powder of *Spirulina platensis* had seven essential amino acids; histidine, lysine, methionine, tryptophan, phenylalanine, isoleucine, leucine and arginine with the amount of 11.9932, 32.0069, 15.2869, 5.9791, 37.9350, 10.7335, 45.8113 and 79.0797 ($\mu\text{g/g}$); respectively. Threonine and valine were not determined in our laboratory because it have not standard for them. These results are in line with the findings of Campanella *et al.* (1999) which stated that the *Spirulina platensis* had eight essential amino acids.

At the same time; nine of non-essential amino acids were presented in dried *Spirulina platensis* powder. These amino acids are aspartic, glutamate, asparagine, serine, glycine, arginine, alanine, tyrosine and cysteine. (Campanella *et al.* 2002) and Sharoba (2014).

3: Fat soluble and water-soluble vitamins profile of dried *Spirulina platensis*:

Table (3) shows the fat soluble and water-soluble vitamins in dried *Spirulina platensis*. Data revealed that the *Spirulina platensis* powder have a moderate amount of vitamin B group such as riboflavin (B2), folic acid, cyanocobalamin (B12). It contained 40.5, 74.62and 282.0 ($\mu\text{g/g}$); respectively. From these data the spirulina considered as a good source of vitamin B12.

Table (2): Amino acids profile of dried *Spirulina platensis* ($\mu\text{g/g}$)

Essential amino acid		Non-Essential amino acid	
Histidine	11.9932	Aspartic	1302.6306
Lysine	32.0069	Glutamic	6662.6633
Methionine	15.2869	Asparagine	4.4996
Tryptophan	5.9791	Serine	29.2218
Phenylalanine	37.9350	Glycine	16.8425
Isoleucine	10.7335	Arginine	79.0797
Leucine	45.8113	Alanine	39.3662
Threonine	ND	Tyrosine	12.9680
Valine	ND	Cysteine	556.9145
		Proline	ND

ND :Not determined

Table (3): Fat soluble and water-soluble vitamins profile of dried

<i>Water-soluble vitamins</i>	
Vitamin B2	40.45
Folic acid	74.62
Vitamin B12	282.06
Vitamin C	1817.42
<i>Fat soluble vitamins</i>	
Vitamin A	N. D
Vitamin D3	N. D
Vitamin E	96.06

Spirulina platensis powder ($\mu\text{g/g}$)

ND :Not determined

Also, results in Table (3) proved that the dried spirulina had a valuable amount of ascorbic acid (vitamin C); it was 1817.42 ($\mu\text{g/g}$). The dried *Spirulina platensis* have vitamin E (Table 3) with amount 96.06 $\mu\text{g/g}$. This vitamin is an antioxidant that can help the body destroy free radicals which cause oxidative stress. Oxidative stress can lead to cell damage and this can result in cancer and other diseases. For that reason's *Spirulina platensis* may help protect the body from a range of health issues. While vitamins A and D3 were not detected. These results were agreement with those obtained by Srivastava (2017) and Shao *et al.* (2019).

4: Phenolic compound in dried *Spirulina platensis* powder:

Bioactive compounds such as phenolic acids and flavonoids are considered as an antioxidant group of compounds. They have antioxidant activity as chelators and free radical scavengers with special impact over hydroxyl and peroxy radicals.

Data in Table (4) shows the phenolic compounds in dried *Spirulina platensis* powder; to study this ability to act as antioxidant. From this Table; there was only five compounds were present. They were gallic acid (75.27 μ g/mg) followed by chlorogenic acid (12.12 μ g/mg), naringenin (1.18 μ g/mg), methyl gallate (0.96 μ g/mg) and caffeic acid (0.58 μ g/mg) in decreasing order. Meanwhile catechins, syringic acid, pyrocatechol, rutin, ellagic acid, coumaric acid, vanillin, ferulic acid, taxifolin, cinnamic acid and kaempferol are not detected in spirulina powder. These results are in agreement with those obtained by Abd El-Baky *et al.* (2009).

Table (4): Phenolic compound in dried *Spirulina platensis* powder (μ g/mg).

Compound phenolic	Concentrate μ g/mg	Compound Phenolic	Concentrate μ g/mg
Gallic acid	75.27	Ellagic acid	N.D
Chlorogenic acid	12.12	Coumaric acid	N.D
Catechin	N.D	Vanillin	N.D
Methyl gallate	0.96	Ferulic acid	N.D
Caffeic acid	0.58	Naringenin	1.18
Syringic acid	N. D	Taxifolin	N.D
Pyro catechol	N. D	Cinnamic acid	N.D
Rutin	N. D	Kaempferol	N.D

N. D: Not Detected

5.The GC-MS analysis of fatty acids of dried *Spirulina* powder:

The application of fatty acids and/or unsaturated fatty acids in medical and dietary area has led to the demand for better methods to extract and purify the lipid from *Spirulina platensis*.

Data in Table (5) shows the fatty acids profile by the GC-MS of *Spirulina platensis* powder (μ g/g). From this table and it can be noticed fifteen fatty acids five of them were a high relative content (%); they were hexadecanoic, palmitic,

Table (5): The GC-MS analysis of fatty acids of dried *Spirulina platensis* powder

Compounds name	Retention time (min)	Relative content (%)
9-Hexadecenoic acid, methyl ester, Z	14.524	0.41
7-Hexadecenoic acid, methyl ester, Z	14.59	2.83
Hexadecanoic acid, methyl ester	14.935	23.53
palmitoleic acid 1tmS	16.747	0.55
Palmitelaidic acid, trimethylsilyl ester	16.85	1.94
Palmitic Acid, TMS derivative	17.219	20.5
.Gamma.-Linolenic acid, methyl ester	17.901	13.86
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.179	13.65
9-Octadecenoic acid (Z)-, methyl ester	18.276	3.48
7-Hexadecenoic acid, methyl ester, (Z)-	18.373	1.61
Octadecanoic acid, methyl ester	18.729	0.93
.Alpha.-Linolenic acid, trimethylsilyl ester	20.052	6.96
9,12-Octadecadienoic acid (Z,Z)-, trimethylsilyl ester	20.415	6.42
Oleic Acid, (Z)-, TMS derivative	20.512	2.28
Stearic acid, TMS derivative	21.037	1.05

gamma linolenic, 9,12 -octadecadienoic, and alpha- linolenic acids; they had a relative content 23.53,20.5,13.86,13.65 and 6.96%; respectively. The retention time varied between 14.9 to 20.4min .The other fatty acids are minor compounds; their relative content (%)ranged between 0.41to 6.42.%.These results were in agreement with the data obtained by Jubie and Dhanabal (2012) and Zheng *et al.* (2012), they reported that the amount of gamma linoleic acid (C18:3),stearic (C18:0) , linoleic (C18:2) , palmatic acid (C16:0) palmitlic acid (C16:1) and oleic acid (C18:1) were the major fatty acids extracted and identified from spirulina micro algae.

6: Minerals content of dried *Spirulina platensis* powder:

Table (6) shows the minerals and trace elements in dried *Spirulina platensis*. Data showed that *Spirulina platensis* contains significant amount of

Table (6): Minerals content of dried *Spirulina platensis* powder.

Minerals	Value g/100g
Potassium (K)	1.44
Sodium (Na)	1.53
Phosphorus (P)	0.75
Iron (Fe)	0.049
<i>Heavy metals (p.p.m)</i>	
Copper (Cu)	9.83
Nickel (Ni)	Nil
Mercury (Hg)	Nil
Cadmium (Cd)	Nil
Lead (Pb)	3.58

sodium, potassium, phosphorus and iron (1.53,1.44,0.75 and 0.049%; respectively). These results are in agreement with those obtained by Campanella *et al.*, (1999); Lu *et al.* (2006); Morsy *et al.* (2014); and Soni *et al.* (2017) who stated that *Spirulina platensis* rich in calcium and iron it containing 922.2 and 273 mg/100g ;respectively.

Heavy metals content (p.p.m) of dried *Spirulina platensis* powder are also illustrated in Table (6). Copper, nickel, cadmium, mercury and lead. nickel, mercury and cadmium were not found in spirulina powder. Meanwhile, copper and lead were noticed in *Spirulina platensis* powder at 9.83 and 3.05 p.p.m., respectively. Johnson and Shubert (1986) revealed high concentration of mercury and lead as well as copper, iron, manganese and zinc. Food and Drug Administration (FDA) recorded that upper limit of lead for adults not more than 90 mg/day while children 5-30mg /day.

Conclusively, the chemical, biochemical and nutritional analysis of dried spirulina showed a gross chemical composition (protein, lipids, carbohydrate, ash and fiber) and bioactive compounds (Gallic acid, chlorogenic acid, naringenin.... *etc.*). For these reasons may be use the spirulina to fortified some food products were suitable for human nutrition.

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دراسات كيميائية وتكنولوجية علي الطحالب المجهرية ومستخلصاتها وإستخدامها في بعض منتجات الاغذية.

١. الخواص الكيميائية والكيميائية الحيوية والغذائية لطحلب الاسبرولينا.

هند محمد فرج محمد - عطية محمد عطية المخزنجي - عبد الجواد محمد محمد الشواف
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هدفت هذه الدراسة الي معرفة الخواص الكيميائية والحيوية والغذائية لمسحوق طحلب الاسبرولينا (*Spirulina platensis*) وقد تبين أنه يحتوي علي بروتين بنسبة ٥٣.٨% ودهون بنسبه ٥.٩٢%، كربوهيدرات كليه، الرماد وألياف خام بنسب ١٥.١٣، ٣.٣٢، ٩.٧٦% علي التوالي. كما احتوي مجفف الطحلب علي أحماض امينية اساسية : الهستيدين ،الليسين ،الميثايونين ، التربتوفان ، الفينايل الانين ، الايزوليوسين وليوسين
كما أظهرت نتائج البحث احتواء مسحوق الطحلب علي كميات ملموسة من الفيتامينات مثل فيتامين B2 ،حامض الفوليك، فيتامين B12 ، فيتامين C ،فيتامين E كما احتوي مسحوق الطحلب علي المواد عديده الفينول والفلافونات التي لها دور مهم كمضادات للأكسدة مثل حمض الجاليك (75.27ميكرو جرام /ملجم) ،حمض الكلوروجينيك (12.12 ميكروجرام /ملجم) ،نارنجين (1.14 ميكروجرام /ملجم)، جالات ميثايل (0.96 ميكروجرام /ملجم) ، وحمض الكافين (0.45 ميكروجرام /ملجم) . كما تم تقدير بعض المعادن الثقيلة لتحديد نسب تواجدها ودرجة الأمان لها و وقد تبين من النتائج عدم احتواء المسحوق المجفف للطحلب علي النيكل ،الكادميوم و الزئبق بينما تواجد النحاس والرصاص بنسب ٩.٨ و ٣.٥٨ جزء في المليون علي التوالي .
التوصية: أظهر التحليل الكيميائي والكيميائي الحيوي والتغذوي للسبيرولينا المجففة تركيباً كيميائياً إجمالياً (البروتين ، الدهون ، الكربوهيدرات ، الرماد والألياف) ومركبات حيوية نشطة مثل(حمض الجاليك ، حمض الكلوروجينيك ، النارنجين ... إلخ). لهذه الأسباب قد يكون استخدام السبيرولينا مناسبة لتعزيز بعض المنتجات الغذائية لتغذية الإنسان.