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Chlorella and *Spirulina* dietary supplements as sources of biologically active lipids

V. Panayotova*, A. Merdzhanova, D. A. Dobreva, K. Peycheva, T. Stoycheva

Department of Chemistry, Faculty of Pharmacy, Medical University - Varna, 84 Tzar Osvoboditel Blvd., 9000 Varna, Bulgaria

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Increasing the knowledge about the relationship between the lipid quality of foods and health benefits suggests the need to utilize sustainable alternative food resources that impact human health beyond basic nutrition. Aquatic organisms, such as microalgae, are promising sources of bioactive compounds. Freshwater microalgae, namely *Spirulina spp* and *Chlorella spp*, contain a variety of functional compounds, such as polyunsaturated fatty acids (PUFAs), carotenoids and vitamins with antioxidant, antihypertensive and cardiovascular protective effects. In recent years, these species have been largely promoted as dietary supplements in Bulgaria.

The aim of the present study was to analyse total lipids, lipid classes, fatty acids, carotenoids, total cholesterol and fat-soluble vitamins (α-tocopherol and ergocalciferol) contents in dietary supplements (*Spirulina platensis* and *Chlorella vulgaris* powders) available on the Bulgarian market. Lipids were extracted according to Bligh and Dyer method and separated into neutral lipids, glycolipids and phospholipids by column chromatography. Cholesterol, carotenoids and fat-soluble vitamins were simultaneously analysed by HPLC/UV/FL. Total lipids accounted 3.8 g/100 g dry Spirulina powder and 5.1 g/100 g dry Chlorella powder. Glycolipids were the main lipid class in Spirulina (over 69% of TL), while neutral lipids predominated in Chlorella (over 58% of TL). In both species polyunsaturated fatty acid presented 50% of total lipids, whereas cholesterol showed a significantly low content – up to 7.3 mg/100 g dw. The results showed that Spirulina and Chlorella powders available in Bulgaria contain high amounts of unsaturated fatty acids and astaxanthin (67.93 – 127.56 μg/100 g dw) and low cholesterol levels which could confirm the high nutritional values of both species.

Keywords: microalgae, fatty acids, fat-soluble vitamins, carotenoids

INTRODUCTION

Microalgae are photosynthetic eukaryotic organisms rich in functional nutrients [1-3] used to provide nourishment to humans and animals since ancient times. In the early 1940s microalgae emerged as attractive and promising alternative lipid, protein, pigment, and polymer sources [4]. Spirulina, or correctly *Arthrospira* spp., and Chlorella are the two most important algal groups that deserve a thorough exploration for nutritional value. Spirulina and Chlorella are a renewable and sustainable source of high-value bioactive compounds. They are well-known as high-protein sources (55–60% of dry weight) with all essential amino acids and a high digestibility coefficient [2, 3, 5]. They are also a rich source of lipids (e.g., n-3 polyunsaturated fatty acids (PUFA), vitamins (B group, ascorbic acid, A, D, E, K), functional components (e.g., chlorophylls, which have antioxidant activity), as well as minerals (Ca, Cr, Cu, Fe, K, Na, P, Se, Zn) [6]. Lipid nutritional value of Spirulina and Chlorella is comparable to that of fish oil, which makes them alternative sources of n-3 fatty acids [7]. The interest in n-3 PUFA as health-promoting nutrients has expanded dramatically in recent years. During the process of photosynthesis, some microalgae can accumulate large amounts of lipids in their cells [8]. Considering their beneficial chemical composition, microalgae are increasingly utilized as food additives, functional foods and dietary supplements.

The aim of the present study was to analyse total lipids, lipid classes, fatty acids, carotenoids, total cholesterol and fat-soluble vitamins (α-tocopherol and ergocalciferol) contents in dietary supplements (*Spirulina platensis* and *Chlorella vulgaris* powders) available on the Bulgarian market.

MATERIALS AND METHODS

Spirulina platensis and *Chlorella vulgaris* dietary supplements were purchased from health-food stores in Varna, Bulgaria. The samples were stored in plastic bags and kept refrigerated (4 °C) until analysis.

Lipid classes separation and fatty acids analysis

Total lipids were extracted according to Bligh and Dyer method [9] and were separated into neutral lipids, glycolipids and phospholipids by column chromatography using a glass column (10 × 200 mm) packed with silica gel 60 [10]. Individual lipid fractions were transmethylated using 2% H_2SO_4 in anhydrous methanol [11]. Analysis of fatty acids

* To whom all correspondence should be sent:
E-mail: veselina.ivanova@hotmail.com

methyl esters (FAME) was carried out using a Thermo Scientific FOCUS GC gas chromatograph equipped with a 30 m × 0.25 mm × 0.25 µm TR-5 MS capillary column and Polaris Q mass detector. Helium was used as a carrier gas at a flow rate of 1 ml/min and a temperature program – initial oven temperature of 40°C for 4 min, followed by a rate of 20°C/min from 40°C to 150°C, raised from 150°C to 235°C at a rate of 5°C/min, and next from 235°C to 280°C a rate of 10°C/min for 5 min. Corresponding peaks were identified based on the comparison of the retention times with the authentic standards (Supelco 37 Component FAME Mix and PUFA №3 from Menhaden oil). The results are expressed as the weight percent of an individual fatty acid to the total fatty acid (TFA) content.

Carotenoids, fat-soluble vitamins and total cholesterol analysis

Fat-soluble vitamins (A, D_3, E), astaxanthin, β-carotene and cholesterol were extracted and analysed by the method of Dobreva *et al*. [12].

RESULTS AND DISCUSSION

The results for the fatty acid profiles of total lipids (TL), neutral lipids (NL), glycolipids (GL) and phospholipids (PL) in Spirulina and Chlorella supplements are presented in Tables 1 and 2, respectively. Total lipid contents were 3.8 g/100 g in Spirulina powder and 5.1 g/100 g in Chlorella powder which is lower than data reported in the scientific literature [13, 14]. The distribution of the fatty acid groups differs between microalgae species and among the lipid subclasses. In Spirulina the most abundant fatty acid group was the PUFA group in TL and GL, and SFA in NL and PL. In Chlorella supplements PUFA was the most abundant group in TL and the lipid classes, except PL. The analysis of FAME showed that the major fatty acid in Spirulina was palmitic acid (C16:0) followed by γ-linolenic (GLA, C18:3 n-6), comprising together about 75% of the FAME in the TL fraction. The third main fatty acids in Spirulina lipids were palmitoleic (C16:1) and linoleic acid (LA, C18:2 n-6). Fatty acid profile of Spirulina is consistent with the literature data [3, 13].

Table 1. Fatty acids (as relative %) composition of TL, NL and PL in Spirulina

FA, % of TFA	TL	NL	GL	PL
C 8:0	tr	nd	tr	nd
C 10:0	tr	tr	tr	tr
C 12:0	tr	0.27±0.02	0.22±0.02	nd
C 14:0	1.39±0.15	0.48±0.03	1.06±0.05	1.73±0.20
C 16:0	35.47±1.67	27.54±0.85	31.17±2.04	42.57±2.50
C 17:0	tr	0.45±0.03	tr	0.20±0.01
C 18:0	2.69±0.05	11.65±0.64	2.81±0.30	12.14±0.55
C 20:0	tr	0.11±0.01	tr	0.23±0.01
C 21:0	nd	0.10±0.02	nd	nd
C 22:0	tr	3.43±0.10	0.31±0.02	5.42±0.65
∑SFA	39.55	44.03	35.57	62.29
C 14:1	tr	0.41±0.03	nd	0.35±0.03
C 16:1	8.50±0.73	4.25±0.40	9.48±0.69	5.95±0.60
C 17:1	nd	0.45±0.02	0.26±0.02	0.29±0.01
C 18:1 n-9	3.50±0.20	12.67±0.75	4.21±0.34	3.21±0.50
C 24:1	tr	nd	nd	nd
∑MUFA	12.00	17.78	13.95	9.80
γ-C 18:3 n-6	39.49±2.30	20.80±1.55	37.53±2.40	16.39±0.80
C 18:2 n-6	5.80±0.55	8.10±0.68	7.55±0.80	4.66±0.23
α-C 18:3 n-3	0.50±0.02	0.82±0.03	0.10±0.01	tr
C 20:4 n-6	0.65±0.03	nd	0.20±0.01	nd
C 20:3 n-6	0.92±0.01	3.92±0.30	1.56±0.40	nd
C 20:2	tr	nd	0.18±0.02	nd
C 20:3 n-3	0.10±0.01	nd	0.20±0.01	nd
C 22:2	0.27±0.02	4.48±0.29	2.98±0.34	6.74±0.45
∑PUFA	47.73	38.12	50.30	27.79
∑n-3	0.60	0.82	0.30	0.09
∑n-6	46.86	32.82	46.64	21.05
PUFA/SFA	1.20	0.86	1.41	0.45

Results represent mean values ± standard deviation (n = 3); SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; TFA – total fatty acids; TL – total lipids; NL – neutral lipids; GL – glycolipids; PL – phospholipids; nd—not detected; tr—trace levels were <0.1% of TFA.

Table 2. Fatty acids (as relative %) composition of TL, NL and PL in Chlorella

FA, % of TFA	TL	NL	GL	PL
C 8:0	tr	tr	tr	0.25±0.02
C 10:0	tr	0.26±0.02	tr	0.28±0.03
C 12:0	tr	tr	0.11±0.02	0.33±0.01
C 14:0	0.25±0.02	0.25±0.03	0.51±0.04	0.75±0.05
C 16:0	28.09±0.54	27.78±1.38	37.28±2.42	32.91±2.10
C 17:0	tr	0.10±0.02	0.15±0.02	0.94±0.32
C 18:0	5.12±0.07	3.13±0.30	1.86±0.04	6.87±0.56
C 20:0	tr	tr	tr	0.43±0.02
C 21:0	nd	nd	0.02±0.01	nd
C 22:0	0.48±0.03	0.38±0.04	0.16±0.01	2.32±0.30
C 24:0	tr	nd	0.26±0.02	0.70±0.04
∑SFA	33.94	31.90	40.35	45.78
C 14:1	tr	0.10±0.02	0.19±0.03	1.31±0.20
C 16:1	5.26±0.21	2.85±0.35	7.72±0.34	3.74±0.40
C 17:1	nd	0.10±0.02	tr	tr
C 18:1 n-9	16.21±0.67	20.29±1.39	10.31±0.45	11.81±0.60
C 20:1	nd	nd	tr	nd
C 24:1	0.13±0.02	nd	0.13±0.01	nd
∑MUFA	21.60	23.34	18.35	16.86
C 18:3 n-6	1.21±0.03	1.74±0.20	2.59±0.15	nd
C 18:2 n-6	9.90±0.43	5.65±0.37	3.47±0.13	2.85±0.40
C 18:3 n-3	30.48±2.54	32.26±2.10	18.71±1.20	23.57±0.95
C 20:5 n-3	nd	nd	2.52±0.06	nd
C 20:4 n-6	0.55±0.02	0.50±0.03	6.68±0.37	3.66±0.30
C 20:3 n-6	0.62±0.03	3.50±0.15	4.18±0.55	7.28±0.64
C 20:3 n-3	tr	nd	0.38±0.03	nd
C 22:2	0.40±0.02	0.94±0.05	2.58±0.15	nd
∑PUFA	43.16	44.59	41.11	37.36
∑n-3	37.56	32.26	21.61	23.57
∑n-6	12.68	11.39	19.50	13.79
PUFA/SFA	1.55	1.53	1.01	0.75

Results represent mean values ± standard deviation (n = 3); SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; TFA – total fatty acids; TL – total lipids; NL – neutral lipids; GL – glycolipids; PL – phospholipids; nd—not detected; tr—trace levels <0.1% of TFA.

Table 3. Fat-soluble vitamins, carotenoids and cholesterol contents in Spirulina and Chlorella dietary supplements

	Spirulina	Chlorella
Astaxanthin, µg/100 g dw	127.56 ± 11.16	67.93 ± 7.16
β–Carotene, mg/100 g dw	18.80 ± 2.4	5.73 ± 0.44
Ergocalciferol, mg/100 g dw	0.30 ± 0.07	0.31 ± 0.02
α-Tocopherol, mg/100 g dw	1.03 ± 0.09	1.30 ± 0.07
Cholesterol, mg/100 g dw	7.31 ± 0.81	3.64 ± 0.74

Results represent mean values ± standard deviation (n = 3)

Previous studies have reported predominance of PUFAs (in particular C18:3 n-3) over SFA (in particular C16:0) grown under autotrophic conditions [14,15]. A striking feature of the Spirulina lipids was the relatively high level of γ-linolenic acid, while of the Chlorella lipids – α-linolenic acid. Other authors reported the presence of the long chain PUFAs arachidonic acid (ARA, C20:4 n-6), eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3) in Spirulina and Chlorella tissues [16,17], but EPA and DHA were not detected in our study.

Fatty acids patterns of NL and PL lipids of Spirulina did not differ significantly from each other with palmitic acid (27.54 – 42.57%) being the most abundant fatty acid. The GL fraction showed fatty acid profile similar to the TL. In Chlorella, NL fraction showed similar fatty acid profile as the TL, while GL – similar to PL, wherein palmitic acid was the main fatty acid.

The PUFA/SFA ratio was considerably higher in Chlorella supplements, in particular in the NL fraction. Spirulina had the highest PUFA/SFA ratio in the GL fraction than in the corresponding neutral fraction.

Fatty acid profiles of Spirulina and Chlorella supplements evince the lipids as alternative sources of the nutritionally essential fatty acids – γ-linolenic acid and α-linolenic acid. Besides being an energy source and a metabolic precursor, γ-linoleic acid also exhibits anti-inflammatory, antioxidant, and anti-allergic properties [18, 19].

The results for the fat-soluble vitamins, cholesterol and carotenoids contents in Spirulina and Chlorella supplements are presented in Table 3. Astaxanthin is a xanthophyll carotenoid naturally synthesized by algae. Its antioxidant activity is 10 times higher than β-carotene and 100 times higher than α-tocopherol. Higher amounts of astaxanthin were detected in Spirulina supplements (127.56 μg/100 g dw). β-carotene is the primary plant-based source of dietary vitamin A. However, β-carotene is only partially able to ensure an optimal total vitamin A intake. In our study, 3 times higher amounts of β-carotene were found in Spirulina (18.80 mg/100 g dw) supplements compared to Chlorella supplements (5.73 mg/100 g dw). According to previous studies, Spirulina exhibited high vitamin A equivalency ratios for β-carotene to vitamin A conversion – from 2.3:1 to 7:1 [20, 21]. α-tocopherol, the most bioactive form of vitamin E, was detected in higher amounts in Chlorella supplements – 1.3 mg/100 g dw. The main role of vitamin E is as an antioxidant, scavenging free radicals that can damage living cells. Total cholesterol content was higher in Spirulina supplements – 3.64 and 7.31 mg/100 g dw, respectively. Although recent evidences suggest that dietary cholesterol does not significantly increase low-density lipoprotein cholesterol [22], consumers are advised to follow healthy dietary patterns, which are inherently low in cholesterol [23].

CONCLUSIONS

The results showed that Spirulina and Chlorella dietary supplements available in Bulgaria contain high amounts of unsaturated fatty acids, astaxanthin, β-carotene and low cholesterol levels which could confirm the high nutritional value of both microalgae species. From this study, it is safe to conclude that Spirulina and Chlorella can be good sources of these nutritionally essential components and used as food additives, functional foods and dietary supplements.

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