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ORIGINAL PAPER

Comparison of the biochemical composition of different *Arthrospira platensis* strains from Algeria, Chad and the USA

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Abstract *Arthrospira platensis* (*Spirulina*) is a widely known microalga with nutritional and therapeutic applications due to its richness in nutrients and bioactive elements. The aim of this research was to characterize the phytochemical and nutritional compounds of five different *Spirulina* strains from the USA, Chad and Algeria. Concentrations of carbohydrates and fats, fatty acid profile, and the total proteins were determined through biochemical and chromatographic methods. The concentration of phytochemical compounds was determined by using UV–Vis spectrophotometry. The *Spirulina* from the USA had the highest amounts of lipids, proteins, phycocyanin and chlorophyll *a* content compared to the others strains tested. The strains from Algeria had the highest amount of carotenoids (5.50 mg/g) among the strains investigated. In all the strains, the main fatty acid compound was the palmitic acid (16:0) with the highest concentration of 71.15% in the *Spirulina* from Chad. All the *Spirulina* strains tested contained high quantities of ω-6-fatty acids (avg. 24%) except in the *Spirulina* strain from Chad, which had only 10.59%. All the *Spirulina* strains investigated were rich in polyphenols with the highest value of 67.52 mg GAE/g in the strains from Algeria. The *Spirulina* from the USA has more potential to be used in the nutraceutical and a functional food area since it is very rich in bioactive compounds.

Keywords *Arthrospira platensis* (*Spirulina*) · Biochemical composition · Phytopigments · Phycobiliproteins

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Introduction

The *Arthrospira platensis* (commercial name '*Spirulina*') is a phototrophic, filamentous and multicellular blue-green microalga which belongs to the Phylum Cyanobacteria. It converts sunlight, water and carbon dioxide into algal biomass. It is an important source of nutrients such as proteins, minerals, carbohydrates, and many phytopigments that can be used as food supplements. In addition, this microalga cells have high digestibility due to the lack of cellulose which facilitates their use for human consumption [1–3]. The most important species used for consumption are *Spirulina maxima* and *Arthrospira platensis* (*Spirulina*) [4, 5]. The *Spirulina* is produced naturally in alkaline water of volcanic lakes and warm brackish waters. However, this alga could also be grown for higher biomass production, under controlled conditions in saline water (>30 g/L) at a pH in the range of 8.5–11.0 with high levels of solar radiation (2500 Lux) and at temperatures in the range of 35–39 °C [2, 6, 7]. The current production of *Spirulina* in the world is estimated at 3000 metric tons and more than 70% of it is marketed for human consumption, mainly as

health food [7]. The *Spirulina* is mostly consumed as tablets, capsules and powder or added into various kinds of foods like pasta, cakes and health drinks which are used as nutritional supplements or as natural colorants [8].

Spirulina is well known for its high content of proteins (60–70%), vitamins such as vitamin B_{12} (8 ppm) and provitamin A (0.2%), minerals such as iron (0.1%), and polyunsaturated fatty acids especially the ω-6-fatty acids (up to 29.4–31.5% of the total fatty acids) [5, 9–11]. In addition to its nutrients richness, it is also a source of phytopigments like chlorophylls, carotenoids and phycobiliproteins, which are the light harvesting pigments in *Spirulina*. The phycobiliproteins that can be obtained from *Spirulina* are phycoerythrin (PE), allophycocyanin (A-PC) and phycocyanin (C-PC). However, several studies showed that the C-PC is the main phycobiliprotein in this microalga and it contains up to 50% of the phycobiliproteins in it [12–14].

The phytopigments play an important role in the photosynthetic metabolism of *Spirulina* and have anti-obesity, anti- inflammatory, anti-carcinogenic, antioxidant and neuroprotective effects on human health [15–17]. These effects have been attributed to the presence of components such as phycobiliproteins, phenolic compounds, beta-carotene and polyunsaturated fatty acid (PUFA) in the *Spirulina* [7, 18–20]. Mühling et al. [11] reported the significant difference in the fatty acid composition especially in the PUFA fraction, among the *Spirulina* strains. This difference in fatty acid composition is due to the differences in the growing conditions that have an influence on the fatty acids content of the algal strains [11]. In addition, many authors have reported that *Spirulina* is one of the best whole food sources for ω-6-fatty acids [7].

Many strains of *Arthrospira* have been identified as having different shapes: spiral, straight and wavy, under different culture conditions [21]. However, only a few of these strains are commercially exploitable due to their different productivities and qualities. Also, the available data on the nutrient composition and bioactive compounds of *Spirulina* are highly varied, and the values are dependent on the growing, harvesting and drying conditions of the microalgae. Hence, information on the phytochemical and biochemical composition of *Spirulina* is important for their use as food supplements. Therefore, this study compared and characterized the biochemical compositions of different *A. platensis* strains from Algeria, the USA and Chad.

Materials and methods

Algal strains

Five *Spirulina* strains were obtained from Algeria, the USA and Chad. Three of them were purchased as powder (*Spirulina* 1–3), whereas, the two other *Spirulina* strains (*Spirulina* 4 and 5) were obtained as fresh starter cultures. The latter strains were cultivated, harvested and dried to preserve them for further use. Table 1 presents the characteristics of all the *Spirulina* strains studied.

The morphology of fresh *Spirulina* (4 and 5) was observed with 10 and 40 times magnifications using an optical microscope (Optech Linear Biostar B4, München, Germany).

Culture conditions

The fresh cells of *Spirulina* strain samples 4 and 5 were separately grown under specific conditions in bath cultures with Zarrouk's medium [22] (Table 2). The pH of the medium was maintained to be in the range of 8.5–10. The culture temperature was 30 ± 2.0 °C, exposed under cool-white fluorescent tubes with 2500 Lux (35 µmol/m^2/s) light intensity with light/dark cycles of 12:12. A steady-state agitation was applied by bubbling air using an aquarium pump to aerate the cells, ensuring even exposure of the algae to the light for optimal growth and nutrition. The pH was measured using a pH-meter (WTW, 315i /SET, Germany). The culture purity assessment, identification of the cells and the calculation of the concentration were carried out using a Nageotte cell under an optical microscope (Optech Linear Biostar B4, München, Germany). After 3 weeks of culturing, the algal cells were harvested by filtration through silkscreen canvas (150–200 mesh), washed with distilled water and finally dried for 3 days in shadow at ambient temperature (~20 °C). All the chemicals used to

Table 1 Characteristics of *Arthrospira platensis* strains

Designation	Name	Origin	Culture medium	Shape
Spirulina 1 (dry)	Hawaiien Spirulina Pacifica (HSP)	Cyanothec Corporation Hawaii, USA	Pacific ocean	Spiral
Spirulina 2 (dry)	Hiri-Tamenrasset (HTAM)	Hiri company, (Tamanrasset, South of Algeria)	Hiri medium (Table 2)	Wavy
Spirulina 3 (dry)	Dihé	Chad	Lack Chad	Spiral
Spirulina 4 (fresh)	M2	University of Science and Technology (Ouargla, Algeria)	Zarrouk medium (Table 2)	Straight
Spirulina 5 (fresh)	Hiri-Tamenrasset (HTAM)	Hiri company (Tamanrasset, South of Algeria)	Zarrouk medium	Wavy

Table 2 Chemical composition of Hiri medium and Zarrouk's medium

Component (g/L)	Hiri medium (HM)	Zarrouk's medium (ZM)
$NaHCO_3$	16.00	16.80
NaCl	1.00	1.00
$NH_4H_2PO_4$	0.10	
$NaNO_3$		2.50
$CaCl_2/2H_2O$	0.10	0.02
EDTA		0.08
$FeSO_4/7H_2O$	0.01	0.01
$MgSO_4/7H_2O$	0.10	0.20
K_2SO_4	0.50	1.00
K_2HPO_4		0.50
Urée azotée $CO(NH_2)_2$	0.10	
Micronutrient[a]		1 mL

[a]Micronutrient solution: H_3BO_3, 2.86; $MnCl_2/4H_2O$, 1.81; $ZnSO_4/4H_2O$, 0.222; Na_2MoO_4, 0.0177; $CuSO_4/5H_2O$, 0.079 (g/L) [23]

prepare the culture medium and for analysis were of analytical grade obtained from the Biochem Chemopharma (France).

The growth rate of *Spirulina* strains was determined by following the dry weight method of Madkour et al. [23]. In this method, 10 mL of algal culture samples were filtered through dried and pre-weighed Whatman filter membrane (Ø 47 mm and nominal pore size 0.45 µm) and washed twice with distilled water. The algal cells on the filter membranes were then dried for 4 h at 80 °C and were cooled in desiccators before weighing them.

Biochemical analysis

The moisture content was determined by drying 2 g of alga sample at 105 °C in a hot air oven until constant weight was obtained. The dietary fiber contents were determined by the method described by Van Soest (1963) [24]. Total ashes were determined by incinerating 5 g sample at 550 °C for 3 h using an incinerator (Linn Electro Therm, model, Germany). This method consisted of measuring the weight of samples before and after the incineration.

The total carbohydrate content was determined according to the methodology described by Dubois et al. [25] using glucose as a standard to plot the calibration curve.

The total protein concentration was determined using Kjeldahl method [26]. The mineralization and distillation of the samples were carried out using an incineration and distillation apparatus (Behr Labor-Technik, Behrosog and Behr S2, Germany).

The lipid contents were extracted by following a modified method of Xu et al. [27], using chloroform:methanol (2:1, v/v) mixture solvent. After refluxing the sample in a Soxhlet apparatus, the solvent was then evaporated under pressure by rotary vacuum evaporator (Rotavapor, Heidolph G1; Germany) and the residue was dried and weighted.

The algae fatty acid profile was determined by using Gas Chromatography (GC, Chrompack CP 9002, Netherlands) according to the methodology described by Tokusoglu and Ünal [28]. The fatty acids from the algae were converted to their corresponding fatty acids methyl esters (FAME) by methylation through Wolff method [29]. The separation of FAME was conducted using a capillary GC-column (DB-23, 50% Cyanopropyl, 30 m × 0.32 mm ID; film thickness 0.25 µm). Methyl-esters were injected (Split 1/100 injector) and detected (FID detector) at a temperature of 250 °C. A standard mixture of FAME (purity 99% by GLC, sigma Co.) was used for the identification of the major fatty acids in the algal strains.

The microalgae total calorific strength was determined by multiplying the total lipid, protein and carbohydrate content values by 9, 4 and 4 Kcal respectively, and then summing the obtained results.

Phytochemical analysis

Determination of Chlorophyll a, b and carotenoids

Chlorophyll *a, b* and carotenoids were determined according to the modified method described by El-Sheekh and Fathy [30]. In this method, 1 g of each sample was suspended in 50 mL of acetone and stirred vigorously with magnetic stirrer (Stuart stir SB161, UK). The solutions were then placed in the dark at 4 °C and centrifuged at 4000×*g* for 10 min using centrifuge (Kseroa Monaco, No. 4222). The obtained supernatants were used to determine the concentration of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and total carotenoids (Car). The concentration of Chl *a*, Chl *b* and Car were determined spectrophotometrically at 645, 662 and 470 nm respectively using a UV/Vis spectrophotometer (Jenway Genova plus, Staffrdshire, UK). The contents (mg/g) of each pigment were quantified using Eqs. (1–3) [31].

$$Chl_a = 13.75 \cdot A_{664} - 5.19 \cdot A_{649}; \quad (1)$$

$$Chl_b = 27.43 \cdot A_{649} - 8.12 \cdot A_{664}; \quad (2)$$

$$Car = (1000 \cdot A_{470} - 2.13 \cdot Chl_a - 97.64 \cdot Chl_b/209 \quad (3)$$

where, A_{662}, A_{645} and A_{470} are absorbance wavelengths at 662, 645 and 470 nm respectively by the sample.

Determination of water soluble pigments (phycobiliproteins)

The extraction and estimation of phycobiliprotein components including phycocyanin (C-PC), allophycocyanin (APC) and phycoerythrin (PE) were determined by using spectrophotometer with wavelengths 620, 652 and 562 nm respectively according to the method described by Anamika et al. [32]. The contents of each component were measured using Eqs. (4–6).

$$C\text{-}PC = [A_{620} - (0.474 \cdot A_{652})]/5.34 \quad (4)$$

$$APC = [A_{652} - (0.208 \cdot A_{620})]/5.09 \quad (5)$$

$$PE = [A_{562} - (2.41 \cdot C\text{-}PC) - (0.849 \cdot APC)]/9.62 \quad (6)$$

where, A_{620}, A_{652} and A_{562} are the absorbance of light at 620, 652 and 562 nm respectively by the sample.

The percentage of crude phycocyanin was estimated by using the method described by Jourdan [33] and calculated by using Eq. (7). The experiment consists of dissolving 4 g of *Spirulina* into 100 mL distilled water.

$$\%PC = 1.873 \cdot [A_{615} - (0.474 \cdot A_{652})] \cdot DIL/C \quad (7)$$

where, DIL is the dilution factor and C is the percentage of the dry powder.

Determination of total phenols

The dry algal samples were ground to fine powder. A known powder amount (0.5 g) was used for the extraction of phenols using 10 mL of methanol (80%) at 35 °C for 24 h according to method described by Cai et al. [34]. The methanol samples were then cooled at room temperature and centrifuged at 4000×*g* for 10 min using a centrifuge (Kseroa Monaco, No. 4222). The obtained supernatant was used for the estimation of total phenolic content.

The total phenolic content present in microalgae was determined using Folin-Ciocalteu reagent [35]. The results were expressed as gallic acid equivalent (GAE)/g dry weight of microalgae.

Statistical analysis

The analyses were conducted in triplicate and the mean values are presented and expressed as mean value ± standard derivation (Mean ± SD). Analysis of variance (ANOVA) was performed using the General Linear Models of the STATGRAPHICS Centurion XVI.II software (STATPOINT Technologies, Inc. http://www.STATGRAPHICS.com). The multiple comparisons of means of each analysis were determined using the least significant difference (LSD) test at the confidence level of 95% and the difference between mean values greater than the LSD (0.95) was determined as significant.

Results and discussion

Strain morphology

The fresh *Spirulina* strain cells (Fig. 1) were multi-cellular and filamentous with cylindrical blue-green trichomes, forming an open helix which is the characteristic of the *Arthrospira* genus [3]. However, the *Spirulina* cells had different shapes. For example, the *Spirulina* 4 was straight filament, whereas, the *Spirulina* 5 (B and B′, Fig. 1) had a wavy shape. One of the main characteristics of this genus is the variable morphology under different environmental conditions [36]. Also, the degree of spiralisation show great variation and the *Spirulina* cells sometimes appear as straight trichomes in the culture [21]. These straight filaments are the *Spirulina* which need to be harvested [33].

The *Spirulina* B′ had changed from wavy to straight form, which is probably due to the change of the culture medium condition (Hiri medium vs Zarrouk medium). The physical and chemical conditions of the growing medium could alter the filamentous shape of the *Spirulina* strains

Fig. 1 Morphological variability of *A. platensis*. **A** Straight filament (Spirulina 4); **B, B′** Wavy shape (Spirulina 5). *Bar* represents 500 µm

[6]. According to Dhiab et al. [37], the change in the morphology of *Spirulina* trichome (from helical to straight shape) is due to the change in NaCl concentrations of the growing medium.

Biomass productivity

Different authors have reported that, in microalgae cultures, the nutritional condition is the main factor that can affect the growth and productivity [3, 6, 22, 23, 33, 38]. In this study, the *Spirulina* was cultivated and grown for a period of 23 days. The growth rate is expressed as dry weight (g/L) in ZM medium (Fig. 2). For the two fresh strains (*Spirulina* 4 and 5), the maximum biomass concentration and cell density were 0.60 g/L and 2.16×10^7 cell / mL respectively. These results are in accordance with those reported by Kumar et al. [6] and Aouir et al. [39].

The physicochemical composition

Table 3 presents the nutrient profiles and biochemical composition of the five *Spirulina* powders. The moisture contents of the samples were 4.70, 5.17, 8.40, 8.63 and 9.77% for *Spirulina* 1, 2, 3, 4 and 5 respectively. For all *Spirulina* samples, the moisture content was less than 10%, which is the recommended condition for long-term storage of *Spirulina* powders [40].

The total ash contents of the studied *Spirulina* varied significantly ($p<0.05$) and were in the range of 8.23–18.11%. Except for *Spirulina* 3, for all strains, the ash contents were in the range of 7–10% and similar results were reported by Tokusoglu and Ünal [28]. The high ash content in *Spirulina* 3 cells can be attributed to the presence of sand and some impurities resulting from the traditional harvesting and drying process of Chad *Spirulina* which is called "Dihé" [41]. Recently, Bensehaila et al. [42] reported 6.88% of ash in *A. platensis* from Tamenrasset (South of Algeria). In our study, for the same strain (*Spirulina* 2), we found 9.3% of ash, which is probably due to the difference in growing conditions.

The total carbohydrate content of the *Spirulina* samples was almost the same (avg. 15%) except for *Spirulina* 3 or "Dihé" sample which had lower concentration (3.64%). The carbohydrate content of *Spirulina* 1, 2, 4 and 5 were almost the same (15–25%) and similar values were reported by Quillet [43].

The protein contents of the *Spirulina* strains studied were varied significantly. The samples had very high protein concentrations (avg. 60%) except in *Spirulina* 3, which had only 23.78% of protein. This could be attributed to the poor nutritive growing media condition (Lake Chad) of this species (*Spirulina* 3); while the other strains were grown in monitored conditions (pond cultures). According to Sorto and Gonnet [41], the presence of sand (over 30%),

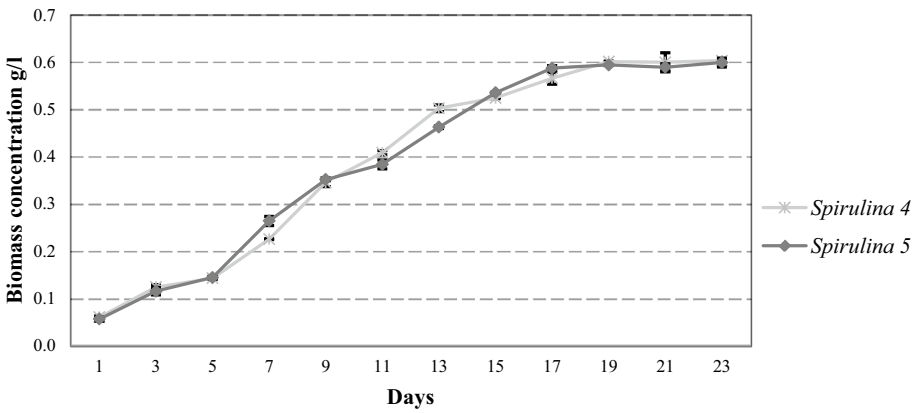

Fig. 2 Biomass concentration (g/L) of *Spirulina* 4 and 5 grown in Zarrouk medium

Table 3 The proximate composition parameters of different *Spirulina* strains

Parameters	*Spirulina* 1	*Spirulina* 2	*Spirulina* 3	*Spirulina* 4	*Spirulina* 5
Moisture (%)	4.70 ± 0.025^{a}	5.17 ± 0.072^{b}	8.40 ± 0.054^{c}	8.63 ± 0.028^{d}	9.77 ± 0.011^{e}
Total ash (%)	9.20 ± 0.113^{b}	9.30 ± 0.028^{c}	18.11 ± 0.076^{e}	8.23 ± 0.006^{a}	11.10 ± 0.092^{d}
Carbohydrate (%)	15.63 ± 0.024^{c}	15.82 ± 0.087^{c}	3.64 ± 0.052^{a}	13.68 ± 0.091^{b}	15.26 ± 0.056^{c}
Crude protein (%)	62.86 ± 0.009^{c}	59.23 ± 0.103^{b}	23.78 ± 0.027^{a}	59.80 ± 0.037^{bc}	59.35 ± 0.013^{bc}
Crude lipid (%)	8.08 ± 0.056^{e}	2.38 ± 0.027^{b}	1.50 ± 0.34^{a}	2.95 ± 0.064^{c}	4.79 ± 0.075^{d}
Crude fiber (%)	3.05 ± 0.025^{a}	3.58 ± 0.047^{c}	3.54 ± 0.075^{bc}	3.79 ± 0.058^{d}	3.52 ± 0.048^{b}
Energy (kJ)	1602.59 ± 2.25^{d}	1329.93 ± 1.85^{b}	511.80 ± 2.48^{a}	1327.34 ± 3.51^{b}	1413.1 ± 2.35^{c}

Means with different superscript across rows are significantly different at $p<0.05$

worms, plants debris, animals and insects in Lake Chad affect the nutritional and hygienic qualities of the *Spirulina* from there. Bensehaila et al. [42] reported 60.32% proteins content, which is higher than the 59.33% observed in our study, for the same strain (*Spirulina* 2). This slight difference could be due to the difference in analysis conditions. Nevertheless, the protein content of the *Spirulina* strains investigated still shows higher value than those of many other food protein sources (vegetables: 22%, meat: 25%, fish: 20%). *A. platensis* protein contents have shown very high digestibility (75–83%) due to the lack of cellulose cell walls in *Spirulina*. Therefore, it is a preferred source of food for major protein intake [10].

The highest total lipid content was observed for *Spirulina* 1 (8.08%). The concentrations for the other strains were 2.38, 1.5, 2.95 and 4.79% for *Spirulina* 2, 3, 4 and 5 respectively (Table 3). Bensehaila et al. [42] found 7.28% lipid content in *Spirulina* 2 which is very high compared to the result observed in this study for the same *Spirulina* strain (2.38%). This difference is closely related to the differences in pH, temperature and lighting in the culture conditions [6]. From the several microalgae species selected by Franke et al. [44], the Cyanophytes contained very low fat content (4.4–7.4%); which is in accordance with our results. According to Tokusoglu and Ünal [28] and Capelli and Cysewski [45], the lipid content in *Spirulina* varies between 5 and 8%. Tomabene et al. [46] found a higher content of lipids in *S. platensis* (16.6% of dry matter). However, Hudson and Karis [47] reported 11% of lipids in *Spirulina maxima*. Under different growth conditions, the lipid content in *Spirulina* was significantly different. For example, high light intensities and temperature of 38 °C don't affect the fatty acid composition but had a drastic effect on the fatty acid content, reducing it by as much as 46% [48]. In addition, when there is a nutrient deficiency in term of nitrogen and phosphorus, the lipid accumulation in *Spirulina* was increased [49]. Hence, according to Orcutt and Patterson [50], light and pH parameters can also affect the lipid metabolism. The total lipid content increased at low pH and it decreased at high pH in *Chlamydomonas sp* [51]. However, Richmond [2] reported that microalgae lipid production depends on the species and their culture conditions such as nutrients, salinity, light intensity, temperature, pH and even the association with other microorganisms.

The fiber content in all the *Spirulina* was about 3%. These results are similar with those reported by Koru [9], who found 3% fiber content in *Spirulina*. These results confirm that the lower fiber content suggests an easily digestible biomass for human use [2, 10].

Finally, all the strains showed higher values of total energy contents in the range of 1327.34–1602.59 kJ, except for *Spirulina* 3, with lower energy (511.80 kJ), which is due to its poor carbohydrate, protein and lipid compositions (Table 3). This could be explained by the fact that the traditional "Dihé" production process is not much suitable to obtain a high *Spirulina* nutritional value, because algal growing conditions are not enriched and/or controlled. The harvesting and drying conditions are done traditionally, which could affect the nutrients and microbiological quality [41].

Fatty acids profile

The values obtained for fatty acids (FA) analyzed were expressed in percentages of total fat and are presented in Table 4. The major FA compound for all *Spirulina* strains is palmitic acid (16:0) with the mean of 43.60, 46.45, 71.15, 51.33 and 48.19% for *Spirulina* 1, 2, 3, 4 and 5 respectively. The highest amount was found for *Spirulina* 3 which is probably due to the lack of phosphorus in the growing medium [49]. Experiments carried out by Tokusoglu and Ünal [28] and Piorreck et al. [52] show that the palmitic acid (PA) content in *Spirulina* is between 26 and 39%. Falquet and Hurni [53] also reported a relatively high proportion of PA, in the range of 25–60% which probably reflects the variation in fatty acid content among the samples. Several other authors have also reported that the predominant fatty acid in *Spirulina* is a palmitic acid and are in the range of 35–54.1% [1, 53].

The statistical analysis shows significant differences ($p < 0.05$) in the stearic acid (SA) content of the samples. The highest and the lowest values were 3.33 and 0.69% for *Spirulina* 1 and 5, respectively. According to Falquet and Hurni [53], the SA content in *Spirulina* was between 0.5 and 2%. Oliveira et al. [54] found that the highest C18:0 content was 2.76% obtained in *Spirulina* grown in a medium with temperature of 30 °C.

The oleic acid (OA, 18:1n-9) content in our samples was in the range of 4.02–5.99% which is almost in the range (5–16%) reported by Hudson and Karis [47]. According to Habib et al. [1], oleic acid in *Spirulina* was varied from 1 to 15.5%.

Essential FAs means that the human body does not synthesize them in enough amounts and thus they need to be obtained through diet [55]. Therefore, the estimation of these FAs is very important for evaluating the potential of microalgae for human health. Linoleic acid (LA, 18:2n-6) levels of *Spirulina* were high, with a mean of 19–20% except for *Spirulina* 3 which had only 7.86%. These results are in accordance with the $17.6 \pm 2.3\%$ value reported by Xue et al. [56]. Several authors have reported that the linoleic acid content of *Spirulina* were in the range of 27% [45] to 30.7% [1].

Spirulina samples investigated in this study contained high amounts of γ-linolenic acid (GLA, 18:3n-6), with a mean concentration of 4.32%, except in *Spirulina* 3 that has

Table 4 Fatty acids composition of 5 *Spirulina* samples as percent of total lipid

Fatty acids	*Spirulina* 1	*Spirulina* 2	*Spirulina* 3	*Spirulina* 4	*Spirulina* 5
C13:0	ND	ND	ND	ND	ND
C14:0	0.42 ± 0.01^{a}	1.46 ± 0.03^{b}	3.25 ± 0.19^{c}	3.26 ± 0.21^{c}	3.65 ± 0.02^{d}
C14:1	0.06 ± 0.00^{a}	0.14 ± 0.01^{b}	0.23 ± 0.02^{d}	0.23 ± 0.01^{c}	0.07 ± 0.01^{a}
C15:0	0.15 ± 0.01^{b}	0.13 ± 0.01^{b}	0.37 ± 0.01^{c}	0.08 ± 0.00^{a}	0.09 ± 0.01^{a}
C16:0	43.60 ± 0.01^{a}	46.45 ± 0.08^{b}	71.15 ± 0.02^{e}	51.33 ± 0.02^{d}	48.19 ± 006^{c}
C16:1	7.60 ± 0.00^{d}	5.49 ± 0.03^{c}	4.36 ± 0.16^{a}	4.83 ± 0.07^{b}	4.75 ± 0.01^{b}
C17:0	0.32 ± 0.00^{d}	0.24 ± 0.00^{b}	0.37 ± 0.01^{e}	0.16 ± 0.01^{a}	0.27 ± 0.01^{c}
C17:1	0.21 ± 0.01^{c}	0.21 ± 0.01^{c}	0.50 ± 0.02^{d}	0.18 ± 0.00^{b}	0.13 ± 0.01^{a}
C18:0	3.33 ± 0.16^{e}	1.67 ± 0.04^{d}	1.42 ± 0.03^{c}	1.10 ± 0.01^{b}	0.69 ± 0.03^{a}
C18:1n-9	4.71 ± 0.23^{b}	4.73 ± 0.18^{b}	4.02 ± 0.19^{a}	5.23 ± 0.01^{c}	5.99 ± 0.04^{d}
C18:2n-6	20.15 ± 0.00^{c}	19.26 ± 0.02^{b}	7.86 ± 0.22^{a}	18.94 ± 0.22^{b}	19.82 ± 0.07^{c}
C18:3n-6	4.32 ± 0.01^{b}	4.22 ± 0.07^{b}	2.73 ± 0.02^{a}	4.19 ± 0.00^{b}	4.50 ± 0.04^{c}
C18:3n-3	0.01 ± 0.00^{b}	ND^{a}	ND^{a}	ND^{a}	0.02 ± 0.01^{c}
C20:0	15.12 ± 0.03^{d}	15.71 ± 0.16^{e}	3.75 ± 0.11^{a}	10.24 ± 0.14^{b}	11.65 ± 0.01^{c}
C20:3n-6	ND^{a}	0.29 ± 0.00^{b}	ND^{a}	ND^{a}	ND^{a}
C24:1	ND^{a}	ND^{a}	ND^{a}	0.23 ± 0.02^{b}	ND^{a}

ND not detected

Means with different superscript across rows are significantly different at $p < 0.05$

only 2.73%. According to Kent et al. [57], *Spirulina* contain high quantities of γ-linolenic acid (9.42%) than all others microalgae strains (*Nannochloropsis* sp., *Scenedesmus* sp., *Chlorella* sp. and *Dunaliella* sp.). Tokusoglu and Ünal [28] have compared the GLA content of three kinds of algae (*Spirulina platensis, Isochrisis galbana and Chlorella vulgaris*) and they reported that *Spirulina platensis* species has a much higher γ–linolenic acid content (3.64–5.52%) compared to *Isochrisis galbana* (0.54%) and *Chlorella vulgaris* (traces %). In addition, Franke et al. [44] selected several species of microalgae and defined their fatty acid composition; they found that the GLA in *Spirulina* was in the range of 16–23%. Whereas, Habib et al. [1], reported that GLA in *Spirulina* was in the range of 8–31.7%. Tomaselli et al. [58] and Quoc and Dubacq [59] have reported that one of the reasons of these variations in FA content could be attributed to the difference in temperature used for cultivation of the *Spirulina*. Therefore, when the temperature of the medium increases, the lipid cells content increases considerably and the FA composition changed towards a higher degree of saturation (GLA biosynthesis was progressively hampered and LA was accumulated).

According to Mühling et al. [11] there are considerable differences in the FA composition, especially in the PUFA fraction, among the *Spirulina* strains. Cohen et al. [48], studied the FA distribution in 19 strains of *Spirulina* grown under various environmental conditions. They found that the fatty acid content increased with the cultivation temperature and the amount of PUFA was decreased. Also, the highest content of GLA was found in the strains grown at 30–35 °C.

Regarding the alpha-linolenic acid (ALA, 18:3n-3) or (ω-3), it was found that only *Spirulina* 1 and 5 contain a trace amount of it. It is reported in the literature that *Spirulina* in general do not have ω-3 fatty acids compared to other microalgae [57].

The levels of arachidic acid (AA, 20:0) in the strains investigated were high and the contents were varying significantly ($p < 0.05$) from 10.24 up to 15.71%. However, the *Spirulina* 3 had a lower nutrient content with 3.75% of AA. Nevertheless, this value of AA in *Spirulina* 3 is of reasonably high quantity, since the *Spirulina platensis* strains studied by Tokusoglu and Ünal [28] contained no amount of AA. In addition, the amount of AA of all the *Spirulina* investigated in this study is very higher than those found in *Chlorella vulgaris* and *Isochrisis galbana* strains (0.19 and 0.74%, respectively).

Mühling et al. [11] have noticed that the algal growing conditions could considerably affect the fatty acids content. For example, high light intensity and temperature favor the accumulation saturated FA [60, 61]. Meanwhile, low light intensities and low temperature promote the synthesis of PUFA [62].

Table 5 presents the sum of monounsaturated (MUFA), polyunsaturated (PUFA) and saturated (SFA) fatty acids in the *Spirulina* strains investigated. *Spirulina* algae contain greater quantities of ω-6 and traces of ω-3 FA. PUFA in the samples investigated were in the range of 23–24.5%, except in *Spirulina* 3 which had only 10.59%. The ω-6 quantities in *Spirulina* 1, 2, 4 and 5 were almost the same as that reported by Kent et al. [57] who found 20.04% of ω-6 in *Spirulina*.

Table 5 SFA, MUFA, PUFA, total ω-3, total ω-6 (as percent of total lipid)

Fatty Acids	*Spirulina* 1	*Spirulina* 2	*Spirulina* 3	*Spirulina* 4	*Spirulina* 5
∑SFA	62.94	65.66	80.31	66.17	64.54
∑MUFA	12.58	10.57	9.11	10.70	10.94
∑PUFA	24.48	23.77	10.59	23.13	24.52
∑ω-3	0.01	–	–	–	0.20
∑ω-6	24.48	23.77	10.59	23.13	24.32

Results do not include SD

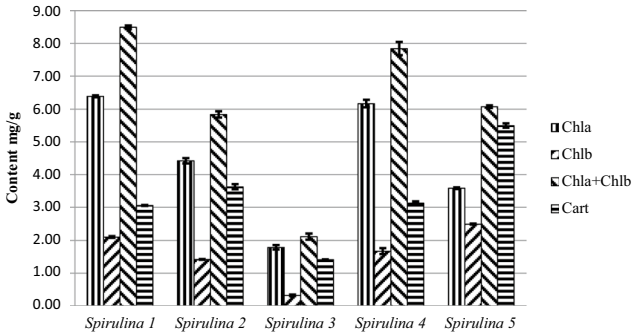

Fig. 3 Content of chlorophyll *a, b* and carotenoids (mg/g) in different *Spirulina* strains

According to the obtained result, the most important PUFA in *Spirulina* samples studied in this present work are linoleic acid and Gamma-linolenic acid (ω-6). Simopoulos [63] showed that the balance between ω-6 and ω-3 FA in foods is essential for human health benefits, and high ω-6/ω-3 ratios have been implicated in the higher chances of cancer, cardiovascular and inflammatory diseases. The same author recommended an optimal ratio of ω-6/ω-3 in the range of 1/1–4/1 as the most beneficial for human health, whereas the human diets have a ratio in the range of 15/1–16.7/1. However, in our case, we can't define the ω-6/ω-3 ratio, since the ω-3 was almost undetectable in the *Spirulina* strains studied (Table 5). The same observations were reported by Kent et al. [57]. However, the deficiency of ω-3 in human diets has to be complemented by other foods rich with mostly PUFA such as nuts, seeds, and fish. Nevertheless, many researchers have reported that *Spirulina* is one of the best ω-6 whole food sources [7, 45].

Phytopigments composition

Chlorophyll a, b and carotenoids content

The amounts of chlorophylls and carotenoids observed in this study are shown in Fig. 3. The content of Chl *a* in *Spirulina* strains is significant ($p < 0.05$) with values of 6.39, 4.42, 1.79, 6.17 and 3.58 mg/g for *Spirulina* 1, 2, 3, 4 and 5, respectively. The Chl *a* is the major photosynthetic pigments in microalgae compared to Chl *b* [2].

According to Kumar et al. [6] the temperature condition affects the metabolic processes and biochemical composition of cells, and the optimal growth temperature is usually depending on the strain. In their study, the highest value of Chl *a* and carotenoids accumulation observed are 1.54 and 0.27%, respectively, obtained at a growth temperature of 35 °C.

El-sheikh and Al-fathy [30] reported that the autotrophic and heterotrophic growing conditions may considerably affect the phytopigments content. They found that, the Chl *a* in *Chlorella vulgaris* cells was higher in autotrophic and lower in heterotrophic growing conditions; whereas, the opposite trend was observed for Chl *b*.

In this study, the chlorophylls content was measured by summing Chl *a* and Chl *b*. The *Spirulina* 1 and 4 contained significantly higher mean values ($p < 0.05$) of chlorophylls and were 8.49 and 7.84 mg/g, respectively.

Carotenoids content were higher in *Spirulina* 5 (5.50 mg/g) than the other samples. This is in accordance with the values reported by M'baye et al. [64] that were in the range of 5.43–8.93 mg/g of carotenoids in 51 Mauritanian *Spirulina* samples.

Pierlovisi [65] and Ripley [3] suggested that the average phytopigments content in *Spirulina* are 11.5, 6.8 and 3.3 mg/g for chlorophylls, Chl *a* and carotenoids, respectively. In addition, Capelli and Cysewski [45] found that total chlorophylls and carotenoids in *Spirulina* are in the range of 10–5 mg/g, respectively. However, Kent et al. [57] found 12.33 and 1.45 mg/g of chlorophylls and carotenoids, respectively, in the same strain. As mentioned above, these differences may be due to the *Spirulina* growing conditions. According to the obtained results, compared to carotenoids, chlorophylls are the major pigment in the *Spirulina* microalgae.

Phycobiliprotein content

The amount of water soluble phycobiliprotein in *Spirulina* strains are shown in Table 6. The phycocyanin is the major phycobiliprotein in *A. platensis*. The phycoerythrin (PE) contents were very low in all the *Spirulina*

Table 6 Phycobiliproteins (CPC, APC and PE) content (%), crude phycocyanin (%) and purity

Strains	C-PC	A-PC	PE	Crude phycocyanin	Purity
Spirulina 1	8.70 ± 0.001^{d}	7.20 ± 0.001^{e}	2.80 ± 0.001^{c}	29.03 ± 0.001^{d}	1.80 ± 0.002^{d}
Spirulina 2	6.40 ± 0.001^{b}	3.10 ± 0.000^{c}	2.50 ± 0.000^{c}	21.36 ± 0.001^{b}	1.35 ± 0.001^{b}
Spirulina 3	1.80 ± 0.002^{a}	2.20 ± 0.001^{a}	1.30 ± 0.001^{a}	5.33 ± 0.002^{a}	0.40 ± 0.007^{a}
Spirulina 4	5.90 ± 0.002^{b}	1.10 ± 0.001^{b}	2.00 ± 0.001^{b}	19.86 ± 0.002^{b}	1.30 ± 0.006^{b}
Spirulina 5	7.20 ± 0.001^{c}	5.10 ± 0.000^{d}	3.30 ± 0.001^{d}	24.18 ± 0.001^{c}	1.44 ± 0.005^{c}

Means with different superscript across rows are significantly different at $p < 0.05$

strains investigated with a significant difference ($p < 0.05$) in the phycobiliprotein content among all the samples. The *Spirulina* 1 contains the highest C-PC and A-PC amounts with an average of 8.7 and 7.2% respectively, followed by *Spirulina* 5 (C-PC: 7.2%, APC: 5.1%), *Spirulina* 2 (C-PC: 6.4%, APC: 3.1%) and *Spirulina* 4 (C-PC: 5.9%, APC: 1.1%). These results are in accordance with those reported by Kumar et al. [6], who studied the effect of different temperature and light intensities on the pigments composition of *S. platensis*. They reported that the highest phycobiliproteins content (C-PC: 7.73%, APC: 3.46%, PE: 1.8%) was obtained when the *Spirulina* were grown at 35 °C temperature and with 2000 Lux light intensity. However, Anamika et al. [32] reported higher *Spirulina* phycobiliprotein content values (C-PC: 17.5%, APC: 3.8%, PE: 1.2%), confirming that growth conditions have significant effect on phytopigment content.

The values of crude *Spirulina* phycocyanin content are also shown in Table 6. The highest value was obtained for *Spirulina* 1 (29.03%). Except for *Spirulina* 3, the phycocyanin content values of all the *Spirulina* samples were more than 10% than the values reported by Jourdan [33]. According to Cuellar-Bermudez et al. [15], the phycocyanin is the major phycobiliprotein in *A. platensis* and can constitute up to 20% of its dry weight.

In this study, the average purity grade of the *Spirulina* strain, except for *Spirulina* 3, was more than 0.7 which is considered as food grade purity. According to Rito-Palomares [66], a purity grade of 0.7 is considered as food grade, 3.9 as reactive grade, and above 4 as analytical grade.

Total phenolic content

Figure 4 shows the total phenolic content of *Spirulina* strains expressed as a Gallic Acid Equivalent (GAE). The results showed that *Spirulina* 5 have the highest total phenolic content with 67.52 mg GAE/g of dry weight followed by *Spirulina* 1 (48.93 mg GAE/g), *Spirulina* 2 (45.22 mg GAE/g), *Spirulina* 3 (19.61 mg GAE/g) and *Spirulina* 4 (39.33 mg GAE/g). These results are in accordance with those reported by Benahmed et al. [67] who found 51.325 mg GAE/g in *Spirulina*. The work carried out by

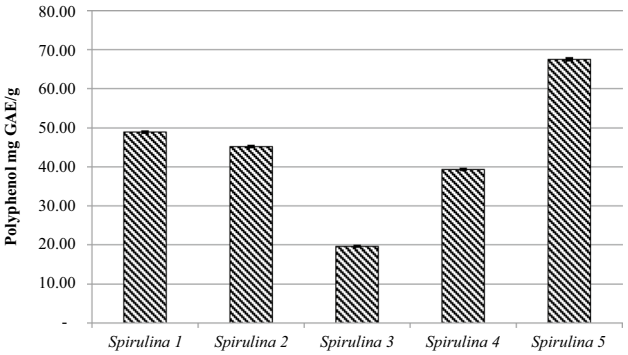

Fig. 4 Polyphenols content of different *Spirulina* strains

Harwati [68] on microalgae like *Chlorococcum* sp. and *Nannochloropsis* sp. showed that total phenolic content of these species were 2.41 and 2.05 mg GAE/g dry weight respectively. Whereas, Li et al. [69] reported that, *Chlamydomonas rivialis* and *Chlorella vulgaris* had relatively high total phenolic contents (>15 mg GAE/g). According to them, the medium composition had a significant influence on the total phenolic content of microalgae. According to our results *Spirulina* microalgae contain higher polyphenols content compared to other microalgae and fruits such as grapes, apples, pears, cherries and berries which contain only 2–3 mg/g of polyphenols [70]

Conclusion

This study has characterized different strains of *Spirulina* from Algeria, Chad and the USA towards their use in the nutraceutical and functional food area. The nutrient composition parameters and antioxidants compounds are more important in *Spirulina* samples 1 and 5 compared to the others. The strains growing in different culture media do not present the same nutrient composition. Hence, different strains with different shapes (straight vs wavy shape) cultivated in the same medium present difference in nutrient composition. Culture and nutrient conditions affect the cell composition and the biomass productivity. The majorities of *Spirulina* strains tested show an excellent nutritional profile and are rich in phytonutrients (phycocyanin,

carotenoids and polyphenols) that have potential health benefits. Nevertheless, *Spirulina* 1 has higher nutritional contents, and a great potential for use in human nutrition. *Spirulina* 3 or "Dihé" has a lower nutritional composition. Therefore, it is highly recommended to use *Spirulina* strains grown under controlled growth conditions for production of nutrient and bioactive compounds for nutraceutical purposes.

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References

1. M.A.B. Habib, M. Parvin, T.C. Huntington, M.R. Hasan, A review on culture, production and use of *Spirulina* as food for humans and feeds for domestic animals and fish. FAO Fisheries and Aquaculture Circular No. 1034, Rome, 2008), p. 33
2. A. Richmond, *Handbook of Microalgal Culture: Biotechnology and Applied Phycology* (Blackwell, Oxford, 2004), p. 566
3. R.D. Fox, *Spiruline, Technique pratique et promesse* (Edisud, Aix-en-Provence, 1999), p. 246
4. M. Abu Affan, D.W. Lee, S.M. Jeon, J.H. Noh, S.J. Heo, C. Oh, H.S. Park, H.S.A. Khomayis, D.H. Kang, Bituminous coal and sodium hydroxide-pretreated seawater stimulates *Spirulina* (*Arthrospira*) *maxima* growth with decreased production costs. Aquaculture **436**, 121–126 (2015)
5. A. Vonshak, Spirulina platensis (*Arthrospira*) *Physiology, Cell-Biology and Biotechnology* (Taylor & Francis, London, 2002), p. 252
6. M. Kumar, J. Kulshreshtha, G.P. Singh, Growth and biopigment accumulation of Cyanobacterium *Spirulina platensis* at different light intensities and temperature. Braz. J. Microbiol. **42**, 1128–1135 (2011)
7. T.S. Vo, D.H. Ngo, S.K. Kim, Nutritional and pharmaceutical properties of microalgal Spirulina, in *Handbook of Marine Microalgae: Biotechnology Advanced* (Elsevier, Amsterdam, 2015), p. 585
8. I. Priyadarshani, B. Rath, Commercial and industrial applications of microalgae. J. Algal Biomass Util. **3**(4), 89–100 (2012)
9. E. Koru, Earth food Spirulina (*Arthrospira*): Production and quality standards, in *Food Additive*, ed. Y. El-Samragy (In Tech, Rijeka, 2012), p. 256
10. S. Sguera, *Spirulina platensis* et ses constituants intérêts nutritionnels et activités thérapeutiques. Thèse de Doctorat. Université Henri Poincaré - Nancy 1 Faculté De Pharmacie, 2008, p. 162
11. M. Mühling, A. Belay, A.B. Whitton, Variation in fatty acid composition of *Arthrospira* (*Spirulina*) strains. J. Appl. Phycol **17**, 137–146 (2005)
12. R. Rodríguez-Sánchez, R. Ortiz-Butrón, V. Blas-Valdivia, A. Hernández-García, E. Cano-Europa, Phycobiliproteins or C-phycocyanin of *Arthrospira* (*Spirulina*) maxima protect against $HgCl_2$-caused oxidative stress and renal damage. Food Chem. **135**, 2359–2365 (2012)
13. K. Brejc, R. Ficner, R. Huber, S. Steinbacher, Isolation, crystallization, crystal structure analysis and refinement of allophycocyanin from the Cyanobacterium *Spirulina platensis* at 2.3 A resolution. J. Mol. Biol. **249**, 424–440 (1995)
14. S. Boussiba, A. Richmond, Isolation and purification of phycocyanins from the blue-green alga *Spirulina platensis*. Arch. Microbiol. **120**, 155–159 (1979)
15. S.P. Cuellar-Bermudez, I. Aguilar-Hernandez, D.L. Cardenas-Chavez, N.O. Soto, M. A. Romero-Ogawa, R. Parra-Saldivar, Extraction and purification of high-value metabolites from microalgae: essential lipids, astaxanthin and phycobiliproteins. Microb Biotechnol. **8**(2), 190–209 (2015)
16. N.T. Eriksen, Production of phycocyanin—a pigment with applications in biology, biotechnology, foods and medicine. Appl. Microbiol. Biotechnol. **80**, 1–14 (2008)
17. G. Penton-Rol, J. Martin-Prida, G. Pardo-Andreu, G. Martínez-Sánchez, E.F. Acosta-Medina, A. Valdivia-Acosta, N. Lagumersindez-Denis, E. Rodríguez-Jiménez, A. Llópiz-Arzuaga, P.A. López-Saura, G. Guillén-Nieto, E. Pentón-Arias, C-Phycocyanin is neuroprotective against global cerebral ischemia/reperfusion injury in gerbils. Brain Res. Bull. 86, 42–52 (2011)
18. Y. Ou, S. Zheng, L. Lin, Q. Jiang, X. Yang, Protective effect of C-phycocyanin against carbon tetrachloride-induced hepatocyte damage in vitro and in vivo. Chem. Biol. Interact. **185**, 94–100 (2010)
19. Y. Ou, L. Lin, X. Yang, Q. Pan, X. Cheng, Antidiabetic potential of phycocyanin: Effects on K KAy mice. Pharm. Biol. **51**(5), 539–544 (2013)
20. P.C. Dartsh, Antioxydant potential of selected *Spirulina platensis*. Phyother. Res. **22**, 627–633 (2008)
21. L. Tomaselli, Morphology, ultrastructure and taxonomy, in *Spirulina platensis (Arthrospira): Physiology, Cell-Biology and Biotechnology*, ed. A. Vonshak (Taylor & Francis, London, 1997), pp. 1–15
22. C. Zarrouk, Contribution à l'étude d'une cyanophycée. Influence de divers facteurs physiques et chimiques sur la croissance et photosynthèse de *Spirulina maxima*. (Setchell et Gardner) Geitler. PhD Thesis, University of Paris, Paris, 1966, p. 455
23. F.F. Madkour, A.E. Kamil, H.S. Nasr, Production and nutritive value of *Spirulina platensis* in reduced cost media. Egypt. J. Aquat. Res. **38**, 51–57 (2012)
24. P.J. Van Soest, Use of detergents in the analyses of fibrous feeds. II. A rapid method for the determination of fiber and lignin. J. Assoc. Off. Agric. Chem. **46**, 829 (1963)
25. M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, F. Smith, Colorimetric method for determination of sugars and related substances. Anal. Chem. **28**, 350–356 (1956)
26. J. Kjeldahl, A new method for the estimation of nitrogen in organic compounds. Anal. Chem. **22**, 366 (1883)
27. X. Xu, J. Beardall, D.N. Hallam, Modification of fatty acid composition in halophilic Antarctic microalgae. Phytochemistry **49**, 1249–1252 (1998)
28. Ö. Tokusoglu, M.K. Ünal, Biomass nutrient profiles of three microalgae: *Spirulina platensis, Chlorella vulgaris*, and *Isochrisis galbana*. J. Food. Sci. **68**(4), 1144–1148 (2003)
29. J.P. Wolff, *Manuel d'analyse des corps gras* (Edit. Azoulay, Paris, 1968), p. 552
30. M.M. El-Sheekh, A.A. Fathy, Variation of some nutritional constituents and fatty acid profiles of *Chlorella vulgaris* Beijerinck grown under auto and heterotrophic conditions. Int. J. Bot. **5**, 153–159 (2009)
31. H.K. Lichtenthaler, A.R. Wellburn, Determination of total carotenoids and chlorophyll a and b of leaf in different solvents. Biol. Soc. Trans. **11**, 591–592 (1985)
32. P. Anamika, M. Sandhya, P. Richa, P.K. Ghosh, Purification and characterization of C-phycocyanin from Cyanobacterial species of marine and freshwater habitat. Protein Expr. Purif. **40**, 248–255 (2005)
33. J.P. Jourdan, Cultivez votre spiruline: manuel de culture artisanale de spiruline (2011), p. 223

34. Y.Z. Cai, Q. Luo, M. Sun, H. Corke, Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci. **74**, 2157–2184 (2004)
35. V.L. Singleton, J.A. Rossi, Colorimetry of total phenolics with phosphomolybdic- phosphotungstic acid reagents. Am. J. Enol. Vitic. **16**, 144–158 (1965)
36. P. Scheldeman, D. Baurain, R. Bouhy, M. Scott, M. Muhling, A.B. Whitton, A. Belay, A. Wilmotte, *Arthrospira* (*Spirulina*) strains from four continents are resolved into only two clusters, based on amplified ribosomal DNA restriction analysis of the internally transcribed spacer. FEMS. Microbiol. Lett. **172**(2), 213–222 (1999)
37. R.B. Dhiab, H.B. Ouada, H. Boussetta, F. Franck, A. Elabed, M. Brouers, Growth, fluorescence, photosynthetic O_2 production and pigment content of salt adapted cultures of *Arthrospira* (*Spirulina*) *platensis*. J. Appl. Phycol. **19**(4), 293–301 (2007)
38. T. Ould Bellahcen, A. Bouchabchoub, M. Massoui, M. El Yachioui, Qualité nutritionnelle de *Spirulina platensis* en croissance dans les eaux usées domestiques. Larhyss. J. **14**, 122–129 (2013)
39. A. Aouir, M. Amiali, T. K. Gachovska, A. Benchabane, A. Bitam, The effect of pulsed electric field (PEF) and ultrasoud (US) technologies on the extraction of phycopiliproteins from *Arthrospira Platensis*. IEEE Canada International Humanitarian Technology Conference (IHTC), IEEE Communications Letters, 2015, pp. 1–4
40. E.W. Becker, *Microalgae Biotechnology and Microbiology* (Cambridge University Press, Cambridge, 1994), pp. 1–293
41. M. Sorto, J. Gonnet, Pilot project for the development of the Dihé food chain in Chad. (GCP/CHD/029/EC), INRAN, Rome, 29 Nov 2007, pp. 1–15
42. S. Bensehaila, A. Doumandji, L. Boutekrabt, H. Manafikhi, I. Peluso, K. Bensehaila, A. Kouache, A. Bensehaila, The nutritional quality of *Spirulina platensis* of Tamenrasset. Algeria. Afr. J. Biotechnol. **14**(19), 1649–1654 (2015)
43. M. Quillet, Research on carbohydrate substances produced by *Spirulina*. Ann. Nutr. Alim. **29**, 553–561 (1975)
44. H. Franke, M. Springer, O. Pulz, U. Tietz, U. Mueller, Polyunsaturated fatty acids from microalgae. Int. Food Ingred. **4**, 41–45 (1994)
45. B. Capelli, R.G. Cysewski, Potential health benefits of *Spirulina* microalgae. A review of the existing literature. Nutra Foods **9**(2), 19–26 (2010)
46. T.G. Tornabene, T.F. Bourne, S. Raziuddin, A. Bem-Amotz, Lipid and lipopolysaccharide constituents of Cyanobacterium *Spirulina platensis* (*Cyanophyceae, Nostocales*). Mar. Ecol. Prog. Ser. **22**, 121–125 (1985)
47. B.J.F. Hudson, I.G. Karis, The lipids of the alga *Spirulina*. J. Sci. Food Agric. **25**, 759–763 (1974)
48. Z. Cohen, A. Vonshak, A. Richmond, Fatty acid composition of *Spirulina* strains grown under various environmental conditions. Phytochemistry **26**, 2255–2258 (1987)
49. R.N. Bhakar, B.S. Pabb, Total lipid accumulation and fatty acid profiles of microalga *Spirulina* under different nitrogen and phosphorus concentrations. Egypt. J. Biol. **16**, 57–62 (2014)
50. D.M. Orcutt, G.W. Patterson, Effect of light intensity upon *Nitzschia closternium* (*Cylindrotheca fusiformis*). Lipids **9**, 1000–1003 (1974)
51. H. Tatsuzawa, E. Takizawa, M. Wada, Y. Yamamoto, Fatty acid and lipid composition of the acidophilic green alga *Chlamydomonas* sp. J. Phycol. **32**, 598–601 (1996)
52. M. Piorreck, K.H. Baasch, P. Pohl, Biomass production, total protein, chlorophylls, lipids and fatty acids of freshwater green and blue-green algae under different nitrogen regimes. Phytochemistry **23**(2), 207–216 (1984)
53. J.J. Falquet, P. Hurni, The nutritional aspects of *Spirulina*. Antenna Technologies (2006), p. 25, http://www.antenna.ch/documents/AspNutr2006.pdf
54. M.A.C.L. Oliveira, M.P.C. Monteiro, P.G. Robbs, S.G.F. Leite, Growth and chemical composition of *Spirulina maxima* and *Spirulina platensis* biomass at different temperatures. Aquac. Int. **7**, 261–275 (1999)
55. C.Y. Chen, C.Y. Chen, H.C. Huang, C.C. Huang, W.L. Lee, J.S. Chang, Engineering strategies for enhancing the production of eicosapentaenoic acid (EPA) from an isolated microalga *Nannochloropsis oceanica* CY2. Bioresour. Technol. **147**, 160–167 (2013)
56. C. Xue, Y. Hu, H. Saito, Z. Zhang, Z. Li, Y. Cai, C. Ou, H. Lin, A. Imbs, Molecular species composition of glycolipids from *Spirulina platensis*. Food Chem. **77**, 9–13 (2002)
57. M. Kent, H.M. Welladsen, A. Mangott, Y. Li, Nutritional evaluation of Australian microalgae as potential human health supplements. PLoS ONE **10**(2), 1–14 (2015)
58. L. Tomaselli, L. Giovannetti, G. Torzillo, Physiology of stress response in Spirulina spp., in *Spirulina Algae of Life*, ed. F. Doumenge, H. Durand-Chastel, A. Toulemont (Bulletin de l'Institut Océanographique, Spécial. 12, Monaco, 1993), pp. 65–75
59. K.P. Quoc, J.P. Dubacq, Effect of growth temperature on the biosynthesis of eukaryotic lipid molecular species by the Cyanobacterium *Spirulina platensis*. Biochim. Biophys. Acta 1346, 237–246 (1997)
60. E.A.T. Floreto, H. Hirata, S. Ando, S. Yamasaki, Effects of temperature, light intensity, salinity and source of nitrogen on the growth, total lipid and fatty acid composition of *Ulva pertusa Kjellman* (Chlorophyta). Bot. Mar. **36**, 149–158 (1993)
61. J. Van Wagenen, T.W. Miller, S. Hobbs, P. Hook, B. Crowe, M. Huesemann, Effects of light and temperature on fatty acid production in *Nannochloropsis salina*. Energies **5**, 731–740 (2012)
62. I.A. Guschina, J.L. Harwood, Lipids and lipid metabolism in eukaryotic algae. Prog. Lipid Res. **45**, 160–186 (2006)
63. A.P. Simopoulos, The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exp. Biol. Med. **233**, 674–688 (2008)
64. B.K. M'Baye, B. Lô, E. Bassene, Etude quantitative de quelques pigments de la Spiruline cultivée en Mauritanie en vue d'une valorisation nutritionnelle. Int. J. Biol. Chem. Sci. **5**(5), 2035–2038 (2011)
65. C. Pierlovisi, L'Hommeetla Spiruline: Un avenir commun? Composition chimique, intérêts alimentaires et activités biologiques. Thèse de Doctorat, Paris V- René Descartes, Faculté des Sciences Pharmaceutiques et Biologiques, Paris, 2007, p. 162
66. M. Rito-Palomares, L. Nunez, D. Amador, Practical application of aqueous two-phase systems for the development of a prototype process for C-phycocyanin recovery from *Spirulina maxima*. J. Chem. Technol. Biotechnol. **76**, 1273–1280 (2001)
67. D.A. Benahmed, Analyse des aptitudes technologiques de poudres de dattes (*Phoenix-dactilifera* L.) améliorées par la spiruline. Etude des propriétés rhéologique, nutritionnelles et antibactériennes. Thèse de Doctorat, faculté des sciences de l'ingénieur. Univ Mhamed Bougara-Boumerdes, Algeria, 2012, pp 1–188
68. T.U. Harwati, Cultivation of microalgae: lipid production, evaluation of antioxidant capacity and modeling of growth and lipid production. PhD Thesis, Faculty of Life Sciences at the Technical University Carolo-Wilhelmina at Brunswick, 2013, pp. 1–130
69. H.B. Li, K.W. Cheng, C.C. Wong, K.W. Fan, F. Chen, Y. Jiang, Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. Food Chem. **102**, 771–776 (2007)
70. A. Scalbert, I.T. Johnson, M. Saltmarsh, Polyphenols: antioxidants and beyond. Am. J. Clin. Nutr. 81, 215S–217S (2005)