Fatty Acids Profile of *Spirulina platensis* Grown Under Different Temperatures and Nitrogen Concentrations

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The influence of culture temperature and the concentration of sodium nitrate ($NaNO_3$) on the gas-chromatographic profile of the fatty acids of the filamentous cyanobacterium *Spirulina platensis* was evaluated. We found that temperature was the most important factor and that the greatest amount of gamma-linolenic acid (GLA) was obtained at 30 °C, the fatty acid profile of the *Spirulina* cultivated showing that (in order of abundance) palmitic, linolenic and linoleic acids were most prevalent.

Key words: Spirulina, Fatty Acids, Temperature

Introduction

The filamentous cyanobacterium *Spirulina platensis* has been used as a food for centuries by native peoples from Lake Chad in Africa and Lake Texcoco in Mexico (Vonshak, 1997; Henrikson, 1994), an observation which has led to the use of *Spirulina* as a food supplement for undernourished people in many parts of the world (Henrikson, 1994) due to its high protein content (65%), high digestibility (Henrikson, 1994) and specific amino acid content.

Preparations of *Spirulina*, sold in capsule form or in foods such as beverages and pastes, have been shown to have therapeutic properties in the treatment of conditions such as hypercholesterolemia and atherosclerosis (Ramamoorthy and Premakumari, 1996), pre-menstrual tension and arthritis and as an auxiliary in weight loss (Henrikson, 1994). The *Spirulina* components which are responsible for these therapeutic properties are thought to be compounds with antioxidant abilities such as polyunsaturated fatty acids, phycocyanin (Estrada *et al.*, 2001) and phenolics (Miranda *et al.*, 1998). Of the compounds present in *Spirulina*, gamma-linolenic acid (C18:3, ω6, GLA) and phycocyanin are those which have received most attention from researchers. Phycocyanin was first studied as a food colorant (Sarada *et al.*, 1999), while GLA has mainly been studied in respect to its therapeutic properties such as its ability to decrease blood cholesterol levels (Ishikawa *et al.*, 1989).

There has been much research on *Spirulina* as a potential source of GLA (Alonso and Maroto, 2000; Quoc *et al.*, 1994; Cohen *et al.*, 1987, 1993) and the growth conditions needed to increase GLA content, *e.g.* supplementation of culture media with exogenous fatty acids (Quoc *et al.*, 1994), low growth temperatures (Tedesco and Duerr, 1989; Cohen *et al.*, 1987), depletion of nitrogen source (Tedesco and Duerr, 1989; Piorreck *et al.*, 1984) and culture age and illumination (Olguín *et al.*, 2001; Quoc *et al.*, 1994; Cohen *et al.*, 1987). However, the production of purified GLA is costly (Cohen *et al.*, 1993; Roughan, 1988), and it appears that the most cost effective way to use the compounds present in this cyanobacterium is by direct consumption of *Spirulina* as capsules or a food additive.

The objective of the research presented in this paper was to evaluate the influence of growth conditions (nitrogen concentration and temperature) on the fatty acid profile of *Spirulina platensis*.

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Materials and Methods

Microorganism and culture medium

In this study we used *Spirulina platensis* strain LEB-52 (Costa *et al.*, 2000, 2003). Zarrouk's synthetic medium (Zarrouk, 1966) was used for culture maintenance and to prepare the inoculum as well as to study the growth of *S. platensis* in batch culture, the concentration of sodium nitrate (the nitrogen source) in this medium being modified according to the experimental design explained below in section *Experimental design.* All the reagents used were analytical grade, obtained from either the Merck Chemical Co. (Darmstadt, Germany) or the Synth Chemical Co. (São Paulo, Brazil).

Cultivation

Cultures were grown in 20-l photo-bioreactors maintained in a greenhouse with an initial volume of 14 l and an initial biomass concentration of 0.15 g/l. The cultures were air-mixed using diaphragm pumps to produce an air flux of 170 l/h and illuminated with 40 W daylight-type fluorescent lights (Osram, Brazil) at an intensity of 1900 lux, a 12 h photoperiod (Costa *et al.*, 2002) and at 30 or 35 °C.

After 648 h the three replicas of each experiment were mixed and the biomass removed by filtration, washed with distilled water to remove culture medium salts, centrifuged at 15000 rev/min, lyophilized and stored at – 20 °C until need.

Experimental design

For this study a Multilevel Factorial Design was used, in which the temperature for runs 1–4 was 30 °C, and 35 °C to runs 5–8. Sodium nitrate concentration in Zarrouk's medium was 0.625, 1.250, 1.875 and 2.500 g/l respectively for runs 1–4, the same concentrations have been used in the same order for runs 5–8, respectively. All experiments were carried out in triplicate.

Fatty acids methyl ester analysis

Lipids were obtained from the lyophilized biomass sample according to the method of Folch (Folch and Lees, 1957), *i.e.* lipids were extracted with chloroform/methanol (2:1 v/v), purified in methanol/water (2:1 v/v) containing 9 g/l NaCl (to remove sugars, salts and proteins) and concentrated in a rotary evaporator, residual solvent was evaporated with nitrogen. Fatty acid methyl esters (FAME) were prepared from the lipid samples using the method of Metcalfe and Schmitz (1966), gas chromatography was used to determine the fatty acid profile for each of the growth conditions of Multilevel Factorial Design. For gas chromatography 1 ml of esterified sample was injected into a Varian 3400CX-FID gas chromatograph (detector temperature 270 °C, injection temperature 250 °C, carrier gas nitrogen at a flow rate of 2 ml/min) fitted with a 30 m long, 0.545 mm diameter DB-FFAP Megabore capillar column (JW Scientific, Folson, USA) with a 1 mm film thickness and a polyethylene glycol modified nitroterephthalic acid stationary phase. Chromatography conditions were: initial column temperature 120 °C for 1 min, a 5 °C/min rise to 170 °C, held for 1 min, a 2 °C/min rise to 190 °C, held for 2 min, and a 5 °C/min rise to 220 °C, held for 20 min (total 50 min). Three replicas were analysed for each lyophilized biomass sample. Fatty acids were identified by comparing the retention times with FAME standards (Sigma, Supelco) and were quantified by normalization of the area under relevant peaks using Varian Star software version 4.51.

Statistical analysis

Analysis of variance (ANOVA) and Tukey honest significant difference (HSD) test were used to determine whether there was any significant difference in fatty acid composition of *Spirulina* grown under the different Multilevel Factorial Design conditions.

Results and Discussion

The major FAMEs extracted from the *Spirulina* are shown in Table I. Other fatty acids (*e.g.* C12:0, C14:0, C16:2) and fatty acids with longer chains (> 20 carbons) are not shown because they were present only in traces.

The percentages of the major FAMEs in the *Spirulina* cultivated by us were in accordance with previous works by other authors (Olguín *et al.*, 2001; Quoc *et al.*, 1994; Cohen *et al.*, 1987), the principal fatty acids present were palmitic, gamma-linolenic and linoleic acid (Table I). However, it may be possible to increase the content of gamma-linolenic acid, because Olguín *et al.* (2001) obtained 26–31% of gamma-linolenic acid (C18:3) in a study on the effect of low light flux and nitrogen deficiency on the chemical composition of

Table I. Fatty acid composition of *Spirulina platensis* cultivated at 30 °C (runs 1 to 4) and 35 °C (runs 5 to 8). Sodium nitrate concentration was 0.625, 1.250, 1.875 and 2.500 g/l, respectively, for runs 1–4. The same concentrations were used for runs 5–8, respectively.

Run/	Fatty acid concentration[a] (%)					
Sample	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3
1	45.92 ± 1.23	2.74 ± 0.26	0.89 ± 0.12	7.77 ± 0.74	11.95 ± 0.91	20.63 ± 0.86
2	44.89 ± 0.39	2.54 ± 0.20	0.95 ± 0.01	8.57 ± 0.06	11.93 ± 0.08	20.10 ± 0.20
3	45.97 ± 0.71	1.67 ± 0.07	0.90 ± 0.12	6.84 ± 0.53	12.29 ± 0.83	20.38 ± 1.46
4	44.59 ± 1.11	1.81 ± 0.11	0.86 ± 0.04	8.56 ± 0.68	12.73 ± 0.45	20.92 ± 0.70
5	45.78 ± 1.51	2.83 ± 0.18	1.57 ± 0.62	9.29 ± 0.92	13.00 ± 0.62	18.51 ± 1.24
6	48.27 ± 0.86	2.46 ± 0.03	0.91 ± 0.03	7.50 ± 0.41	12.30 ± 0.47	18.34 ± 1.08
7	45.57 ± 1.64	2.12 ± 0.13	1.09 ± 0.04	8.34 ± 0.22	14.54 ± 0.31	19.53 ± 0.59
8	45.31 ± 1.12	2.01 ± 0.10	1.24 ± 0.02	8.02 ± 0.11	14.68 ± 0.13	19.51 ± 0.34

[a] Mean ± standard deviation.
C16:0 = palmitic acid; C16:1 = palmitoleic acid; C18:0 = stearic acid; C18:1 = oleic acid; C18:2 = linoleic acid and C18:3 = gamma-linolenic acid.

Spirulina cultivated in sea-water supplemented with anaerobically digested pig waste. The analyses of variance shown that the variation of temperature and nitrogen content caused significant effects on the concentration of palmitoleic (C16:1) and linoleic (C18:2) acids. Changing the temperature from 30 °C to 35 °C had a positive effect and increased the concentration of both palmitoleic and linoleic acid, but increasing the concentration of sodium nitrate had a negative effect on the concentration of palmitoleic acid while the interaction of both factors (sodium nitrate concentration and temperature) was significant ($p < 0.01$) in the case of linoleic acid and should be taken into account instead of the individual effects of the two factors. Fig. 1 shows linoleic acid content as a function of sodium nitrate concentration at 30 and 35 °C; an increase in linoleic acid content with increasing sodium nitrate concentration occurs only at 35 °C. The relationship between the concentration of the nitrogen source in the culture medium and the fatty acid content of *Spirulina* has been studied by Rijn and Shilo (1986), who have shown that reserve compounds accumulate during nitrogen depletion. However, Piorreck *et al.* (1984) showed that such reserve compound accumulation occurs mainly in green algae metabolism, while in the cyanobacterium *Spirulina* the fatty acids of the lipid polar fraction remain constant for potassium nitrate concentrations between 0.001% and 0.1%, similar results have been found by Tedesco and Duerr (1989). Even though, Olguín *et al.* (2001) have observed an increase in the concentration of linolenic acid in *Spirulina* growing in a nitrogen-deficient medium compared to *Spirulina* growing in Zarrouk's medium.

Besides the effect of temperature on linoleic acid content, we also verified with that increasing the incubation temperature the amount ($p < 0.10$) of palmitic acid increased, but palmitoleic acid and stearic acid decreased the amount of GLA. It has been reported that the content and composition of the fatty acids are temperature-dependent in *S. platensis*, an increase in temperature reduces the composition of polyunsaturated fatty acids in membrane lipids. This regulation of fatty acid saturation by desaturase enzymes is known as homeoviscous adaptation (*i.e.* the adjustment of the membrane fluidity needed to maintain the optimal function of biological membranes), although another explanation for this change in lipid satura-

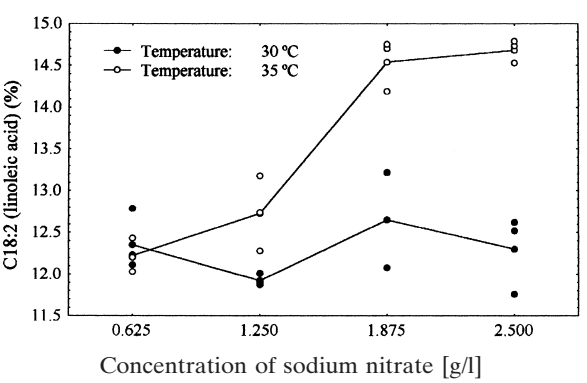

Fig. 1. Linoleic acid concentration obtained at 30 and 35 °C by *Spirulina platensis* grown in Zarrouk's medium with different concentrations of sodium nitrate.

tion with temperature is that at lower temperatures more dissolved oxygen is available in the culture medium for desaturase enzymes that are oxygen dependent (Cohen *et al.*, 1987).

The mechanisms by which polyunsaturated fatty acids are synthesized are not fully understood (Alonso and Maroto, 2000), although many studies have been undertaken to identify the desaturases responsible for the introduction of double bonds into saturated fatty acids (Meesapyodsuk *et al.*, 2001). It is known that the *desC* and *desA* desaturases responsible for the first and second double bonds in the fatty acids of the lipidic membranes of *S. platensis* (*Arthospira* sp. PCC 9438) are temperature-independent while the *desD* desaturase, responsible for the desaturation of C18:2 (9,12) to C18:3 (6,9,12), is temperature dependent (Meesapyodsuk *et al.*, 2001), suggesting that C18:2 content can vary because it is a substrate for the *desD* desaturase.

The percentage ratio of gamma-linolenic acid (GLA) to total fatty acids (TFA), unsaturated fatty acids (UFA) to TFA, and GLA to oleic plus linoleic (O+L) acid in the samples we studied is shown in Table II. The GLA/TFA and UFA/TFA ratios obtained for the cultivated *Spirulina* using the Tukey HSD test shown (Table II) that increasing the culture temperature from 30 to 35 °C resulted in a decrease ($p < 0.001$) in the GLA/O+L ratio.

The GLA/TFA ratio varied from 20 to 23% in the *Spirulina* cultivated by us (Table II), supporting the view of other researchers (Alonso and Maroto, 2000; Quoc *et al.*, 1994; Cohen *et al.*, 1987, 1993) that *Spirulina* is a potential source of this fatty acid. However, according to Roughan (1988), the cost of obtaining GLA from *Spirulina* are 4–6 times higher than from evening primrose (*Oenothera biennis*) and Cohen *et al.* (1993) emphasized the importance of the extraction of other compounds (*e.g.* phycocyanin and xanthophylls) with high nutritional (and monetary) value to decrease the overall costs associated with the extraction and production of GLA.

Table II. Percentage ratio of fatty acids in *Spirulina* cultivated at 30 °C (runs 1 to 4) and 35 °C (runs 5 to 8). Sodium nitrate concentration was 0.625, 1.250, 1.875 and 2.500 g/l, respectively, for runs 1–4. The same concentrations were used for runs 5–8, respectively.

Run	Fatty acid ratio[a] (%)		
	GLA/TFA	UFA/TFA	GLA/O+L
1	23.10 ± 0.73	48.69 ± 1.45	1.02 ± 0.02
2	22.59 ± 0.26	48.48 ± 0.24	0.98 ± 0.01
3	22.55 ± 0.79	45.87 ± 1.17	1.05 ± 0.03
4	23.38 ± 0.74	49.20 ± 1.35	0.98 ± 0.07
5	20.60 ± 1.61	47.69 ± 20.1	0.86 ± 0.06
6	20.42 ± 0.96	45.21 ± 1.39	0.93 ± 0.04
7	21.39 ± 0.75	48.90 ± 1.51	0.85 ± 0.01
8	21.50 ± 0.46	48.73 ± 0.92	0.86 ± 0.01

[a] Mean ± standard deviation.
GLA = gamma-linolenic acid; TFA = total fatty acids; UFA = unsaturated fatty acids; O+L = oleic + linoleic acid.

Because of the high costs of extraction of polyunsaturated fatty acids (especially GLA) from *Spirulina* it seems that the best way to use *Spirulina* is by its direct consumption as a nutritional supplement, especially because in this way the consumer will benefit not only from the beneficial properties ascribed to GLA but also to other nutritionally active components. *Spirulina* can be used either as a food supplement or taken in capsule form, capsules appearing to be the preferred form at present. It is important to know the fatty acid profile *Spirulina* preparations, our results (Table II) showing that different *Spirulina* preparations have different fatty acid profiles. The fatty acid profile of the *Spirulina* cultivated by us demonstrate that palmitic acid is the most abundant followed by linolenic and linoleic acids. Temperature was more important than sodium nitrate concentration, with greater amounts of GLA being obtained at 30 °C. It seems that the *Spirulina* produced under the culture conditions described in this paper is a potential source of GLA for use as a food additive or in capsule form as a nutritional supplement.

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