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α-tocopherol and Fatty acids of *Spirulina platensis* Biomass in Glass Panel Bioreactor

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Abstract: The objective of this study was to investigate the effect of temperature, at 26°C, on growth, α-tocopherols and fatty acid composition of *S. platensis*, grown in laboratory batch cultures with glass panel bioreactor. α-tocopherol among of *S. platensis* was 63.48±10.73 μg g^{-1} in the dried samples. The cell density of *S. platensis* increased rapidly to 32.38±3.58×10^4 cells mL^{-1} and the maximum specific growth rate was 0.36 day^{-1}. The percentage of total SFA, total MUFAs and total PUFAs of dry weight were 70.3, 1.9 and 18.6%, respectively. The predominant fatty acids were by order of abundance: palmitic acid (16:0), palmitoleic acid (16:1(n-7)), oleic acid (18:1 (n-9)), linoleic acid (18:2(n-6) and γ-linoleic acid (18:3(n-6)). Among the PUFAs, 18:3 (n-6) was the major fatty acids reaching 18.5±0.7% of total fatty acids followed by 18:2 (n-6) (13.8±0.5%). The concentrations of γ-linolenic acid varied from 12-20% for *S. platensis*, at the optimum growth temperatures and it was concluded that the temperature did not affect γ-linolenic acid. Finally, the major goal of the present study was to assess the effectiveness of the supplementation procedure together with low growth temperatures on improving the γ-linoleic acid level and α-tocopherol in *S. platensis*.

Key words: Microalgae, *Spirulina platensis*, fatty acids, γ-linoleic acid, tocopherol

INTRODUCTION

Microalgae are able to produce important biomolecules such as fatty acids, vitamins, sterols, carotenoids, amino acids, minerals and pharmaceuticals, which are affected by environmental conditions, such as temperature, media composition, light intensity and age of the culture. The composition of microalgae species can also change significantly under different culture conditions, including temperature and light (Richmond, 1986; Renaud *et al*., 1995; Vonshak, 1997; Richmond, 1992), particularly their gross composition and content on fatty acids (Thompson *et al*., 1992).

Spirulina sp. contains high quantities of protein, along with good amounts of essential fatty acids, polysaccharides, phycobiliproteins, carotenoids, vitamins and minerals, making it a desired food source (Cohen, 1997; Vonshak, 1997; Hu, 2004). Furthermore, *Spirulina* is of widespread utilization in aquaculture for feeding of tropical fish due particularly to its unique pigment content (Vonshak and Richmond, 1988; Jimenes *et al*., 2001).

Many microalgae have a high content of polyunsaturated fatty acids (PUFAs) (Yongmanitchai and Ward, 1991). *S. platensis* contains high level of γ-linoleic acid (GLA, 18:3 (n-6)) that is associated with pharmaceuticals and nutraceuticals (Cohen *et al*., 1987). PUFAs of the omega-3 and omega-6 series in human health are recognized to be important in human health. The γ-linoleic acid is claimed to have medicinal properties. It has been used for the treatment of atopic eczema (Biagi *et al*., 1988). It is also thought to have a positive effect in heart diseases, Parkinson's disease and multiple sclerosis (Dyerberg, 1986).

Other important bioactive metabolites such as tocopherols vitamin E indicates different compounds with antioxidant activity. Vitamin E is as considered an essential constituent because of its well documented ability to protect membrane lipids from oxidative damage (Huo *et al*., 1997). Animal cells are unable to synthesize vitamin E and must obtain it from plant sources (Vismasa *et al*., 2003). Vitamin E comprises a group of lipid-soluble compounds from which α-tocopherol is the most abundant and has the highest antioxidant activity *in vivo*.

The objective of this study was to investigate the effect of temperature, with 26°C, on growth, α-tocopherols and fatty acid composition of *S. platensis*, grown in laboratory batch cultures. The results are discussed to consider possible differences in the nutritional value of the grown under the high temperature culture conditions.

MATERIALS AND METHODS

Microorganism: *Spirulina platensis* was used in this study. The starter culture was obtained from Ben Gurion University of the Negev, The Jacob Blaustein Institue for Desert Research, Israel.

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Growth Conditions: S. platensis were grown in batch culture in Zarrouk's medium. Glass panel bioreactors was constructed from sheets of glass (100×50×10 cm), which was 10 cm light path. Culture temperature was kept at 26±1°C. The culture was illuminated with florescent lamps (Philips TLM 40W/54 RS) at a photon flux density 116 μ mol photons m^{-2} s^{-1} and was continuously mixing with air bobble. Cell numbers were measured using Neubauer hemacytometer and instantaneous growth rates (μ) were calculated with this equation:

$$\mu = \frac{\ln(N_t) - \ln(N_0)}{t - t_0}$$

where N_t is the cell number at time t and N_0 is the cell number at time t_0.

Samples of microalgae were collected at the end of culture. Harvesting by centrifugation with separtor (Cream Sperator, Turkey). It was working 12000 rpm and collected to algal paste in the collector. The obtained pellet was dried at 60°C for further analysis.

Analytical methods

Tocopheros analysis: Alpha-tocopherol was immediately analysed after freeze-drying. The extraction was carried out following a method adapted from Chen *et al.* (1998). The organic phases recovered were pooled and a 20 μL aliquot was injected in a HPLC JASCO model 980 (Japan) equipped with an automatic injector (JASCO Model AS-950-10 (Japan)) and a fluorescent detector (JASCO Model FP-1520 (λ_{exc} = 290 nm and λ_{em} = 300 nm)). The separation was carried out in a Lichrosorb Si 60-5 (250×3 mm i.d) column from Chrompack (Walnut Creek, CA) protected by a silica pre-column S2-SS (10×2 mm i.d) from Chrompack (Walnut Creek, CA). The mobile phase was a mixture of n-hexane and isopropanol (99.3:0.7 v/v) degassed in the Gastor Model GT-104 System (Japan) and eluted at a constant flow of 1 mL min^{-1}. The data was recorded and analysed using Borwin chromatographic software (version 1.21, JMBS Developpements, France).

Fatty acids analysis: Fatty acid methyl esters were prepared modified by Cohen *et al.* (1988). The fatty acid methyl ester preparation was carried out using 0.3 g of freeze-dried material and 5 mL of the acetylchloride:methanol reagent (1:19 v:v). Esterification was at 80°C for 1 h. After cooling, 1 mL of water and 2 mL of n-heptane were added to the mixture, stirred and centrifuged at 2150 g for 10 min. The organic phase was collected, filtered and dried with anhydrous sodium sulphate. Solvent was removed under nitrogen and the methyl esters solubilized in 0.1 mL of n-heptane. The analysis was performed using a gas chromatograph (Varian Star 3400 Cx (Walnut Creek, CA)) equipped with an auto-sampler and fitted with a flame ionisation detector at 250°C using a polyethylene glycol capillary column with 30m length 0.25 mm i.d and 0.25 μm film thickness (DB-WAX, JandW Scientific (USA)). The column was subjected to a temperature program starting at 180°C for 5 min, heating at 4°C min^{-1} for 10 min and then held at 220°C for 25 min. The injector (split ratio 100:1) and detector temperatures were kept constant at 250°C during the 40 min analysis. Heneicosanoic acid ($C_{22}H_{44}O_2$, Methyl ester (C_{21})) was used as internal standard for quantification of fatty acids.

RESULTS AND DISCUSSION

Growth of culture: The growth *S. platensis* at 26°C is shown in Fig. 1. The cell density of *S. platensis* increased rapidly to 32.38±3.58×10^4 cells mL^{-1} on day 28 without any apparent lag phase. The maximum specific growth rate was 0.36 divide day^{-1}. Optimum temperature of *S. platensis* is reported in the range of 35-38°C by many authors (Richmond, 1992; Vonshak, 1997; Koru and Cirik, 2003). *S. platensis* can reach sufficient biomass density at cold culture conditions.

α-tocopherols: Vitamin E level was evaluated in *S. platensis.* α-tocopherol level was 63.48±10.73 μg g^{-1} in the dried samples. α-tocopherol results of *S. platensis* was lower than for *Euglena gracilia*, *Dunaliella salina* and *Tetraselmis suecica* which was recorded by Vismasa *et al.* (2003) 283.6, 153.2 and 157.7 μg g^{-1}, respectively. α-tocopherol levels recorded in this microalgae cultivated at 26°C were superior to that registered in foods known as rich in α-tocopherol, e.g., carrot with 39.47 μg g^{-1} (Fαbregas and Herrero, 1990). This variability is not only inherent in the species but also depends on environmental conditions, e.g., temperature

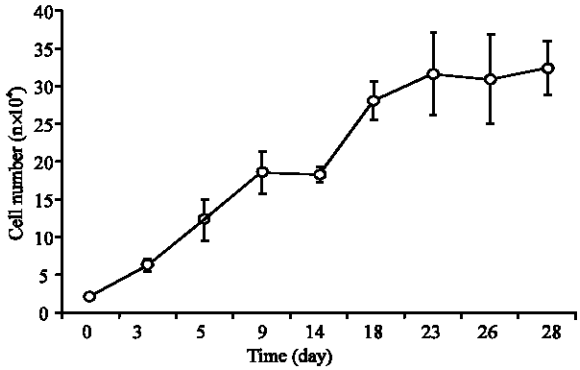

Fig. 1: Cell number of *S. platensis* at temperature of 26°C

and light (Huo *et al*., 1997). On the other hand for the microalgae, vitamin E is an antioxidant and the sample stayed the before analysis to avoid oxidation reaches is an important factor. This shows that *S. platensis* can be used as a supplement of vitamin E.

Fatty acids: The fatty acids composition of *S. platensis* cultured at 26°C is shown in Table 1. The most important saturated fatty acid was 16:0 (palmitic acid). Its proportion was recorded as 33.1±2.1% which had the highest amount among all the fatty acid groups. The most important monounsaturated fatty acid (MUFAs) was (18:1(n-7) with the value 2.4%). Among the PUFAs, 18:3 (n-6) was the major fatty acids reaching 18.5±0.7% of total fatty acids followed by 18:2 (n-6) (13.8±0.5%. The percentage of total saturated fatty acids (SFAs), total MUFAs and total PUFAs of dry weight were 70.3, 1.9 and 18.6%, respectively. The predominant fatty acids were by order of abundance: palmitic acid (16:0), palmitoleic acid (16:1 (n-7)), oleic acid (18:1(n-9)), linoleic acid (18:2 (n-6)) and γ-linoleic acid, which were more abundant in previously reported data for *Spirulina* strains, were analysed and expressed as weight percentage of total fatty acids (Ahlgren *et al*., 1992; Ortega *et al*., 1993; Cohen, 1997; Campanella *et al*., 1999).

Table 1: Fatty acids content of *S. platensis* at temperature of 26°C

Fatty acid (%)	Spirulina platensis
12:0	7.6±2.3
16:0	33.1±2.1
16:0 isobr.	4.0±3.1
20:0	5.8±0.5
Other SFA	1.4±0.7
∑ÓSFA	70.3±3.7
16:1 (n-7)	1.8±0.2
18:1 (n-7)	2.4±0.9
18:1 (n-9)	1.6±0.5
Other MUFA	1.9±0.7
∑MUFA	6.1±1.8
18:2 (n-6)	13.8±0.5
18:3 (n-3)	2.4±0.2
18:4 (n-3)	0.8±0.2
18:3 (n-6)	18.5±0.7
20:5 (n-3)	0.5±0.2
22:6 (n-3)	0.8±0.2
Other PUFA	1.6±0.6
∑ÓPUFA	18.6±1.5

Table 2: Analytical data on fatty acid composition of *S. platensis* at different temperature of culture

	Culture temperature						
Fatty acid (%)	26°C	20°C	25°C	30°C	35°C	28°C	35°C
6:0	37.17	39.73	44.92	36.39	46.5	52.68	45.5
16:1	10.02	9.11	6.78	3.39	2.73	2.95	09.6
18:0	2.19	1.29	1.56	2.76	2.28	1.93	1.3
18:1	5.87	6.63	11.51	20.92	14.69	4.10	3.8
18:2	13.83	14.72	12.26	8.69	11.41	10.40	14.5
18:3	20.97	17.61	14.06	13.65	12.50	12.63	21.0

Source this study De Oliveira *et al*. (1999). Koru and Cirik (2003). Becker (2004)

Analytical data on fatty acid composition of *S. platensis* at different temperature of culture is shown in Table 2. Among polyunsaturated fatty acids, γ-linoleic acid reached the highest percentage, which was already reported by Vonshak and Richmond, (1988), Vonshak (1997) and De Oliveira *et al*. (1999). Koru and Cirik (2003) found highest amount of γ-linoleic acid (12.63%) at 28°C. Other reseachers, such as De Oliveira *et al*. (1999) investigated high production of the γ-linolenic acid at the temperature of 20°C (17.61%), 25°C (14.02%), 30°C (13.65%) and 35°C (12.50%) for *S. platensis*. The concentrations of γ-linolenic acid varied from 12-20% for *S. platensis*, at the optimum growth temperatures and they concluded the temperature did not affect γ-linolenic acid. On the other hand, Colla *et al*. (2003) found that temperature was the most important factor and that the greatest amount of γ- linolenic acid was obtained at 30°C. In addition, Becker (2004) investigated high production of the γ-linoleic acid (21.10%) at the temperature 35°C. From a general point of view, in the range 20-35°C, temperature changes did not affect fatty acids ratios extensively.

CONCLUSIONS

In conclusion, growth phase and growth temperature had a significant effect on the fatty acid and biochemical composition in the culture of *S. platensis*. This microalgae can be easily grown as a photooutotroph and low temperature. Finally, the major goal of the present study was to assess the effectiveness of the supplementation procedure together with low growth temperatures on improving the γ-linoleic acid level and α-tocopherol in *S. platensis*. The results of tocopherols shows that *S. platensis* can be used as a supplement of vitamin E. The high levels registered for the bimolecular studied namely ω3 fatty acids and vitamin E, well known for health properties and survival of fish and shrimp, gives to this species a special interest for being used in food as supplements/nutraceuticals.

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