Functional Bioactive Compounds and Biological Activities of *Spirulina platensis* Lipids

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Abstract

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The compositions and concentrations of lipid classes, fatty acids, tocopherols were determined in the lipids from blue-green microalga *Spirulina platensis*. Total lipids (TL) recovered using chloroform: methanol (2:1, v/v) were found to be 163.5 g/kg (on dry weight basis). The level of neutral lipids was the highest, followed by glycolipids and phospholipids, respectively. Among TL and lipid classes, palmitic, γ-linolenic and linoleic acids were the dominating fatty acids. Compared to the neutral lipids, the polar fractions were generally characterised by higher percentages of saturated fatty acids. The recovered lipids were characterised by high percentage of tocopherols, wherein α-tocopherol constitutes about 73% of total tocopherols present, the rest being γ-tocopherol. *Spirulina platensis* lipids exhibited a strong radical scavenging activity towards stable DPPH free radicals, whereas 27% of DPPH radicals were quenched after 2 h incubation. TL and lipid classes inhibited the growth of different microorganisms except gram-negative bacteria. At high concentrations, the tested lipids appeared more effective against *A. niger* (28.3 ± 1.53 mm). The information obtained in the present investigation is useful for lipid characterisation and further chemical and nutritional investigations of *Spirulina platensis*.

Keywords: *Spirulina platensis*; blue-green microalga; lipids; fatty acids; tocopherols; antiradical action; antimicrobial activity

The cyanobacteria represent a large group within the prokaryotic kingdom. They are the oldest oxygenic photosynthetic organisms known so far and they also serve as a rich source of novel bioactive metabolites, including many cytotoxic, antifungal and antiviral compounds (Patterson *et al.* 1994). *Spirulina platensis*, a blue green microalga, has been used since ancient times as a source of food because of its high nutritional value (Dillon *et al.* 1995). The cyanobacterium *Spirulina platensis* is rich in nutrients, such as proteins, vitamins, minerals, carbohydrates, and γ-linolenic acid. It is gaining more and more attention, not only for the foods aspects but also for the development of potential pharmaceuticals (Quoc & Pascaud 1996). This alga is being widely studied, not only for nutritional reasons but also for its reported medicinal properties; thus, several studies have shown that *Spirulina* or its extracts could prevent or inhibit cancer in humans and animals, and recent works have indicated that this species has immuno-promoting effects (Qureshi & Ali 1996;

Hayakawa *et al.* 1997; Kim *et al.* 1998; Miranda *et al.* 1998; Mishima *et al.* 1998; Hirahashi *et al.* 2002; Subhashini *et al.* 2004). *Spirulina platensis* was also reported to present antimicrobial activity (Demule *et al.* 1996; Ozdemir *et al.* 2004) as well as to inhibit the the replication of several viruses, such as Herpes simplex and HIV-1 (Ayehunie *et al.* 1998; Hernández-Corona *et al.* 2002). *Spirulina* contains a whole spectrum of natural mixed carotene and xanthophyll phytopigments which, together with phycocyanin, seem to be related to its antioxidant activity (Miranda *et al.* 1998; Bhat & Madyastha 2000; Pineiro Estrada *et al.* 2001). Recent studies reported that *Spirulina platensis* could be used as a matrix for the production of selenium-containing compounds and proved to be successful in transforming inorganic selenium to organic selenium *in vivo* when cultivated in selenium-rich medium (Li *et al.* 2003). The antioxidant activities of selenium-containing phycocyanin and its different aggregates (monomer, trimer, and hexamer) against free radicals of superoxide, hydrogen peroxide, and 2,2-diphenyl-1-picryl-hydrazyl (DPPH) were found to be variable (Huang *et al.* 2007).

Cyanobacteria may contain significant quantities of lipids with the composition similar to those of vegetable oils (Singh *et al.* 2002). The lipids of some cyanobacterial species are rich in essential fatty acids such as linoleic 18:2n6 and α-linolenic 18:3n3 acids and their C_{20} derivatives, eicosapentaenoic acids 20:5n3 and arachidonic acids 20:4n6 (Otheș & Pire 2001). Some of the filamentous cyanobacteria tend to contain large quantities (20–60% of the total of fatty acids) of polyunsaturated fatty acids (PUFA) (Parker *et al.* 1967; Holton & Blecker 1972; Kyle *et al.* 1992). Where microalgae can be cultured, PUFA in algae have profound benefits and functions in dietetics and therapeutic uses (Carlson & Salem 1991; Innis 1991; Ogata *et al.* 1996; Otheș & Pire 2001). They are believed to have positive effects for the treatment of hypertension, premenstrual tension, various atopic disorders, diabetes and a number of other cases (Roughan 1989; Saito *et al.* 1992). Cyanobacteria have a glycerolipid composition very similar to that of the chloroplasts of leaves, the major lipids being monoglactosyl diacylglycerols, digalactosyl diacylglycerols, sulfoquinovosyl diacylglycerols and, to a minor degree, phosphotidylglycerol. Glycolipids molecular species and their fatty acid composition in *Spirulina platensis* were recently reported (Xue *et al.* 2002) and more recently Mendiola *et al.* (2007) applied supercritical fluid extraction to obtain functional extracts with antioxidant and/or antimicrobial activities from *Spirulina platensis.*

The data about the detailed composition of *Spirulina platensis* lipids are scare; on the other hand, no reports exist in literature about antiradical and antimicrobial action of *Spirulina platensis* lipids. In this work, the microalga *Spirulina platensis* lipids were investigated as a natural source of functional bioactives because of its usefulness for human health. In addition, antiradical and antimicrobial activities of the lipid from *Spirulina platensis* were also studied.

MATERIALS AND METHODS

Neutral lipid (NL) standards were from Sigma Chemical Co. (St. Louis, MO, USA). Standards used for glycolipids (GL) identification, monogalactosyl diacylglycerol (MGD), digalactosyldiacylglycerol (DGD), cerebrosides (CER), steryl glucoside (SG), and esterified steryl glucoside (ESG) were purchased from Biotrend Chemikalien GmbH (Köln, Germany). Standards used for phospholipids (PL) identification, phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylinositol (PI) from bovine liver, phosphatidylcholine (PC) from soybean, phosphatidylglycerol (PG), *lyso*-phosphatidylcholine (LPC) and *lyso*-phosphatidylethanolamine (LPE) from egg yolk were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Standards used for vitamin E (α-, β-, γ- and δ-tocopherol) characterisation were purchased from Merck (Darmstadt, Germany). 1,1-Diphenyl-2-picrylhydrazyl (DPPH, approximately 90%) was from Sigma (St. Louis, MO, USA). The reagents and chemicals used were of the highest purity available.

The biological material used in this investigation was the cyanobacterium *Spirulina platensis*. This organism was obtained from an algal collection (Mansoura University, Egypt). The axenic culture of *Spirulina platensis* was maintained in 2 l Erlenmeyer flasks containing the derided nutrient medium. Six to seven-day-old culture was used as inocula. The culture flasks were incubated for 15 days at 25 ± 2°C under continuous illumination (using florescent lamps) at 4000 lux. At the expiry of the experimental period (15 days), the culture biomass was separated from its me-

dium by centrifugation at 10 000 rpm for 20 min (Sigma-Laborzentrifugen, 2K 215) and washed several times with bi-distilled water to dissolve the soloing salts.

Extraction of total lipids (TL)

The wet mass was extracted twice by shaking with 720 ml chloroform/methanol 2:1 (v/v) at 37°C for 18–24 h and the extract was then filtered through 47 µm diameter GF/C whatman glass microfibre filters (Novik *et al*. 2006). Chloroform (10 ml) and distilled water (10 ml) were added sequentially to the filtrate and shaking was carried out for 10 minutes. The resultant solution was filtered under vacuum through a 25 mm diameter whatman glass filter microfiber. The filtrate was washed with 30 ml of 5% sodium chloride solution, the lower layer of chloroform was then separated and treated with anhydrous sodium sulphate to remove the traces of water. After filtration, the extract of TL was rotary evaporated at 40°C.

Column and thin-layer chromatography of lipid classes

Fractionation of lipid classes and subclasses. TL were separated into different classes by elution with different polar solvents using a glass column (20 mm dia × 30 cm) packed with a slurry of activated silicic acid (70 to 230 mesh; Merck, Darmstadt, Germany) in chloroform (1:5, w/v) according to Ramadan & Moersel (2003). Neutral lipids (NL) were eluted with 3-times the column volume of chloroform. The major portion of GL was eluted with 5-times the column volume of acetone, and that of PL with 4-times the column volume of methanol. The amount of the lipid classes obtained was determined by gravimetry. By means of thin-layer chromatography (TLC) on Silica gel F_{254} plates (thickness = 0.25 mm; Merck, Darmstadt, Germany), a further characterisation of the GL and PL subclasses was carried out with the following solvent system: chloroform/methanol/ammonia solution 25% (65:25:4, v/v/v). For the characterisation of NL subclasses, Silica gel F_{254} plates were developed in the solvent system *n*-hexane/diethyl ether/acetic acid (60:40:1, v/v/v). For the detection of the lipids, the thin-layer plates were sprayed with the following agents: for the marking of all lipids with sulphuric acid (40%), for the marking of GL with α-naphthol/sulphuric acid, and for the marking of PL with the molybdate-blue reagent (Kates 1972). Each spot was identified by means of lipid standards and their reported retention factor (R_f) values. For fatty acid characterisation of subclasses, the individual bands were visualised under ultraviolet light, scraped from the plate and recovered by extraction with chloroform/methanol (2:1, v/v). Fatty acid compositions of NL, GL and PL were determined by GLC/FID as described below.

Quantitative determination of lipid subclasses. For the quantitative determination of NL subclasses, the individual bands were scraped from the plate and recovered by extraction with 10% methanol in diethyl ether, followed by diethyl ether. The data presented are the average of three gravimetrical determinations. For the quantitative estimation of GL subclasses, the acetone fraction obtained by column chromatography was separated by TLC in the above given solvent system. The silica gel regions with the corresponding GL subclasses were scraped out followed by hexose photometrical measurement at 485 nm using the phenol/sulphuric acid in acid-hydrolysed lipids (Southgate 1976). The percent distribution of each component was obtained from the hexose values. From the extinction values, the quantitative amount was determined and related to the respective portion of the GL fraction. The determined portion was set into relation with the amount of TL, which had been separated by column chromatography into the main lipid fractions. For the determination of PL, the methanol fraction from column chromatography was also separated by TLC in the above given solvent system and, after scraping out of the individual PL subclasses, brought to reaction with the hydrazine sulphate/sodium molybdate reagent at 100°C for 10 min and photometrically analysed at 650 nm according to AOCS method (1990). From the obtained extinction values *via* a calibration chart for phosphorus the amount of PL was calculated. The individual values were put into relation to the PL fraction (methanol fraction from column chromatography) and to the amount of TL.

Gas liquid chromatography (GLC) analysis of fatty acid methyl esters

Fatty acids were transesterified into methyl esters (FAME) using *N*-trimethylsulfoniumhydroxide (Macherey-Nagel, Düren, Germany) according

to the procedure reported by Arens *et al.* (1994). FAME was identified on a Shimadzu GC-14A equipped with flame ionisation detector (FID) and C-R4AX chromatopac integrator (Kyoto, Japan). The flow rate of the carrier gas helium was 0.6 ml per min and the split value with a ratio of 1:40. A sample of 1µl was injected on a 30 m × 0.25 mm × 0.2 µm film thickness Supelco SP^{TM}-2380 (Bellefonte, PA, USA) capillary column. The injector and FID temperature was set at 250°C. The initial column temperature was 100°C programmed by 5°C/min until 175°C and kept 10 min at 175°C, then 8°C/min until 220°C and kept 10 min at 220°C. To facilitate the identification, a comparison was made between the retention times of the samples with those of authentic standard mixture (Sigma, St. Louis, MO, USA; 99% purity specific for GLC), run on the same column under the same conditions.

Normal phase high performance liquid chromatography (NP-HPLC) separation, identification and quantification of tocopherols

Procedure. NP-HPLC was selected to avoid extra sample treatment (e.g., saponification) according to Ramadan *et al.* (2006). The analysis was performed with a solvent delivery LC-9A HPLC (Shimadzu, Kyoto, Japan). The chromatographic system included a model 87.00 variable wavelength detector and a 250 × 4 mm *i.d.* LiChrospher-Si 60, 5 µm, column (Knauer, Berlin, Germany). The separation of tocopherols was based on isocratic elution when the solvent flow rate was maintained at 1 ml/min at a column back-pressure of about 65–70 bar. The solvent system selected for tocopherols elution was isooctane/ethyl acetate (96:4, v/v) with the detection at 295 nm. Twenty µl of the diluted solution of TL in the selected mobile phase were directly injected into the HPLC column. Tocopherols were identified by comparing their retention times with those of authentic standards.

Preparation of standard curves. Standard solutions were prepared by serial dilution to the concentration of approximately 5 mg/ml of vitamin E. Standard solutions were prepared daily from the stock solution which was stored in the dark at –20°C. Among of 20 µl was injected and the peaks areas were determined to generate standard curve data.

Quantification. All quantitation was made by means of the peak areas using Shimadzu C-R6A chromatopac integrator (Kyoto, Japan). Standard curves (concentration versus peak area) were calculated from six concentration levels by linear regression. Based on the established chromatographic conditions, repeated injections of different concentrations of the tocopherols were made 3-times onto the HPLC system. Injections in triplicates were made at each concentration of both standards and the sample. All work was carried out under subdued light conditions. All the experiments were repeated at least thrice when the variation in any one was routinely less than 5%.

Radical scavenging activity (RSA) of total lipids toward DPPH radical

Different solvents were used to assay the RSA of TL, the best results having been achieved with toluene which was able to dissolve completely the hydrophobic and the hydrophilic compounds (Ramadan *et al.* 2003; Ramadan & Moersel 2006). Therefore, the RSA of total lipids and lipid classes was assayed with DPPH radical previously dissolved in toluene. Toluenic solution of DPPH radicals was freshly prepared at a concentration of 10^{-4}M. For the evaluation, 10 mg of total lipids (in 100 µl toluene) were mixed with 390 µl toluenic solution of DPPH radicals and the mixture was vortexed for 20 s at ambient temperature. The decrease in absorption at 515 nm was measured against a blank of pure toluene without DPPH in 1-cm quartz cells after 30, 60 and 120 min of mixing using a UV-260 visible recording spectrophotometer (Shimadzu, Kyoto, Japan). RSA toward DPPH radicals was estimated from the differences in absorbance of toluenic DPPH solution with or without sample (control) and the inhibition percent was calculated according to Ramadan and Moersel (2006) from the following equation:

$$\% \text{ inhibition} = [(\text{absorbance of control} - \text{absorbance of test sample})/\text{absorbance of control}] \times 100$$

All experimental procedures were performed in triplicate and their mean values (± standard deviation) were reported.

Antimicrobial activity

The antimicrobial activities were determined out according to the conventional agar diffusion test (Greenwood 1983) using cultures of *Bacillus*

subtilis NRRL B-94, *E. coli* NRRL B-3703, *Pseudomonas aeruginosa* NRRL, *Staphylococcus aureus* NRRL, *Aspergillus niger* NRRL 313, *Aspergillus flavus* NRC, *Saccharomyces cervisiae* NRC, and *Candida albicans* NRRL 477. The bacterial strains were cultured on nutrient medium, while the fungi and yeast strains were cultured on malt medium and yeast medium containing 1% Tween 20, respectively. Broth media included the same contents except for agar. For bacteria and yeast, the broth media were incubated for 24 hours. For fungi, the broth media were incubated for approximately 48 h, with subsequent filtering of the culture through a thin layer of sterile sintered Glass G2 to remove mycelia fragments before the solution containing the spores was used for inoculation. For the preparation of the plate and inoculation, 0.5 ml of inocula were added to 50 ml of agar media (50°C) and mixed by simple inversion. Agar was poured into 120 mm Petri dishes and allowed to cool to room temperature. Wells (6 mm in diameter) were cut in the agar plates using paper sterile tubes, then fill wells were filled up to the surface of agar with the tested lipids (20, 40, 60, 80 and 100 µl/well). The microbial growth inhibition zone was measured after incubation at 30°C by the appearance of clear microbial free inhibition zones, beginning within 24 h for yeast, 24–48 h for bacteria and 48–72 h for fungus. Antimicrobial activities were calculated as means of three replicates.

RESULTS AND DISCUSSION

Recently, there has been a growing interest in functional foods, that is, foods able to provide additional physiological benefits for human health, other than the basic nutritional and energetic requirements (Goldberg 1996). Often, functional foods are traditional foods enriched with an ingredient able to provide or promote a specific beneficial action for human health. These are called functional ingredients. These ingredients are preferred to have a natural origin, such as plants or perhaps algae and/or microalgae. These types of marine sources are receiving an increasing attention mainly for their content in, for example, polyunsaturated fatty acids (Cohen & Vonshak 1991; Mahajan & Kamat 1995), β-carotene and other pigments (antioxidants) (Bhat & Madyastha 2000; Madhava *et al.* 2000), and sulphated polysaccharides (anti-virals) and sterols (antimicrobials) (Richmond 1988; Otheş & Pire 2001; Xue *et al.* 2002).

In fact, for a biosource to be suitable for the lipid production, it must meet the following two criteria: (*i*) the lipid content must reach the minimum for commercially viable exploitation and (*ii*) the source must be suitable for high acreage cultivation (Bockisch 1998). TL content of dried *Spirulina* powder was found to be 6.38% (Xue *et al.* 2002) while in our study; it was found that *Spirulina platensis* contains a considerable amount of lipids (*ca.* 163.5 g/kg).

Table 1. Levels of subclasses (g/kg TL) in *Spirulina platensis* lipids

Neutral lipid subclass	R_f values × 100[a]	g/kg TL	Glycolipid subclass	R_f values × 100[b]	g/kg TL	Phospholipid subclass	R_f values × 100[b]	g/kg TL
MAG	14	22.5 ± 0.37	SQD	6	198 ± 3.50	PS	4.7	23.5 ± 0.43
DAG	39	22.0 ± 0.28	DGD	17	83.5 ± 2.55	PI	11	15.5 ± 0.25
ST	50	40.5 ± 1.39	CER	29–35	39.0 ± 1.25	PC	20	56.0 ± 0.66
FFA	56	31.5 ± 1.09	SG	41	20.5 ± 0.96	PE	30	64.0 ± 0.80
TAG	79	243 ± 3.16	MGD	64	40.0 ± 0.99			
STE	95	90.7 ± 2.10	ESG	76	19.0 ± 0.57			

[a]Solvent system used in TLC development: *n*-hexane/diethyl ether/acetic acid (60:40:1, v/v/v)

[b]Solvent system used in TLC development: chloroform/methanol/ammonia solution 25% (65:25:4, v/v/v)

Results are given as the average of triplicate determinations ± standard deviation

TL – total lipids; MAG – monoacylglycerols; DAG – diacylglycerols; ST – free sterols; TAG – triacylglycerols; FFA – free fatty acids; STE– sterol esters; SQD – sulphoquinovosyldiacylglycerol; DGD – digalactosyldiacylglycerol; CER – cerebrosides; SG – steryl glucoside; MGD – monogalactosyldiacylglycerol; ESG – esterified steryl glucoside; PS – phosphatidylserine; PI – phosphatidylinositol; PC – phosphatidylcholine; PE – phosphatidylethanolamine

Levels of lipid classes and subclasses

A suitable combination of chromatographic procedures on Silica gel was used to obtain major lipids classes and subclasses of *Spirulina platensis* lipids according to Ramadan *et al.* (2006). The proportion of TL, lipid classes and subclasses presented in *Spirulina platensis* lipids as well as R_f values of these subclasses are shown in Table 1. Among the TL present, the level of NL was the highest (45% of TL), followed by GL (39% of TL) and PL (16% of TL), respectively. The subclasses of NL in the crude extract contained triacylglycerol (TAG), esterified sterols (STE), free sterols (ST), free fatty acids (FFA) diacylglycerol (DAG), and monoacylglycerol (MAG) in decreasing order. A significant amount of TAG was found (ca. 55% of total NL) followed by a relatively high level of STE (ca. 20% of total NL) and ST (ca. 9% of total NL), while FFA, DAG, and MAG were recovered in lower levels. In our investigation, subclasses of GL in *Spirulina platensis* lipids were sulphoquinovosyldiacylglycerol (SQD), digalactosyldiacylglycerol (DGD), cerebrosides (CER), sterylglycosides (SG), monogalactosyldiacylglycerol (MGD), and esterified sterylglycosides (ESG) as shown in Table 1. The proportion of each component was estimated by the lipid-carbohydrate determination. From the various reagents used in total carbohydrate estimation, phenol is most popular and, apart from its high sensitivity, a further advantage is the equal response of hexose and sulpholipids when measuring the absorbance at 485 nm. In contrast, the colour developed with anthrone has different absorption maxima for hexose (620 nm) and sulpholipids (590 nm). SQD and DGD were the prevalent components and made up about a 70% of the total GL followed by MGD as the third major subclass. SG, ESG, and CER formed together about 20% of total GL. Glycolipids are major lipid components of all chloroplast membranes and the photosynthetic membranes of cyanobacteria. MGD and DGD mainly occupy the thylakoid membranes of plants, and SQD is one of the main lipid components. Recently, Xue *et al.* (2002) studied molecular species profile of GL from *Spirulina platensis* and found that TL contained MGD (10.3%), DGD (6.44%), and SQDG (11.4%). C16:0, C18:3n6, and C18:2n6 were the main fatty acids in GL of MGD and DGD, while C16:0 and C18:2n6 were the main fatty acids in SQD. It was reported that natural sulfated polysaccharides separated from *S. platensis* inhibit the replication of several viruses, such as Herpes Simples and HIV-1 (Ayehunie *et al.* 1998). In addition, Gustafson *et al.* (1989) reported that SQD from the cyanobacterium possesses AIDS-antivirus activities. The average daily intake of GL in humans was reported to be 140 mg of ESG, 65 mg of SG, 50 mg of CER, 90 mg of MGD and 220 mg of DGD (Sugawara & Miyazawa 1999). Therefore, it is worthy to point out that *Spirulina platensis* lipid could be an excellent and complete source of GL in the diet. PL subclasses in *Spirulina platensis* extract were separated into four fractions *via* TLC. Phosphorimetry of TLC fractions (Table 1) revealed that the predominant PL subclasses were PE followed by PC, PS, and PI, respectively. More than one-third of total PL was in PE and about 35% was in PC, while PI and PS were isolated in smaller quantities.

Fatty acids and tocopherols profile

Fatty acid profiles of TL lipid classes are presented in Table 2. Seven fatty acids were identified in *Spirulina platensis* extract, wherein the analysis of FAME gave the proportion of palmitic followed by γ-linolenic (GLA, C18:3*n*-6) as the major fatty acids, comprising together more than 67% of the total FAME identified. The third main fatty acid was linoleic acid which amounted to ca. 18% of total fatty acids. A striking feature of the *Spirulina platensis* lipids was the relatively high level of polyunsaturated fatty acids (PUFA), especially trienes, in which γ-linolenic fatty acid was estimated in high levels. Fatty acids in neutral lipids and polar lipids did not differ significantly from each other, wherein palmitic acid was still the main fatty acid in all fractions. The saturation ratio (S/U ratio) was considerably higher in the polar fractions than in the corresponding neutral fraction. Fatty acid profile of *Spirulina platensis* extract evinces the lipids as a good source of the nutritionally essential fatty acids as well as PUFA. The interest in the *n*-3 PUFA as the health-promoting nutrients has expanded dramatically in recent years. A rapidly growing literature illustrates the benefits of PUFA in alleviating cardiovascular, inflammatory, and heart diseases, atherosclerosis, autoimmune disorder, diabetes, and other diseases (Finley & Shahidi 2001; Riemersma 2001). The fatty acid composition and high amounts of PUFA makes the *Spirulina platensis* lipids a special component for nutritional applications.

Table 2. Fatty acid profile of *Spirulina platensis* lipids and lipid classes (relative content in %)

Fatty acid	Total lipids (TL)	Neutral lipids (NL)	Glycolipids (GL)	Phospholipids (PL)
C14:0	nd	nd	nd	nd
C16:0	44.2 ± 0.07	38.5 ± 0.07	48.8 ± 0.07	49.5 ± 0.07
C16:1	6.42 ± 0.07	8.66 ± 0.07	5.37 ± 0.07	4.50 ± 0.07
C18:0	nd	nd	nd	nd
C18:1	0.93 ± 0.07	1.48 ± 0.07	0.92 ± 0.07	0.93 ± 0.07
C18:2	18.7 ± 0.07	19.5 ± 0.07	17.6 ± 0.07	17.8± 0.07
C18:3*n*-6	23.4 ± 0.07	24.5 ± 0.07	21.2 ± 0.07	21.4 ± 0.07
C22:1	5.24 ± 0.07	6.33 ± 0.07	5.04 ± 0.07	4.60 ± 0.07
C24:0	1.11 ± 0.07	1.03 ± 0.07	1.07 ± 0.07	1.27 ± 0.07
Total saturates	45.3	39.5	49.8	50.7
Total monoenes	12.5	16.4	11.3	10.0
Total PUFA	42.2	55.9	38.9	39.3
S/U^{+}	0.82	0.65	0.99	1.02

Results are given as the average of triplicate determinations ± standard deviation
PUFA – polyunsaturated fatty acids; nd – not detected
$^{+}$Saturation ratio = (14:0 +16:0 + 18:0 + 24:0)/(16:1 + 18:1 + 18:2 + 18:3 + C22:1)

The nutritionally important components such as tocopherols improve the oil stability. Tocopherols are the major lipid-soluble, membrane-localised antioxidants in humans. The deficiency of these compounds affects many tissues in mammalian and bird models (Nelson 1980). Vitamin E deficiency in humans causes defects in the developing nervous system of children and haemolysis in the man (Sokol 1996). Epidemiologic studies suggest that people with a lower vitamin E and other antioxidant intake and plasma levels may be at increased risk for certain types of cancer and for atherosclerosis (Gey *et al*. 1991; Rimm *et al*. 1993). It is also suggested that the supplementation with antioxidants may decrease the risk of these and other degenerative processes (Kallio *et al.* 2002). Tocopherols in edible oils, moreover, are believed to protect PUFA from peroxidation (Kamal-Eldin & Andersson 1997). In our investigation, NP-HPLC technique was used to eliminate the column contamination problems and allow the use of a general lipid extraction for tocopherol isolation (Ramadan & Moersel 2003). Thus, saponification of lipid samples was not required, which allowed a shorter analysis time and a greater vitamin stability during analysis. Tocopherols level was high (523 mg/kg oil) in the *Spirulina platensis* lipids, wherein α-tocopherol constituted ca. 73% of the total analytes, the rest being γ-tocopherol (ca. 27%). α-Tocopherol is the most efficient antioxidant of these compounds. β-Tocopherol has 25–50% of the antioxidative activity of α-tocopherol, γ-tocopherol 10–35% (Kallio *et al*. 2002). Despite the general agreement that α-tocopherol is the most efficient antioxidant and vitamin E homologue *in vivo*, studies indicate, however, a considerable discrepancy in its absolute and relative antioxidant effectiveness *in vitro*, especially when compared to γ-tocopherol (Kamal-Eldin & Appelqvist 1996). High amounts of tocopherols detected in the lipids examined may contribute to a great stability towards oxidation of these lipids.

Radical scavenging activity (RSA) of *Spirulina platensis* lipids

The interest has increased in the past few years in the free radical theory of disease causation, particularly vascular diseases and certain forms of cancer. These developments have led to the

investigation into dietary agents, the antioxidant nutrients (mainly vitamins A, C, and E), in a possible prophylactic, even curative, role in the disease process. Closely related to this probable benefit of natural antioxidants is their role in controlling free radicals as these may lead to pathological effects such as vascular diseases and cancer. A free radical is defined as any compound or chemical species that has one or more unpaired electrons. This results in very reactive compounds. Oxidation is a natural and needed reaction in metabolism. A highly reactive hydroxyl radical, $OH^{\bullet}$, results. This can attack DNA, proteins and polyunsaturated fatty acids residues of the membrane phospholipids, among others. With the latter, a peroxyl radical is formed. Antioxidants quench this radical. If the supply of antioxidants is inadequate, a chain reaction takes place that may lead to damaged tissues. The evidence in the literature begins to make an overwhelming case for the existence of a relationship between high blood levels of antioxidant nutrients and a lowered incidence of disease.

Natural antioxidants, on the other side, allow processors to produce stable products with clean labels and tout all-natural ingredients. The tests expressing antioxidant potency can be categorised into two groups: the assays for radical scavenging ability, and the assays that test the ability to inhibit lipid oxidation under accelerated conditions. However, the model of scavenging stable free radicals is widely used to evaluate the antioxidant properties in a relatively short time, as compared to other methods (Ramadan & Moersel 2006).

Oxidative stability of edible oils and fats depends on the fatty acid composition, the presence of minor fat-soluble bioactive, and the initial amount of hydroperoxides. Antiradical properties of the *Spirulina platensis* total lipids were screened using stable DPPH free radicals. DPPH radicals are a purple stable radical that turns yellowish when it reacts with antioxidant analytes, and the degree of discoloration indicates the scavenging potential of the antioxidant extract. After 60 and 120 min incubation, 21 and 27%, respectively, of DPPH radicals were quenched by *Spirulina platensis* total lipids. After 2 h, antiradical action of lipid classes varied, wherein PL exhibited the strongest action (45% inhibition) followed by GL (34% inhibition) and NL (21% inhibition). These data are in agreement with those by Ramadan *et al.* (2003) whereas PL exhibited the strongest radical scavenging activity followed by GL and then NL in crude vegetable oils. The strong antiradical action of *Spirulina platensis* lipids may be due to (*i*) the differences in the contents and compositions of polar lipids and unsaponifiables (*ii*) synergism of polar lipids with other components present, and (*iii*) different kinetic behaviours of potential antioxidants. All these factors may contribute to the radical quenching efficiency of oils and fats.

Antimicrobial activity

Different microbial species, including gram negative bacteria, gram positive bacteria, yeasts, and fungi, were used to screen the possible antimicrobial activity of *S. platensis* lipids. The examination of antimicrobial activity of the TL and lipid classes (NL, GL and PL) by the agar diffusion method revealed that the TL and lipid classes inhibited the growth of all microorganisms tested (Table 3) except the Gram-negative bacteria (*E. coli* and *P. aeruginosa*). The total lipids were more active against fungi and yeasts than the lipid classes. The maximum inhibition zone obtained with 100 µl/well was that against *A. niger* (28.6 ± 1.53 mm). On the other hand, the lipid classes led to the highest inhibition of Gram-positive bacteria, wherein the inhibition zones were between 19.3 ± 0.57 mm and 25.6 ± 1.52 mm at the concentration of 100 µl/well.

Cyanobacteria were screened for potential antimicrobial activity which was attributed to different compounds belonging to a diverse range of chemical classes (Borowitzka 1995; Ozdemir *et al*. 2004). Specifically, the antimicrobial activity of the methanolic extract of *S. platensis* was explained by the presence of γ-linolenic acid (Demule *et al*. 1996), an antibiotically-active fatty acid present in a high concentration in this alga (Xue *et al*. 2002). Since the fatty acid analysis indicated the presence of other fatty acids that had been also reported to have some antimicrobial activity, specifically palmitoleic and oleic acids, the antimicrobial activity found in TL and lipid classes could be linked to the synergic effect of all these fatty acids.

It was hypothesised that lipids kill microorganisms by leading to disruption of the cellular membrane(s) (Lampe *et al.* 1998). Lipids kill the gram-positive bacteria, fungi and yeasts because they can penetrate the extensive meshwork of peptidoglycan in the cell wall without visible changes and reach the bacterial membrane leading to its disintegration. This can probably be explained by the strong fabric of the cell wall of gram-positive

Table 3. Antimicrobial action at different concentrations of total lipids and lipid classes of *Spirulina platensis (*diameter of inhibition in mm)

Lipid fraction	Concentration (µl/well)	Bacteria				Fungus		Yeast	
		B. subtilis	*St. aureus*	*E. coli*	*P. aeruginosa*	*A. niger*	*A. flavus*	*S. cervisiae*	*C. albicans*
TL	20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	40	04.4 ± 1.15	10.3 ± 1.53	0.00	0.00	8.67 ± 1.15	7.3 ± 0.57	4.33 ± 0.57	5.33 ± 0.57
	60	10.7 ± 1.53	15.0 ± 2.00	0.00	0.00	12.3 ± 1.52	9.66 ± 1.15	11.0 ± 0.20	10.3 ± 1.15
	80	14.3 ± 1.15	21.3 ± 1.53	0.00	0.00	23.3 ± 1.53	18.0 ± 1.73	14.6 ± 1.53	15.3 ± 1.53
	100	19.6 ± 1.53	24.6 ± 1.15	0.00	0.00	28.3 ± 1.53	21.0 ± 1.53	23.6 ± 0.57	21.3 ± 0.57
NL	20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	40	10.3 ± 0.57	10.6 ± 0.57	0.00	0.00	9.33 ± 0.57	8.33 ± 0.57	7.67 ± 0.57	6.67 ± 0.57
	60	15.3 ± 0.57	15.6 ± 0.57	0.00	0.00	15.0 ± 1.00	13.3 ± 0.57	14.3 ± 1.15	12.6 ± 1.15
	80	19.6 ± 0.57	20.3 ± 0.57	0.00	0.00	18.3 ± 0.57	17.3 ± 0.57	16.3 ± 0.57	15.3 ± 1.53
	100	25.6 ± 0.57	25.3 ± 0.57	0.00	0.00	23.3 ± 1.15	21.0 ± 1.73	19.6 ± 0.57	20.3 ± 0.57
GL	20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	40	9.6 ± 1.15	9.6 ± 0.57	0.00	0.00	7.66 ± 0.57	6.66 ± 0.57	6.33 ± 0.57	7.33 ± 0.57
	60	15.3 ± 0.57	15.6 ± 1.15	0.00	0.00	11.3 ± 0.57	10.0 ± 1.00	09.3 ± 0.57	09.6 ± 0.57
	80	20.6 ± 0.57	19.3 ± 1.52	0.00	0.00	16.0 ± 1.00	13.3 ± 0.57	12.6 ± 1.15	12.3 ± 0.57
	100	24.6 ± 1015	23.6 ± 1.53	0.00	0.00	22.3 ± 0.57	19.3 ± 0.57	20.3 ± 0.57	18.3 ± 0.57
PL	20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	40	10.3 ± 0.57	10.6 ± 0.57	0.00	0.00	9.33 ± 0.57	8.33 ± 0.57	7.66 ± 0.57	6.67 ± 0.57
	60	15.3 ± 0.57	15.6 ± 0.57	0.00	0.00	15.0 ± 1.00	13.3 ± 0.57	14.3 ± 1.15	12.6 ± 1.15
	80	19.6 ± 0.57	20.3 ± 0.57	0.00	0.00	18.3 ± 0.57	17.3 ± 0.57	16.3 ± 0.57	15.3 ± 1.52
	100	25.6 ± 057	25.3 ± 0.57	0.00	0.00	23.3 ± 1.15	21.0 ± 1.73	19.6 ± 0.57	20.3 ± 0.57

Each value represents mean of sample ± SD for $n = 3$

Diameter of inhibition zone was measured as the clear area centered on the agar well containing the sample

Well with non-inhibition zone were recorded 0.00

bacteria, which maintains its structure in spite of substantial hydrostatic turgor pressure within the bacteria (Bergsson 2005). The difference in the susceptibility of gram-negative bacteria to killing by lipids was notable (Bergsson *et al.* 2002) and is probably due to the differences in the outer membrane or the cell wall of bacteria. The external leaflet of the outer membrane of Enterobacteriacae, such as *E. coli* that lives in the rectum, an environment rich in hydrophobic compounds, is almost entirely composed of lipopolysaccharides and proteins. These bacteria have a hydrophilic surface because of the side chains of lipopolysaccharides, and thus hydrophobic molecules, like lipids, have difficulty in entering the bilayer (Bergsson 2005).

CONCLUSIONS

An improved knowledge of the composition, analysis, and properties of *Spirulina platensis* lipids would assist in efforts for the industrial application of this edible microorganism. It can be said that the *Spirulina platensis* gives considerable yield of lipids which are rich sources of essential fatty acids and lipid-soluble antioxidants. Moreover, the recovered lipids may be suitable for commercial exploitation as a source of lipids for food use and the production of cosmetic. The high level of glycolipids determines that the peel can be a suitable and valuable source to obtain the corresponding glycolipid concentrates. Tocopherols at the level estimated may be of nutritional importance in the application of this blue-green microalga. The data presented in this study illustrate that the lipid extract from *Spirulina platensis* shows antioxidant and antimicrobial activity. Consequently, *Spirulina platensis* lipid extracts present a promising potential as an accessible and safe alternative to synthetic antioxidants and antimicrobials.

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