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Fatty Acid Profile of *Spirulina platensis* Used as a Food Supplement

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Abstract

The commercially produced multicellular microalgae, *Spirulina platensis*, is widely consumed by humans in the Aegean area of Turkey as a food additive or a whole food. The fatty acid profiles of six commercial tablets produced from *S. platensis* in Turkey and one from China were determined. The samples contained 33.68-66.75% saturated fatty acids (SFA) and 28.20-47.78% polyunsaturated fatty acids (n-3 and n-6 PUFA). *Spirulina platensis* is a rich source of gamma linolenic acid (GLA), which accounted for 4.07-22.51% of the fatty acids. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were found in only two samples where they accounted for 1.79 and 7.70%, and 2.28 and 2.88%, respectively. There was a significant (p<0.05) variation among samples in total SFA, monosaturated fatty acids (MUFA), PUFA, GLA, total unsaturated fatty acids (UFA), and ratio of n-3 to n-6 PUFA. The *S. platensis* samples from Turkey were classified by fatty acid profile using two multivariate statistical methods, Principal Component Analysis and Hierarchical Cluster Analysis. Clustering produced defined groups according to production site.

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Introduction

Production of *Spirulina* species, especially *S. platensis* (Parachas strain), is rapidly increasing in the Aegean region of Turkey. Commercially produced *S. platensis* is widely consumed by humans as a food additive or whole food. *Spirulina* sp. are a photosynthetic, filamentous, spiral-shaped, multicellular, blue-green microalgae. The two most important species in culture are *S. maxima* and *S. platensis*. *Spirulina* is an excellent food, lacking toxicity and having protective properties against human viral diseases, anemia, tumors, and malnutrition (Mazo et al., 2004; Sanchez et al., 2006). Preclinical testing suggests *Spirulina* has hypocholesterolemic, immunological, antiviral, and antimutagenic properties (Chamorro et al., 1996). It is also used as a food additive to improve coloration in ornamental fish (James et al., 2006) and as a probiotic agent (Ramakrishnan et al., 2008).

Spirulina species, known as cyanobacteria, can be produced in simple pilot plants or industrial installations (Sanchez et al., 2006). They contain the essential fatty acids, linoleic acid (LA, 18:2 delta-9,12) and gamma-linolenic acid (GLA, 18:3 delta-6,9,12; Cifferi, 1983; Cohen, 1997; Otles and Pire, 2001; Tokusoglu and Unal, 2003; Gupta et al., 2007), high quality proteins, carbohydrates, vitamins (B1, B2, tocopherols), minerals (sodium, potassium, calcium, magnesium, phosphorus, iron), carotenes (especially beta-carotene), chlorophyll *a*, phycocyanin, and some phenolic acids (Miranda et al., 1998; Koru and Cirik, 2002; Tokusoglu and Unal, 2003; Mazo et al., 2004; Sanchez et al., 2006). N-3 polyunsaturated fatty acids (PUFA), particularly linolenic acid which is a precursor for prostoglandin, eicosapentaenoic acid (EPA, 20:5 n-3), and docosahexaenoic acid (DHA, 22:6 n-3), exert beneficial effects on human health by reducing the risk of cardiovascular diseases in clinical and epidemiological studies (Ackman, 1988; Chamorro et al., 1996; Leaf and Weber, 1998; Tokusoglu and Unal, 2003; Sanchez et al., 2006). The beneficial effects have been attributed to an increased ratio of n-3 to n-6 PUFA in human blood lipids and cell membrane lipids. Moreover, DHA has a therapeutic effect on human physiology (Ackman, 1988; Leaf and Weber, 1998). EPA and DHA are found in green leafy vegetables and especially in oily fish such as herring and sardines. N-6 PUFA and n-3 PUFA have different physiological functions and effects. The main n-6 PUFA are LA and its metabolites, GLA and arachidonic acid (ARA), which are found in some vegetable seed oils. Relative to other biosources, *Spirulina* is especially rich in GLA, claimed have to positive medical properties.

The objective of this study was to compare fatty acid profiles, especially n-3 and n-6 PUFA, in *Spirulina* (*Arthrospira*) *platensis* samples from different commercial sources.

Materials and Methods

Spirulina and processing of biomass. The microalgal strain *S. platensis* (Parachas) was obtained from the Plankton Research Laboratory Culture Collection of the Department of Aquaculture of the Fisheries Faculty, University of Ege (Bornova, Izmir, Turkey). Cultures were grown in flasks of various sizes and transferred to large transparent polyethylene bags for upscaling at 35±1°C. The cells were grown in Zarrouk's medium described by Fox (1983) and Dalay et al. (2001). Continuous illumination was provided by cool white fluorescent lamps with a mean light intensity of 150 μmol photon/m^2/s. Light was measured on the surface of the bioreactors with a Li-Cor quantum sensor (model Li-250). When a sufficient volume of *Spirulina* was cultured, the cells were moved to outdoor raceway ponds maintained at ambient temperature (33±1°C), a light density of 7000 lux, and pH of 9.0-10.0, by adding CO_2 to media (Fox, 1983). Fresh *S. platensis* was dried at 60±3°C for 1 h and the thoroughly dried samples were milled. The algae powder was formed into tablets with a press. No chemicals were added.

Five of six algae companies in Turkey are the major producers of *Spirulina* tablets used as food supplements. Their products were selected for analysis together with the *Spirulina* from Ege University and another imported from China. Each sample was examined three times over two months and kept no more than three days at 4°C until analyzed.

Determination of fatty acid composition. About 150 g tablets were sampled from a lot of each brand and ground to powder. About 10 g of the powdered algae samples was immediately extracted, in duplicate, and dissolved in 150 ml hexane at room temperature for 4 h. The hexane was removed with a rotary evaporator (40°C) and the residue was stored in the dark at -10°C until the fatty acid fraction could be determined. During the extraction and evaporation steps, the sample and oil were kept away from light (Qiu et al., 2002; Ozgul-Yucel, 2005; Gupta et al., 2007).

Fatty acid compositions were determined by a standard capillary gas choromatographic method (GC; IUPAC, 1987; Diraman and Hisil, 2004). Analysis was carried out on a GC (HP 6890) using a 30-m capillary column (DB-23; J &W Scientific, Folsom, USA) with an internal diameter of 0.25 mm and a 0.25-μm film thickness of 50% cyanopropyl. The GC conditions were: initial temperature 100°C, 100-175°C at 5°C/min, 175-210°C at 10°C/min, and 210°C for 15 min. The injector and flame ionization dedector (FID) were set at 250°C. The carrier gas was helium at 0.5 ml/min, split ratio was 100:1, hydrogen flow was 30 ml/min, make-up flow (nitrogen) was 24.5 ml/min, and dry air flow was 300 ml/min. Each sample was injected in triplicate (n = 3). Fatty acid standards had linear calibration curves through the origin ($R^2 = 0.99$). The GC method was validated for fatty acid determination of algae samples within the 95% confidence limits. Results were calculated using the HP 3365 Chemistation program and recorded as % peak area.

Chemicals. All standards and reagents were of 99.9% purity and choromatographic grade (Merck, Darmstadt, Germany). Identification of the fatty acid methyl esters (FAME) of the samples was performed by comparison to standard FAME (Sigma-Aldrich Chemicals 189-19, Diesenhofen, Germany).

Chemometric analysis. The samples were characterized and classified using two chemometric methods, Principal Component Analysis (PCA, Ward Method) and Hierarchical Cluster Analysis (HCA, Euclidian Distance). Multivariate analysis was performed using Matlab 7.5.0 (Mathworks, 2007).

Statistical analysis. Duncan's Multiple Range Test was applied when variance analysis indicated significant differences in mean values. Statistical analysis was performed using the SPSS 10 statistical software (SPSS, 2001).

Results

Fatty acids in the *Spirulina platensis* samples are given in Table 1. Most of the samples contained short (4:0-8:0) and long (10:0-18:0) chain saturated fatty acids (SFA) but very-long chain SFA (20:0-24:0) were minor constituents. The samples contained considerable levels of n-3 and n-6 polyunsaturated fatty acids (PUFA), including LA and GLA. Five samples contained stearidonic acid (SDA) or moroctic acid (18:4 n-3), biosynthesized from alpha linolenic acid (ALA), and three samples contained arachidonic acid (ARA, 20:4 n-6), an essential fatty acid required by most mammals. EPA, DHA, and docosapentaenoic acid (DPA) were found in only two samples. The imported sample was similar to the domestic samples in terms of palmitic acid (PA) and LA but contained no n-3 PUFA. The DB column produced clear and excellent separations of all short and long chain fatty acids (Fig. 1).

PCA showed that the samples from China and Menderes were characterized by ARA, eicosadienoic acid (EDA), SDA, EPA, homogamma linoleic acid (DGLA), total n-3 value, and n-3/n-6 ratio (Fig. 2). The Bozdogan-2 sample was characterized by tridecanoic acid (TDA), elaidic acid (EA), arachidic acid (AA), eicosatrienoic acid (ESA), trans-palmitoleic acid (*t*POA), *trans* fatty acids (TFA), myristo oleic acid (MOA), and PUFA. Bozdogan-1 was characterized by very long SFA, n-6, and margaric acid (MG) while the sample from the Fisheries Faculty was characterized by long SFA, GLA, and PA. Variance levels were 39.56% and 65.05% for PC1 and PC2, respectively.

The samples were arranged in three groups according to fatty acid profile on the dendogram produced by Hierarchical Cluster Analysis (Fig. 3).

Table 1. Fatty acid profiles (% of total lipids) of *Spirulina platensis* from different origins (n = 3).

	Sample						
Fatty acid	*Faculty of Fisheries (FCT)*	*Bozdogan-1 Aydin (BZD-1)*	*Seferihisar Izmir (SH)*	*Imported from China (China)*	*Bozdoga-2 Aydin (BZD-2)*	*EBILTEM Izmir (EBT)*	*Menderes Izmir (MND)*
Oil content (%)	7.45±0.04	5.30±0.03	7.15±0.05	4.10±0.04	5.75±0.04	6.04±0.02	4.90±0.05
Short chain saturated fatty acids (SFA)							
4:0 BA*	nd	nd	1.81±0.07	nd	nd	nd	nd
6:0 CA	1.20±0.12	nd	1.62±0.33	6.21±0.23	4.78±2.65	15.18±1.65	6.32±2.23
8:0 CAPY	0.49±0.13	0.27±0.03	1.01±0.05	10.42±0.52	4.68±1.68	15.90±1.86	5.15±0.11
Total short chain	1.69±0.10^{b}	0.27±0.08^{a}	4.44±0.03^{c}	16.63±0.33^{f}	9.46±2.15^{d}	31.08±1.70^{g}	11.36±1.48^{de}
Long chain SFA							
11:0 UDA	1.41±0.22	0.85±0.10	0.86±0.10	2.06±0.50	1.63±0.75	3.35±0.88	2.28±0.73
12:0 LAU	0.22±0.07	nd	0.37±0.05	1.52±0.50	0.71±0.04	0.95±0.07	0.40±0.05
13:0 TDA	nd	6.88±2.19	nd	nd	nd	nd	nd
14:0 MA	1.62±0.08	0.24±0.06	1.74±0.05	nd	0.19±0.02	0.52±0.02	nd
15:0 PDA	0.66±0.05	nd	0.38±0.03	1.40±0.07	0.54±0.06	0.69±0.30	0.41±0.05
16:0 PA	18.02±0.78	38.56±2.10	24.50±0.52	30.13±2.48	33.61±1.54	26.45±1.72	37.01±3.96
17:0 MG	1.63±0.21	0.25±0.02	0.37±0.04	0.58±0.05	0.51±0.21	nd	1.24±0.08
18.0 SA	7.60±0.56	2.33±0.68	5.32±0.37	6.42±0.40	5.15±1.18	3.71±1.02	3.16±0.68
Total long chain	31.13±0.56^{a}	48.91±1.09^{d}	33.54±0.40^{ab}	42.11±1.40^{bc}	42.34±1.66^{bc}	35.67±1.50^{b}	44.50±2.16^{c}
Very long chain SFA							
20:0 AA	0.73±0.05	0.75±0.10	nd	nd	nd	nd	nd
24:0 NA*	0.13±0.03	nd	0.21±0.02	0.83±0.15	0.08±0.02	nd	nd
Total very long chain	0.86±0.00^{c}	0.75±0.02^{c}	0.21±0.01^{c}	0.83±0.03^{c}	0.08±0.00^{b}	0.00±0.00^{a}	0.00±0.00^{a}
Total SFA	33.68±0.21^{a}	49.93±0.78^{b}	38.19±0.21^{b}	59.57 ±0.03^{e}	51.88±0.03^{c}	66.75±0.03^{e}	55.86±0.03^{c}
Monounsaturated fatty acids (MUFA)							
11:1 UDE*	nd	nd	nd	0.78±0.05	0.31±0.05	0.83±0.25	nd
14:1 MOA	nd	0.15±0.10	nd	nd	nd	nd	nd
16:1 n-7 POA	1.18±0.33	2.85±0.96	5.53±0.38	1.86±0.12	4.65±0.65	2.72±0.52	8.18±1.32

17:1 MGO*	nd	0.25±0.03	0.49±0.02	0.70±0.10	0.25±0.10	nd	nd
18:1 n-9 OA	5.82±0.03	2.52±0.90	20.35±0.07	5.95±0.80	6.55±1.80	2.93±0.89	4.74±1.50
20:1 GA	0.44±0.03	nd	1.34±0.20	nd	nd	nd	nd
22:1 DMA*	nd	nd	2.00±0.27	nd	nd	nd	nd
Total MUFA	7.44±0.10^{b}	5.77±0.85^{a}	29.71±0.03^{e}	9.29±0.09^{c}	11.76±0.25^{d}	6.48±0.76^{b}	12.92±0.75^{d}
n-3 polyunsaturated fatty acids (PUFA)							
18:4 n-3 SDA	5.25±1.89	0.69±0.10	0.93±0.15	nd	0.20±0.03	2.15±0.88	nd
20:3 n-3 ESA	1.93±0.70	5.56±2.10	0.28±0.08	nd	0.29±0.05	1.12±0.09	nd
20:5 n-3 EPA	7.70±1.40	nd	1.79±0.20	nd	nd	nd	nd
22:5 n-3 DPA	0.25±0.03	nd	1.37±0.10	nd	nd	nd	nd
22: 6 n-3 DHA	2.88±0.88	nd	2.28±0.96	nd	nd	nd	nd
Total n-3	18.01±0.03^{d}	6.25±0.04^{c}	6.65±0.05^{c}	nd	0.49±0.03^{a}	3.27±0.04^{b}	nd
n-6 PUFA							
18:2 n-6 LA	6.40±1.03	13.38±2.10	13.21±2.21	15.70±2.08	15.82±1.82	12.10±2.75	14.67±0.27
18:3 n-6 GLA	4.07±1.42^{a}	22.51±2.10^{f}	7.12±3.31^{b}	14.65±1.05^{d}	19.15±1.32^{e}	10.77±1.85^{c}	18.91±0.11^{e}
20:2 n-6 EDA	8.29±1.30	nd	0.49±0.03	nd	0.46±0.10	0.59±0.10	nd
20:4 n-6 ARA	9.92±2.40	0.44±0.07	0.34±0.03	nd	nd	nd	nd
20:3 n-6 DGLA	1.09±0.07	nd	0.39±0.05	nd	nd	nd	nd
Total n-6	29.77±0.03^{d}	36.33±0.06^{f}	21.55±0.05^{b}	30.35±0.04^{d}	35.43±0.03^{e}	23.46±0.03^{bc}	33.58±0.03^{e}
Total PUFA	47.78±0.89^{f}	42.58±0.78^{e}	28.20±2.03^{ab}	30.35±1.33^{c}	35.92±1.09^{d}	26.73±1.04^{a}	33.58±0.07^{d}
Total unsaturated fatty acids (UFA)	55.22±0.45^{e}	48.35±0.66^{d}	57.91±1.86^{f}	39.64±0.88^{b}	47.68±0.75^{b}	33.21±0.88^{a}	46.50±0.45^{c}
n-3/n-6	0.60±0.09^{e}	0.17±0.04^{c}	0.31±0.06^{d}	0.00±0.00	0.01±0.00^{a}	0.14±0.03^{b}	0.00±0.00
Total UFA/SFA	1.64±0.06^{f}	0.97±0.03^{d}	1.52±0.03^{e}	0.67±0.03^{c}	0.92±0.05^{d}	0.50 ±0.02^{a}	0.83±0.05^{d}
Trans fatty acids (TFA)							
16:1 *trans* POA	0.36±0.09	0.90±0.42	0.22±0.05	nd	nd	nd	nd
18:1 *trans* EA	0.15±0.03	0.38±0.02	0.08±0.03	nd	nd	nd	nd
Total TFA	0.51±0.09^{a}	1.28±0.21^{b}	0.30±0.03^{a}	nd	nd	nd	nd

Values in the same row with different letters significantly differ (p<0.05). nd = not detected
* BA = butyric acid, NA = nervonic acid, UDE = undecenoic acid, MGO = margaroleic acid, DMA = docosaenoic acid. For other abbreviations, see Fig. 1.

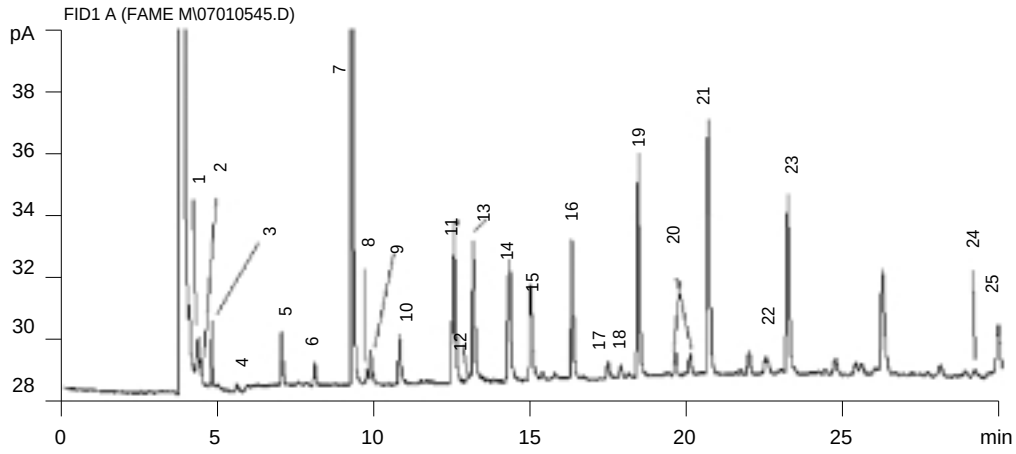

Fig. 1. Fatty acid peaks in *Spirulina platensis* produced in the Faculty of Fisheries of Ege University (from left): 1 = 6:0 (caproic acid; CA); 2 = 8:0 (caprylic acid; CAPY); 3 = 11:0 (undecanoic acid; UDA); 4 = 12:0 (lauric acid; LAU); 5 = 14:0 (myristic acid; MA); 6 = 15:0 (pentadecanoic acid; PDA); 7 = 16:0 (palmitic acid; PA); 8 = 16:1*t* (trans-palmitoleic acid; *t*POA); 9 = 16:1 (palmitoleic acid; POA); 10 = 17:0 (margaric acid; MG); 11 = 18:0 (stearic acid; SA); 12 = 18:1*t* (trans-oleic acid; *t*OA); 13 = 18:1 (oleic acid; OA); 14 = 18:2 n-6 (linoleic acid; LA); 15 = 18:3 n-6 (gamma-linolenic acid; GLA); 16 = 18:4 n-3 (stearidonic acid; SDA); 17 = 20:0 (arachidic acid; AA); 18 = 20:1 (gadoleic acid; GA); 19 = 20:2 n-6 (eicosadienoic acid; EDA); 20 = 20:3 n-3 (eicosatrienoic acid; ESA); 21 = 20:4 n-6 (arachidonic acid; ARA); 22 = 20:3 n-6 (homogamma linoleic acid; DGLA); 23 = 20:5 n-3 (eicosapentaenoic acid; EPA); 24 = 22:5 n-3 (docosapentaenoic acid; DPA); and 25 = 22:6 n-3 (docosahexaenoic acid; DHA).

Discussion

Regarding SFA, our results were generally in agreement with earlier studies. The distribution of short and long chain SFA was similar to 45.51-55.57% for *S. platensis* as found by Otles and Pire (2001). Palmitic acid, the most abundant SFA, and the total SFA level were 23-60% in earlier studies (Tanticharoen et al., 1994; Campanella et al., 1999; Otles and Pire, 2001; Koru and Cirik, 2002; Tokusoglu and Unal, 2003; Colla et al., 2004; Whitton et al., 2005; Gupta et al., 2008). Our results for MUFA and oleic acid (OA) were similar to previous studies in which they ranged 4-12% (Tanticharoen et al., 1994; Cohen, 1997; Campanella et al., 1999; Otles and Pire, 2001; Colla et al., 2004; Gupta et al., 2008) but unlike the 35% reported by Tokusoglu and Unal (2003). No information on *trans* fatty acid (TFA) levels of *Spirulina* species has been reported previously.

Our results on GLA agree with earlier findings. Commercial *Spirulina* samples contain 0.2-13.4% GLA (Campanella et al., 1999) while GLA in *Spirulina* sp. strains vary 8-31.7% (Cohen et al., 1987; Tanticharoen et al., 1994; Mahajan and Kamat, 1995; Otles and Pire, 2001; Koru and Cirik, 2002; Tokusoglu and Unal, 2003; Colla et al., 2004; Whitton et al., 2005). The GLA and n-6 contents varied significantly between samples, perhaps because the *Spirulina* was grown under different growing conditions. GLA in *S. platensis* is influenced by many factors, including temperature (Cohen et al., 1987; Maahjan and Kamat, 1995; Cohen, 1997; Koru and Cirik, 2002; Colla et al., 2004), light/dark cycle, whether the algae is grown indoor or out (Tanticharoen et al., 1994; Mahajan and Kamat, 1995), harvest time (Cohen, 1997; Apt and Behrens, 1998), and age of the culture (Whitton et al., 2005).

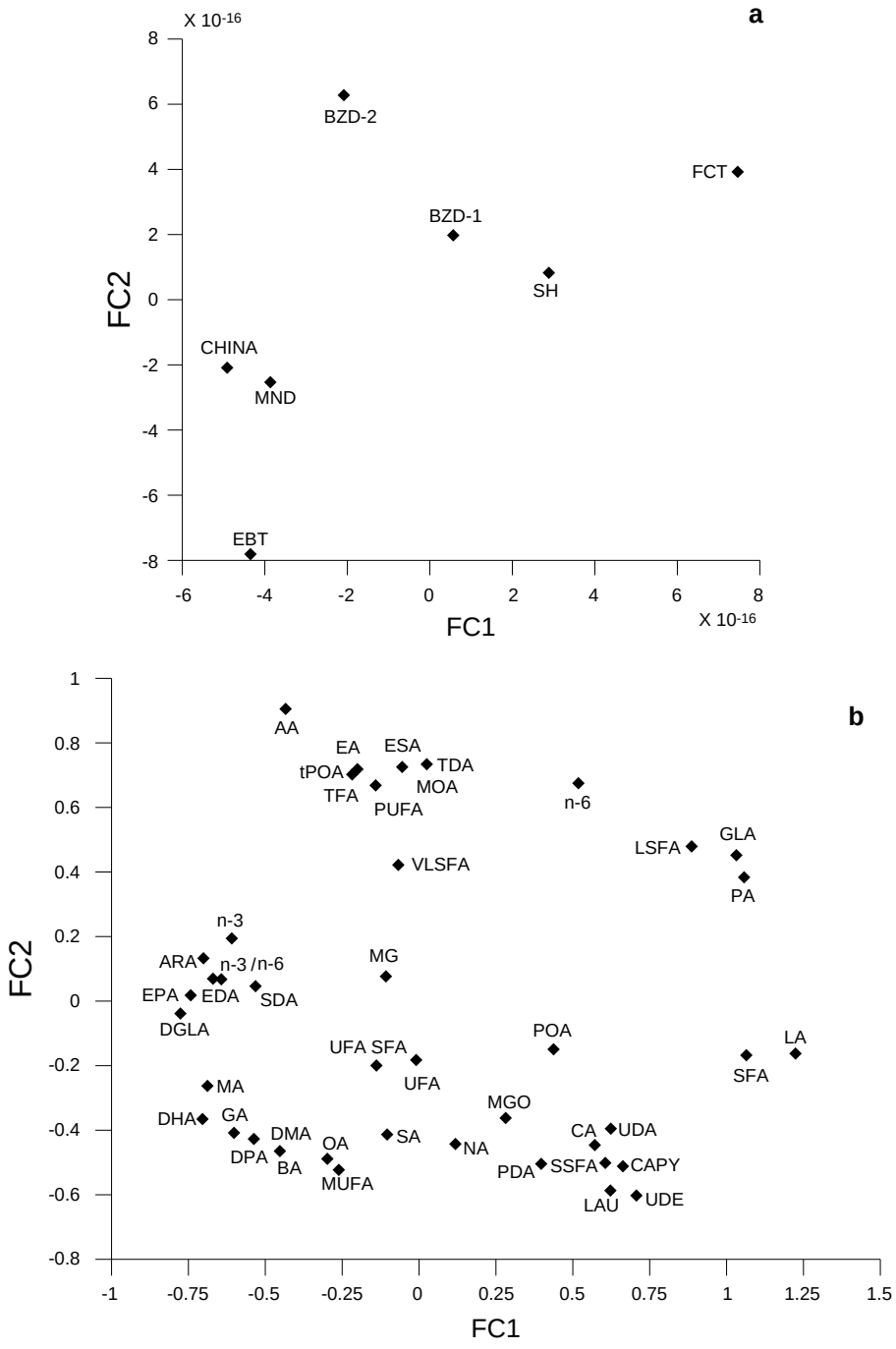

Fig. 2. Distribution of (a) production sites based on their (b) fatty acids as determined by Principal Component Analysis (PCA). See Table 1 and Fig. 1 for explanation of abbreviations.

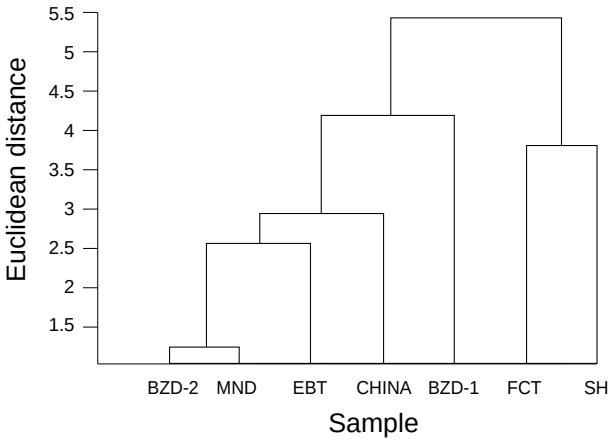

Fig. 3. Dendogram showing relationship of *Spirulina platensis* from different production sites based on fatty acid profiles, as determined by Hierarchical Cluster Analysis (HCA).

Our findings on LA were generally similar to results of previous studies. While LA ranged 10.37-31.5% in many studies (Cohen et al., 1987; Tanticharoen et al., 1994; Otles and Pire, 2001; Tokusoglu and Unal, 2003; Whitton et al., 2005), it varied 0.5-14.2% in others (Koru and Cirik, 2002; Campanella et al., 1999). ARA ranged 0.48-0.81% in *Spirulina* sp. (Tokusoglu and Unal, 2003).

Only two of our samples contained EPA and DHA. In an earlier study, EPA (0.19%) but no ALA or DHA was found in only one sample (Otles and Pire, 2001; Tokusoglu and Unal, 2003) although EPA (2.21-2.91%), DHA (2.30-3.51%), and ALA (0.62-0.71%) were found in all samples of another study and SDA ranged 0.57-0.81% (Tokusoglu and Unal, 2003).

Our n-3/n-6 and UFA/SFA ratios are in accordance with those found earlier, 0.31-0.46 (Tokusoglu and Unal, 2003) and 0.74-1.14 (Otles and Pire, 2001), respectively. A higher PUFA content increases the nutritional value of foods. The minimum recommended PUFA/SFA ratio for humans is 0.45 (Cuthbertson, 1989). Exposure of algae to sunlight for several days may affect PUFA content because of photoxidation, hence, changes during marketing should be considered (Campanella et al., 1999).

In conclusion, the nutritive composition of the final biomass of *Spirulina* sp. produced in the sunny Aegean region of Turkey is of high quality due to its high level of PUFA, in particular GLA. *Spirulina platensis* is used in the food, medicine, and cosmetic industries, and as an additive for chips, fruit juices, sauces, spice mixtures, vegetables, soups, and other products. This investigation contributes to the determination of nutrients in *S. platensis* microalgae used in the Turkish food and aquaculture feed industries.

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