DRUGS, COSMETICS, FORENSIC SCIENCES

Fatty Acid Composition of *Chlorella* and *Spirulina* Microalgae Species

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Two New Age foods which contain high concentrations of whole food nutrients are the single-celled microalgae *Chlorella* and *Spirulina*. They are accepted as functional foods, which are defined as products derived from natural sources, whose consumption is likely to benefit human health and enhance performance. These foods are used as a supplement/ingredient or as a complete food to enhance the performance and state of the human body, or improve a specific bodily function. Functional foods are used mainly as products to nourish the human body after physical exertion or as a preventive measure against ailments. We determined the fatty acid compositions, particularly polyunsaturated fatty acid compositions, of *Chlorella* and *Spirulina* by capillary column-gas chromatography. The data obtained show that *Spirulina* contains unusually high levels of gamma-linolenic acid, an essential polyunsaturated fatty acid.

Spirulina (a Cyanobacteria, *Arthrospira*) is a multicellular, spiral-shaped plant with a prokaryotic cell structure (class: *Cyanophyceae*, order: *Nostocales*), a property that it shares with bacteria; it grows in brackish or salty water. On the other hand, *Chlorella* is a single-celled, round plant with a eukaryotic cell structure (class: *Chlorophyceae*, order: *Chlorococales*) and a well-defined nucleus; it grows in fresh water (Figure 1). Both plants use light energy to break apart the abundant carbon dioxide and water molecules into carbon food components, releasing oxygen (1–3).

Chlorella and *Spirulina* have been used in a variety of practical applications in biotechnology and medical science. *Chlorella*, also known as a nutraceutical, has been used as an alternative medicine in the Far East since ancient times and is known as a traditional food in the Orient. *Spirulina*, also a nutraceutical, was consumed by the Kanembu people near Lake Chad and the Aztecs in Mexico. The term nutraceutical is defined as "a product produced from foods but sold in pills, powders (potions), and other medicinal forms not generally associated with food and demonstrated to have a physiological benefit or [to] provide protection against chronic disease" (4–6).

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Some cultures use *Chlorella* and *Spirulina* as high-value health foods, nutraceutical, functional foods, or dietary supplements. However, the use and cultivation of *Chlorella* have reached a pinnacle in the Far East (Japan and Taiwan). *Spirulina* use and cultivation in the United States, Far East countries (Japan and Taiwan), Israel, and Mexico is increasing (7–9).

Chlorella and *Spirulina* are considered a potential source of a wide spectrum of nutrients, including chlorophyll, carotenoids, minerals, vitamins, and long-chain polyunsaturated fatty acids (PUFA). PUFA, especially w-3 and w-6 series fatty acids, are pharmacologically significant. These long-chain polyunsaturated fatty acids in microalgae are important in dietetics and therapeutics. They are believed to have a positive effect on cardio-circulatory diseases, atherosclerosis, coronary heart diseases, hyperlipidemia, hypertonia, and cancer. They have been used for prophylatic and therapeutic treatment of chronic inflammations, e.g., rheumatism, skin diseases, and inflammation of the mucosa of the gastrointestinal tract (10–15).

Although several companies produce microalgae, very little is known about the various strains used for commercial production of *Spirulina*. Because the classification system used for *Spirulina* is essentially morphological and based mainly on its spiral shape, it is difficult to differentiate the strains used (16, 17).

The aim of this investigation was to observe and compare the fatty acid composition, especially long-chain w-3 and w-6 fatty acids, in *Chlorella* and *Spirulina* species produced commercially.

Experimental

Sampling

Nine powdered samples of *Chlorella* (*C. pyrenoidosa* and *C. vulgaris*) and *Spirulina* (*S. platensis*, *S. maxima*, and *S. pacifica*) were obtained from commercial sources.

Analysis of Fatty Acid Composition

The standardized modified BF_3-method was used for extraction and methylation of fatty acids in microalgae (18, 19). The samples were prepared by homogenizing and transmethylation, followed by extraction of the methyl esters of fatty acids as a combined single step.

Conditions of Gas–Liquid Chromatography (GLC)

Instrument.—Hewlett-Packard 5890 Series II GC with flame ionization detector; GLC column, SP2560 (column

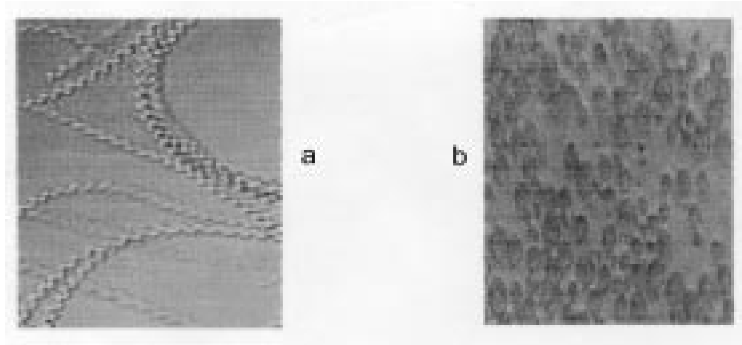

Figure 1. Pictures of microalgae species: (a) *Spirulina*; (b) *Chlorella.*

Table 1. Fatty acid composition of different *Spirulina* species (percent of fatty acids)

Fatty acid	Name	*Spirulina platensis*, bio-organic	*Spirulina platensis*-1	*Spirulina platensis*-2	*Spirulina maxima*	*Spirulina pacifica*, organic
4:0	Butyric	0.12	Nd[a]	Nd	0.57	1.01
6:0	Caproic	0.28	Nd	Nd	0.65	0.95
8:0	Caprylic	3.73	3.72	3.65	4.00	3.81
11:0	Undecanoic	0.58	1.06	1.57	0.68	0.91
11:1	Undecenoic	0.89	1.65	1.36	1.07	1.08
12:0	Lauric	0.49	0.84	1.70	0.52	Nd
12:1	Lauroleic	0.39	Nd	Nd	0.52	Nd
13:0	Tridecanoic	0.86	0.72	Nd	0.95	0.92
14:0	Myristic	0.23	Nd	Nd	Nd	Nd
15:0	Pentadecanoic	1.53	0.70	Nd	1.05	1.10
15:1	Pentadecenoic	3.16	1.26	1.46	3.15	2.90
16:0	Palmitic	46.07	42.30	43.65	35.82	35.34
16:1	Palmitoleic	1.26	1.00	1.50	0.85	1.20
16:2	Hexadecadienoic	3.38	2.43	2.71	4.61	4.06
16:3	Hexadecatrienoic	0.14	Nd	Nd	Nd	Nd
17:0	Margaric	0.27	0.45	Nd	Nd	Nd
17:1	Heptadecenoic	0.27	Nd	Nd	Nd	Nd
18:0	Stearic	1.41	0.95	1.39	1.49	1.47
18:1	Oleic	5.23	1.97	2.05	5.03	4.51
18:2 (w-6)	Linoleic	17.43	16.18	17.19	16.34	16.87
18:3 (w-6)	Gamma linolenic (GLA)	8.87	20.06	21.73	18.16	17.49
18:3 (w-3)	Alpha linolenic (ALA)	Nd	Nd	Nd	Nd	Nd
20:0	Arachidic	Nd	Nd	Nd	Nd	Nd
20:2 (w-6)	Eicosadienoic	0.16	0.94	Nd	0.59	1.01
20:3 (w-8)	Dihomolinolenic	Nd	Nd	Nd	0.66	1.39
20:5 (w-3)	Eicosapentaenoic (EPA)	0.19	Nd	Nd	Nd	Nd
22:1	Docosaenoic	Nd	Nd	Nd	Nd	1.39
22:6 (w-3)	Docosahexaenoic (DHA)	Nd	Nd	Nd	Nd	Nd
23:0	Tricosanoic	Nd	Nd	Nd	Nd	Nd
24:0	Tetracosanoic	0.15	0.90	Nd	0.58	Nd
24:1	Tetracosenoic	Nd	Nd	Nd	0.62	Nd
Others		2.91	2.97	0.04	2.09	2.59

[a] Nd = Not detected.

Table 2. *Spirulina*: Summary of evaluation criteria

Fatty acids[a]	*Spirulina platensis*, bio-organic	*Spirulina platensis*-1	*Spirulina platensis*-2	*Spirulina maxima*	*Spirulina pacifica*, organic
Σ Saturated	55.72	51.64	51.96	46.31	45.51
Σ Short chain (4:0–18:0)	55.57	50.74	51.96	45.73	45.51
Σ Long chain (20:0–24:0)	0.15	0.90	—	0.58	—
Σ Unsaturated	41.37	45.39	48.00	51.60	51.90
Σ Monoens	11.20	5.88	6.37	11.24	11.08
Σ Dienes	20.97	19.45	19.90	21.54	21.94
Σ Trienes	9.01	20.06	21.73	18.82	18.88
EPA	0.19	—	—	—	—
DHA	—	—	—	—	—
Σ Others	2.91	2.97	0.04	2.09	2.59
Linolenic (ALA)	—	—	—	—	—
Linolenic (GLA)	8.87	20.06	21.73	18.16	17.49
Ratio unsat. /sat.	0.74	0.87	0.92	1.11	1.14
Σ PUFA	30.17	39.51	41.63	40.36	40.82
Σ Fat[b]	6.2	8.1	5.1	9.6	6.8

[a] % of fatty acids.
[b] % of material.

length: 100 m; column diameter: 0.25 mm; film thickness: 0.25 µm), cyanopropylpolysiloxane fused silica capillary (FSC) GLC column (Supelco, Bellefonte, PA); flow rates: hydrogen 0.5 mL/min, make-up (He) 35 mL/min; temperatures: injection 220°C, detector 220°C; temperature programming in column, starting temperature, 170°C; final temperature, 220°C; rate of temperature increase, 1°C/min; final time, 10 min; split flow, 50 mL/min; split ratio, 100.0/1; velocity, 15.9 cm/s.

Results and Discussion

Fatty Acids of Spirulina Samples

PUFA can be divided into 2 groups, omega-6 (w-6 or n-6) and omega-3 (w-3 or n-3), which have different physiological functions and effects. The main w-6 PUFAs are linoleic (18:2) and its metabolites, gamma-linolenic acid (GLA) and arachidonic acid (ARA), which are found in vegetable oils.

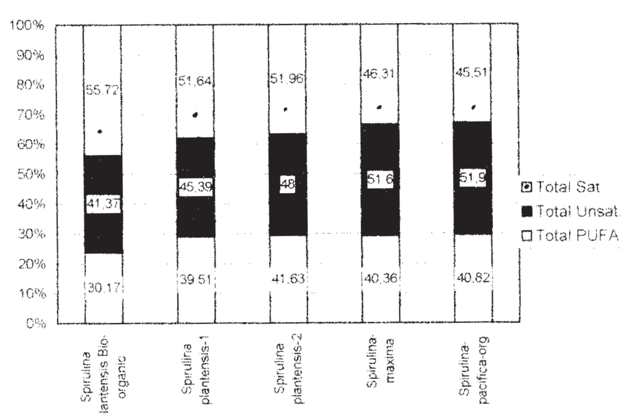

Figure 2. PUFA distribution of *Spirulina*.

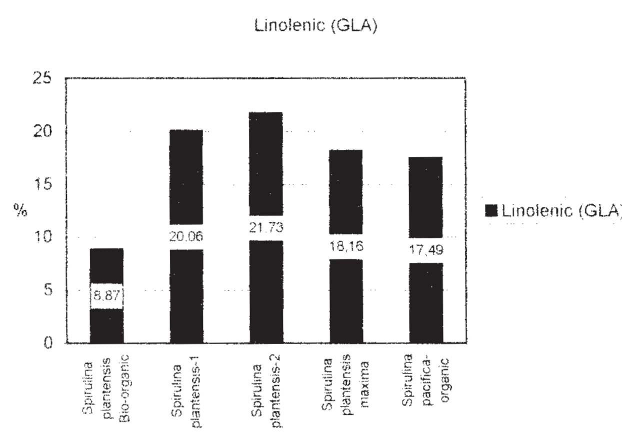

Figure 3. GLA level of *Spirulina*.

Table 3. Fatty acid composition of different *Chlorella* species (percent of fatty acids)

Fatty acid	Name	*Chlorella pyrenoidosa*-1	*Chlorella pyrenoidosa*-2	*Chlorella pyrenoidosa*-3	*Chlorella vulgaris*
4:0	Butyric	0.38	5.88	0.31	0.20
6:0	Caproic	4.91	4.31	1.65	2.77
8:0	Caprylic	3.80	1.85	0.88	0.26
11:0	Undecanoic	1.45	1.19	0.14	1.39
11:1	Undecenoic	2.63	1.90	1.70	2.17
12:0	Lauric	1.44	1.07	1.02	0.87
12:1	Lauroleic	0.45	Nd[a]	0.12	0.41
13:0	Tridecanoic	0.82	0.31	0.15	1.03
14:0	Myristic	0.65	0.58	0.31	0.69
15:0	Pentadecanoic	0.88	0.36	0.12	1.70
15:1	Pentadecenoic	1.79	0.77	0.14	3.53
16:0	Palmitic	14.63	14.60	17.22	14.42
16:1	Palmitoleic	3.70	3.07	3.67	4.04
16:2	Hexadecadienoic	5.44	5.10	2.43	5.34
16:3	Hexadecatrienoic	5.01	3.16	4.45	4.90
17:0	Margaric	0.35	0.51	0.20	0.12
17:1	Heptadecenoic	Nd	Nd	0.19	0.27
18:0	Stearic	1.40	1.93	2.55	1.57
18:1	Oleic	18.05	18.24	19.71	17.62
18:2 (w-6)	Linoleic	12.26	11.24	21.55	11.97
18:3 (w-6)	Gamma linolenic (GLA)	Nd	Nd	0.51	Nd
18:3 (w-3)	Alpha linolenic (ALA)	15.75	15.87	13.81	15.79
20:0	Arachidic	Nd	Nd	Nd	0.14
20:2 (w-6)	Eicosadienoic	Nd	0.71	0.39	Nd
20:3 (w-8)	Dihomolinolenic	Nd	Nd	Nd	Nd
20:5 (w-3)	Eicosapentaenoic (EPA)	Nd	0.40	0.31	Nd
22:1	Docosaenoic	Nd	Nd	Nd	Nd
22:6 (w-3)	Docosahexaenoic (DHA)	Nd	Nd	0.22	0.30
23:0	Tricosanoic	Nd	0.41	0.18	Nd
24:0	Tetracosanoic	Nd	Nd	Nd	0.22
24:1	Tetracosenoic	0.39	Nd	0.38	Nd
Others		3.82	6.54	5.69	8.28

[a] Nd = Not detected.

The main w-3 PUFAs are alpha-linolenic (ALA) and its metabolites, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are found in green leafy vegetables and in the fat of oily fish such as herring, sardines, and tuna. Several companies have recently developed applications of the products for commercial processing of the raw materials (19–21).

The data on fatty acid composition of 5 different *Spirulina* samples is shown in Table 1. The SP-2560 FSC column gave clear and excellent separation of long-chain PUFAs from others. About 30 fatty acids were found in *Spirulina* samples, about 10 of which were major components; the remainder were minor and occurred in small or trace quantities. In the *Spirulina* samples, palmitic acid (16:0) was normally dominant (35–46%), but mono-, di-, and tri-unsaturated fatty acids in the C_{18} series were all significant. These samples also contained quite high caprylic (8:0), palmitoleic (16:1), and hexadecadienoic (16:2) acid contents, 3.65–4.00, 0.85–1.50, and 2.43–4.61%, respectively. Total fatty acid composition was slightly less or higher than those reported in the literature. Previous studies found that the fatty acid composition of algae

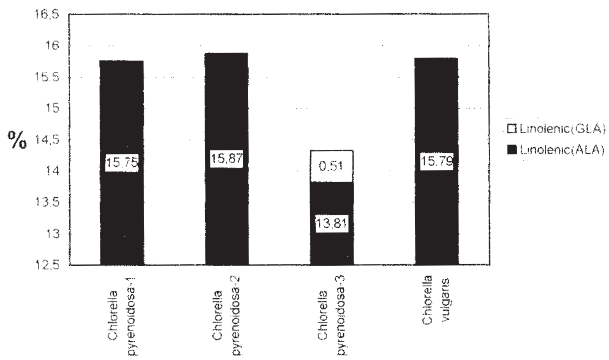

Figure 4. ALA level of *Chlorella*.

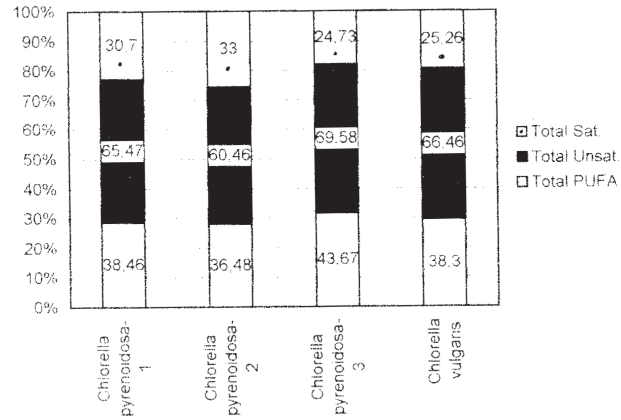

Figure 5. PUFA distribution of *Chlorella*.

is greatly influenced by many factors, including temperature, light intensity, and growth stage, the latter usually being correlated with nutrient availability (22–25).

Although high proportions of GLA were found, no ALA was found in these samples. This observation on GLA was in general agreement with the values reported by others (26–28). Very few PUFAs were detected in some samples. EPA was present only in organic *Spirulina platensis*, and dihomo linolenic acid (20:3 w-8) only in *Spirulina maxima* and organic *Spirulina pacifica*. No traces of DHA and ARA (20:4 w-6) were found in any *Spirulina* samples.

Evaluation Criteria for Spirulina Samples

Table 2 summarizes the data of evaluation criteria that indicate the need for future research (Figure 2). There is a balanced ratio of unsaturated and saturated fatty acids. Most saturated fatty acids were short-chain fatty acids. On the other hand, the 5 *Spirulina* samples had a high PUFA content (30–42%), represented by linoleic acid (18:2 w-6) and GLA, except for organic *Spirulina*, which contained less GLA (Figure 3). GLA, which is a commercially important PUFA, is effective in lowering plasma cholesterol and in stimulating prostaglandins, and has been used as a dietary supplement for

Table 4. *Chlorella*: Summary of evaluation criteria

Fatty acids[a]	*Chlorella pyrenoidosa*-1	*Chlorella pyrenoidosa*-2	*Chlorella pyrenoidosa*-3	*Chlorella vulgaris*
Σ Saturated	30.70	33.00	24.73	25.26
Σ Short chain (4:0–18:0)	30.70	32.59	24.55	24.90
Σ Long chain (20:0–24:0)	—	0.41	0.18	0.36
Σ Unsaturated	65.47	60.46	69.58	66.46
Σ Monodiens	27.01	23.98	25.91	28.04
Σ Dienes	17.70	17.05	24.37	17.31
Σ Trienes	20.76	19.03	18.77	20.69
EPA	—	0.40	0.31	—
DHA	—	—	0.22	0.30
Σ Others	3.82	6.54	5.69	8.28
Linolenic				
(ALA)	15.75	15.87	13.81	15.79
(GLA)	—	—	0.51	—
Ratio unsat./sat.	2.13	1.83	2.81	2.63
Σ PUFA	38.46	36.48	43.67	38.30
Σ Fat[b]	12.5	11.8	11.4	13.3

[a] % of fatty acids.
[b] % of material.

the treatment of various chronic health problems, e.g., arthritis, heart disease, obesity, and zinc deficiency (29–31).

Fatty Acids of Chlorella Samples

Table 3 lists the fatty acid composition of 4 different *Chlorella* samples. All samples displayed almost similar characteristics, with some exceptions. Although no GLA was found in *Chlorella* samples (except *Chlorella pyrenoidosa*-3, in small amounts), they contained high proportions of ALA. On the other hand, oleic acid (18:1) was the most dominating fatty acid in 3 *Chlorella* samples (linoleic acid in *C. pyrenoidosa*-3 sample). This observation on ALA was in general agreement with the values reported by others (13, 16, 32, 33).

The 4 *Chlorella* samples contained a large amount of PUFA (36–43%), represented by linoleic acid and ALA. *C. pyrenoidosa*-3 sample had the highest level of ALA (21.55%; Figure 4). Other PUFAs, e.g., DHA and eicosadienoic acid (20:2 w-6) were present only in small or trace amounts. No trace of dihomo linolenic acid (20:3 w-8) was found in any *Chlorella* samples.

Evaluation Criteria for Chlorella Samples

Table 4 summarizes the evaluation criteria of *Chlorella* samples shown in Figure 5. There is an unbalanced ratio of unsaturated and saturated fatty acids. The ratio for *Chlorella* samples ranged between 1.83 and 2.81. Most of saturated fatty acids were palmitic acid and short-chain fatty acids (24.73–33.00%).

Spirulina versus Chlorella

Spirulina and *Chlorella* displayed different characteristics in fatty acid composition. Total amounts of PUFA in *Spirulina* and *Chlorella* were almost similar, but the total level of unsaturated fatty acids in *Chlorella* was higher than that in *Spirulina*. Although no GLA was found in *Chlorella* samples, high amounts of ALA were found. In contrast, although no ALA was found in *Spirulina*, these samples contained high amounts of GLA. Oleic and hexadecatrienoic acids (16:3) in *Chlorella* were higher than those in *Spirulina*. Other PUFAs in *Spirulina* and *Chlorella* were found in small or trace amounts.

Conclusions

PUFAs are recognized as an essential component in human nutrition, and some edible microalgae, e.g., *Spirulina* and *Chlorella*, may play an important role in meeting nutritional requirements for PUFAs. High levels of these products exert pharmacological effects, and may be of value in the management of certain diseases.

The variability of the species and growing factors of *Spirulina* and *Chlorella* samples analyzed in this study obscures any relationships between fatty acid composition, species-specific factors, and environmental parameters. Although continuous monitoring of commercial products is difficult, it would aid in determining the quality of commercial final products available to consumers at the market.

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