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β-carotene, vitamin C and vitamin E content of the marine microalga *Dunaliella tertiolecta* cultured with different nitrogen sources (Conference Paper)

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Abstract

Variations in the β-carotene, vitamin C and vitamin E content of D. tertiolecta have been shown to result from the nitrogen source used in the culture medium. Differences of 101%, 38% and 69% have been found in β-carotene, ascorbic acid and tocopherol content in mg/g of dry matter, respectively, and differences of 147%, 63% and 37% occurred in β-carotene, vitamin C and E concentrations in mg/litre of culture, respectively. Considering the β-carotene, vitamin C and vitamin E content in mg/g of chlorophyll a, maximum variations occurred in β-carotene content, with differences of 145% among the different nitrogen sources. Maximum β-carotene and vitamin C values were found in urea cultures, whereas urea cultures showed the minimum values for vitamin E.

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D. tertiolecta, β-carotene, vitamin C, vitamin E.

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Engineering controlled terms: Algae - Industrial Applications; Biomass; Cell Culture; Vitamins - Biosynthesis

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EMTREE drug terms: alpha tocopherol; ammonia; ascorbic acid; beta carotene; cell protein; chlorophyll a; nitrate; nitrite; nitrogen; tocopherol; urea

EMTREE medical terms: alga; animal cell; biomass; biotechnology; cell density; conference paper; culture medium; extract; nonhuman

Species Index: algae; Animalia; Dunaliella tertiolecta

INTRODUCTION

Marine micro algal biomass constitutes an important potential (Soeder, 1980). Marine microalgal mass culture has been focused on its use in aquaculture systems, but marine micro algae are considered today to be an important potential for a variety of products.

The marine micro alga *Dunaliella tertiolecta* Butcher is a naked flagellate that provides excellent nutrition in aquaculture systems (Walne, 1974; Bayne, 1976; Laing & Utting, 1980) and can be also used as raw material for Single Cell Protein (SCP) (Fabregas &

Herrero, 1985) or as supply of minerals in fish diets (Fabregas & Herrero, 1986). Besides its high protein content, this marine microalga contains water- and lipid-soluble vitamins (Fabregas & Herrero, 1990) and can constitute, as other *Dunaliella* species (Richmond, 1986), a potential source of some of them.

Great variability in the gross chemical composition of *D. tertiolecta* has been shown as a result of the source and concentration of nitrogen used in the culture medium (Fabregas et *al., 1989a).* This biochemical variability can be used for producing cells with biochemical contents that can be previously determined as a function of nitrogen source and concentration.

The vitamin content of this microalga can be also affected by the source and concentration of nitrogen in the culture medium. Therefore, *β*- carotene, ascorbic acid and tocopherol content of *D. tertiolecta* were determined in cultures with different nitrogen sources.

METHODS

The marine microalga *Dunaliella tertiolecta was* obtained from The Culture Centre of Algae and Protozoa, Cambridge, England. *D. tertiolecta* was cultured with different sources and concentrations of nitrogen. It was cultured in seawater filtered through a 0,45 *µm* Millipore filter, autoclaved at 120'C for 60 min and enriched with $ZnCl_2$, 1 *µ*M; $MnCl_2$, 1 *µ*M; Na_2MoO_4, 1 *µ*M; $CoCl_3$, 0·1 *µ*M; $CuSO_4$, 0·1 *µ*M; ferric citrate, 20 *µ*M; thiamine, 35 *µ*g/litre; biotin, 5 *µ*g/litre; B_{12} , 3 *µ*g/litre; EDTA, 26-4 mM; TRIS-HCl, 15 mM; pH 7·6. Mass cultures of *D. tertiolecta* were carried out with four nitrogen sources: nitrate ($NaNO_3$), nitrite ($NaNO_2$), ammonium (NH_4Cl) and urea ($(NH_2)_2CO$), at a concentration of 2 mg atom N /litre. Culture conditions had previously been optimized for maximum biomass production (Fabregas *et al., 1989b).*

Cultures were carried out at laboratory scale in a 10 litre flask with 9 litres of culture medium. All cultures were maintained in a controlled environmental incubator at 18 ± 1ºC, 35‰ salinity and a light intensity of 120 µ*E* m^{-2}, S^{-1} from fluorescent lamps (Osram daylight L55/10). A 12:12 light-dark cycle was maintained. An inoculum of 6 x 10^5 logarithmic phase cells/ml was used. Cultures had air continuously bubbled through them at a rate of 15 litres/min.

Cellular density was determined by counting culture aliquots in a Thoma chamber.

Chlorophylls were extracted from the cells in acetone-methanol 2:1 at 4ºC for 24 h. The extracts were filtered through a Fluoropore Millipore filter for clarification (Fabregas *et*

af., 1984), and absorbances of the pigment extract at specific wavelengths were recorded. The concentration of chlorophyll *a* was determined by the formula of Parsons and Strickland (1965).

The cells were collected by centrifugation (3000 g) at the end of their logarithmic phase and dried by lyophilisation. Samples for fi-carotene determination were saponified under reflux and extracted with hexane. An aliquot of the hexane extract was chromatographed on alumina and the β-carotene determined by a colorimetric method (De Ritter & Purcell, 1981). Ascorbic acid was determined as described by Roe (1966) and Omaye *et af.* (1979). Vitamin E (tocopherol) was extracted with petroleum ether and diethyl ether, the extract chromatographed by Florisil (Sigma) and the vitamin E determined fluorimetricaliy in the appropriate fraction (Demetriou, 1969).

Results are expressed as contents of vitamin per cell, per gram of dry matter and per mg of chlorophyll *a,* and also yields of vitamins per litre of culture.

RESULTS AND DISCUSSION

The source of nitrogen used in the culture medium affected the biomass production at the end of the logarithmic phase, expressed both as cellular density and as yield in dry matter per litre (Table 1). Maximum cellular density was obtained in urea cultures with values of 7·53 x 10^6 cells/ml wlfereas minimum va lues occurred with nitrite and ammonium cultures, with 5·45 x 10^6 and 5·60 x 10^6 cells/ml, respectively. Variations in cellular density of 38% occurred as a function of the nitrogen source. Maximum yield (dry matter per litre) was also obtained in urea cultures, with 0·76 g of dry matter/litre, whereas minimum yields were obtained in nitrate cultures (0·59 g/litre). Chlorophyll *a* values per ml of culture were very similar among the different nitrogen sources (Table 1), with values about 28 mg/litre.

Table 1. Cellular density, yield and chlorophyll *a* content of *D. tertiolecta* grown with different nitrogen sources

Nitrogen source	*Cellular density (cells × 10^6/ml)*	*Yield dry matter (g/litre)*	*Chlorophyll* a *(mg/litre)*
Nitrate	6·12	0·59	28·32
Nitrite	5·45	0·62	35·00
Ammonium	5·60	0·66	28·56
Urea	7·53	0·73	28·24

The cellular contents of the three vitamins assayed appear very similar among the different nitrogen sources used (Fig. 1), with the exception of the β-carotene content in nitrite cultures and vitamin E content in urea cultures, that were significantly lower than those of the other nitrogen sources.

However, there are significantly differences in each vitamin content per dry matter, per litre and per unit of chlorophyll *a* in function of the nitrogen source used in the culture media (Figs 2, 3, 4). These differences are mainly due to the different biomass production obtained with the different nitrogen sources.

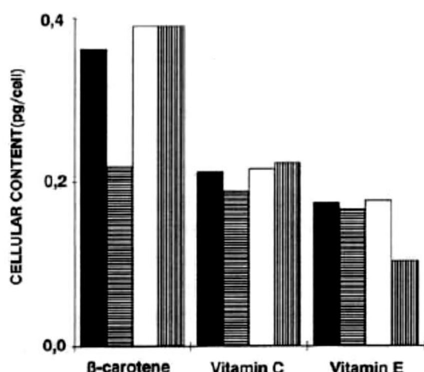

Fig. 1. Cellular content of *β*-carotene, vitamin C and vitamin E of *D. tertiolecta* grown with different nitrogen sources: nitrate (■), nitrite (▤), ammonium (□) and urea (▥).

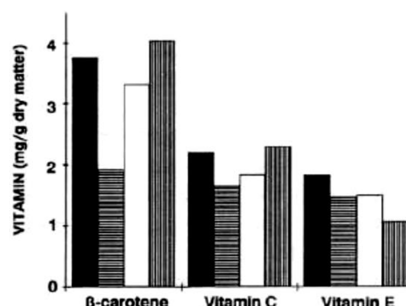

Fig. 3. *β*-carotene, vitamin C and vitamin E contents in the dry matter of *D. tertiolecta* grown with different nitrogen sources: nitrate (■), nitrite (▤), ammonium (□) and urea (▥).

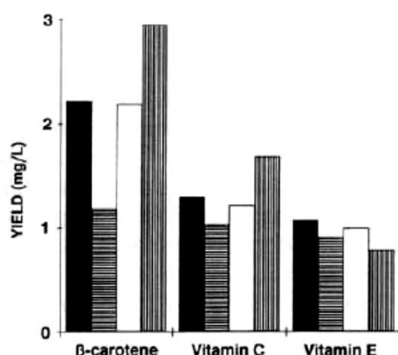

Fig. 2. Production of *β*-carotene, vitamin C and vitamin E per litre of culture of *D. tertiolecta* grown with different nitrogen sources: nitrate (■), nitrite (▤), ammonium (□) and urea (▥).

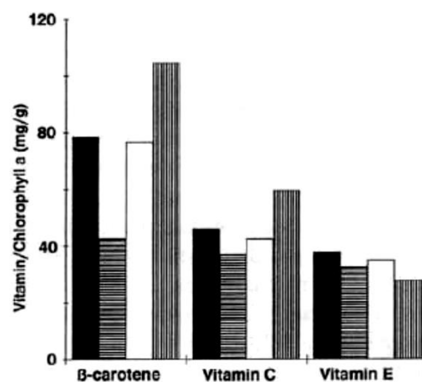

Fig. 4. Ratios *β*-carotene/chlorophyll *a*, vitamin C/chlorophyll *a* and vitamin E/chlorophyll *a* in *D. tertiolecta* cultures grown with different nitrogen sources: nitrate (■), nitrite (▤), ammonium (□) and urea (▥).

β -carotene concentrations per litre of culture obtained with the different nitrogen sources were 2·22 mg/litre in nitrate cultures, 1·19 mg/litre in nitrite cultures, 2·19 mg/litre in ammonia cultures and 2·95 mgjlitre in urea cultures (Fig. 2). Maximum β - carotene values per g of dry matter were also obtained in urea and nitrate cultures, with values of 4·03 and 3·76 *mg/g,* respectively (Fig. 3). Differences higher than 100% occurred between β -carotene content in urea cultures and in nitrite cultures, since in these cases, besides biomass differences, changes in cellular content were also found (Fig. 1).

Different species of the genus *Dunaliella* possess the ability to accumulate large amounts of β -carotene under different conditions. *D. bardawil* was shown to accumulate β -carotene to at least 8% of its dry weight when grown under defined conditions such as high light intensity, high salt concentration, extreme temperatures or nutrient deficiency (Ben-Amotz *et ai.,* 1982).

Other β -carotene non-accumulating strains of *Dunaliella* grown under similar conditions, or *D. bardawil* grown under non-accumulating conditions, contain about 0·3% β -carotene (Ben-Amotz & Avron, *1989a). D. tertiolecta* cultures were carried out under β-carotene non-accumulating conditions and the concentrations obtained amounted to between the 0·2 and 0·4% of the dry matter, showing significant differences in β-carotene content among the different nitrogen sources used in the culture medium.

Significant differences can also be observed (Fig. 4) in β-carotene in mg per gram of chlorophyll *a* among the different nitrogen sources used in the culture medium. Maximum values were obtained in urea cultures with 104·28 mg β-carotene/g chlorophyll *a.* This value is 33-36% higher than those obtained in nitrate and ammonia cultures and 147% higher than that obtained in nitrite cultures. The ratio β-carotene/ chlorophyll *a* had been used for establishing not only the effect of light quality and intensity but also the effect of nitrate concentrations in cultures of different *Dunaliella* species (Ben-Amotz & Avron, 1983, *1989b).* It has been shown that this ratio varies depending upon light (quality and intensity) (Ben-Amotz & Avron, *1989b)* and also depending upon nutrient concentrations, as nitrate and sulphate concentrations (Ben-Amotz & Avron, 1983). The present data show also variations due to the nitrogen source used in the culture medium. The increase in the β-carotene/ chlorophyll *a* ratio can be due to increased net production of β-carotene, a decrease in chlorophyll *a* or both (Ben-Amotz & Avron, 1983). Under the present conditions, chlorophyll *a* concentrations were similar in all the nitrogen sources used (Table 1), therefore changes are due to differences in the β-carotene production in function of the nitrogen source.

Maximum values of vitamin C/litre of culture were obtained in urea cultures, with 1·68 mg/litre, whereas minimum values were found in nitrite cultures, with 1·03 mg/litre (Fig. 2). Results obtained with ammonium and nitrate cultures are very similar (1'21 and 1·30 mg/litre, respectively). Values of ascorbic acid obtained with the different nitrogen sources per dry matter were 2·20 mg/g of dry matter in nitrate cultures, 1·67 mg/g in

nitrite cultures, 1·83 mg/g in ammonium cultures and 2·31 mg/g in urea cultures (Fig. 3).

Variations in vitamin C contents in mg per gram of chlorophyll *a* among the different nitrogen sources used in the culture medium are lower than those found in β-carotene contents (Fig. 4), but results show certain parallelism. Maximum and minimum values occurred in urea and nitrite cultures, respectively, with a 63% difference between them.

Studies carried out with the freshwater microalga *Scenedesmus acutus* show ascorbic acid values between 705 and 1653 mg/kg (Becker, 1980); these studies show a great variability in vitamin C content in function of the processing method. *D. tertiolecta,* under the present conditions, shows higher values of ascorbic acid with all the different nitrogen sources, with values between 1670 and 2310 mg/kg.

Tocopherol values obtained with the different nitrogen compounds were 1·81 mg/g of dry matter in nitrate cultures, 1-47 mg/g in nitrite cultures, 1·50 mg/g in ammonium cultures and 1·07 mg/g in urea cultures (Fig. 2). Concentrations of tocopherol/litre were similar among the different inorganic nitrogen sources, with values between 0·91 and 1·07 mg/litre whereas this concentration is significantly lower in urea cultures, with a value of 0·78 mg/litre of culture (Fig. 3).

Vitamin E/chlorophyll *a* ratios presented lower variations among the different nitrogen sources than variations occurring in β-carotene/ chlorophyll *a* and vitamin C/chlorophyll *a* ratios (Fig. 4). In this case, maximum values appear in nitrate cultures whereas urea cultures show the lowest values. Variations accounted for 36%.

Vitamin E plays an important role as antioxidant, both *in vivo* and *in vitro,* and its increasing importance as stabilizer of membrane lipids and its influence in the stability and permeability of membranes has been reported (Giasuddin & Diplock, 1981). Early studies on vitamin production by microorganisms showed that most of the prokaryotes and the yeasts showed little or no tocopherol (Hughes & Tove, 1982). However, one freshwater microalga, *Euglena gracilis,* resulted in a potential source of tocopherol, with vitamin E values between 1·12 and 7·35 mg/g dry matter, under conditions of oxygen starvation, low temperature and high light intensity (Ruggeri *et al.,* 1985). Vitamin E content of *D. tertiolecta* varies between 1·07 and 2·20 mg/g dry matter under the present conditions, and this content probably can be increased under suboptimum conditions. This content is higher in *D. tertiolecta* than in conventional food traditionally considered rich in it (Geigy, 1975).

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REFERENCES

Bayne, B. L. (1976). The biology of the mussel larvae. In *Marine Mussels: Their Ecology and Physiology.* Cambridge University Press, Cambridge, pp. 81 - 120.

Becker, E. W. (1980). Comparative toxicological studies with algae in India, Thailandia and Peru. In *Algae Biomass: Production and Use.* Elsevier *JNorth* Holland Biomedical Press, Amsterdam, pp. 767-86.

Ben-Amotz, A. & Avron, M. (1983). On the factors which determine massive .B-carotene accumulation in the halotolerant alga *Dunaliella bardawil. Plallf. Physiol, 72 ,* 593-7.

Ben-Arnotz, A. & Avron, M. *(1989a).* The biotechoology of mass culturing *Dunaliella* for products of commercial interest. In *Algal and Cyanobacterial Biotechnology e*d. R. C. Cresswell, T. A. V. Rees & N. Shah. Longman Scientificand Technical Press, London, pp. 90-114.

Ben-Arnotz, A. & Avron, M. (1989b). The wavelength dependence of massive carotene synthesis in *Dunaliella bardawil* (Chlorophyceae). *J. Phycol,* 25, 175-8.

Ben-Arnotz, A., Katz, A. & Avron, V. (1982). Accumulation of β-carotene in halotolerant algae: purification and characterization of , β -carotene-rich globules from *Dunaliella bardawil* (Chlorophyceae). 1. *Phycol ..* 18, 529-3 7.

De Ritter, E. & Purcell, A. E. (1981). Carotenoid analytical methods. In *Carotenoids as Colorants and Vitamin A Precursors,* ed. J. C. Bauernfeind. Academic Press, New York, pp. 815-82.

Demetriou, J. A. (1969). Determinacion de vitamina E. In *Química Clínica. Principios y Técnicas,* JIMS. Barcelona, pp.1 4 15-17.

Fabregas, J. & Herrero, C. (1985). Marine microalgae as a potential source of single cell protein (SCP). *Appl. Microbial. Biotechnol. ,* 23, 110-1 3.

Fabregas, 1. & Herrero, C. (1986). Marine microalgae as a potential source of minerals in fish diets. *Aquaculture,* 51 , 237-43.

Fabregas, J. & Herrero. C. (1990). The potential use of marine microalgae as source of vitamins in nutrition. *J. Ind. Microbial.,* 5, 259- 64

Fabregas, J., Abalde. J. , Herrero. C., Cabezas, B. & Veiga, M. (1984). Growth of the marine microalga *Tetraselmis suecica* in batch cultures with different salinities and nutrient concentrations. *Aqlloculwre,* 42 . 207-15.

Fabregas. J ., Abalde, J. Cabezas, B. & Herrero, C. (1989a). Changes in protein, carbohydrates and gross energy in the marine microalga *Dunaliella tertiolecta* (Butcher) by nitrogen concentrations as nitrate, nitrite and urea. *Aquacul. Engng,* 8, 223-39.

Fabregas, J., Abalde, J. & Herrero, C. *(1989b).* Biochemical composition and growth of the marine microalga *Dunaliella tertiolecta* (Butcher) with different ammonia nitrogen concentrations as chloride, sulphate. nitrate and carbonate. *Aquaculture,* 83, 289-304.

Geigy (1975). *Documenra Geigy.* J. R. Geigy, SA., Basle. Giasuddin A. S. M. & Diplock, A. T. (1981). The influence of vitamin E on membrane lipids of mouse fibroblast in culture. *Arch. Biochem. Biophys.,* 210, 348-62.

Hughes, P. E. & Tove, S. B. (1982). Occurrence of a-tocopherolquinona and a-tocopherolquinol in microorganisms. *J. Bacterial.,* ISI , 1397-402.

Laidman, D. L., Gaunt, J. K., Hall, G. S. & Broad, C. T. (1971). Extraction of tocophenol from plant tissues. In *Methods of Enzymology,* Vol. 18, ed. D. B. McCormick & L. D. Wrigh. Academic Press, New York, pp. 366-9.

Laing, I. & Utting_ S. (1980). The influence of salinity in the production of two commercially important unicellular algae. *Aquaculture,* 21 , 79-86.

Omaye, S. T., Turnbull, 1. D. & Sauberlich, H. E. (1979). Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. 1n *Methods in Enzymology,* Vol. 62, ed. D. B. McCormick & L. D. Wrigh. Academic Press, New York, p. 78.

Parsons, T. R. & Strickland, J. D. H. (1965). Particulate organic matter.lll.I Pigment analysis lllll. Determination of phytoplankton pigments. *J. Fish. Res. Bd. Can.. 18,* 117-27.

Richmond, A. (1986). Microalgae of economic potential. In *Microalgall Mass Culture,* ed. A. Richmond. CRC Press. Boca Ratom, Flo rid a, USA, pp. 199-243.

Roe. J. H. (1966). Chemical determination of ascorbic, dehydroascorbic and diketogulonic acids. In *Methods of Biochemical Analysis,* ed. D. Glick. Interscience Publishers, New York, pp. 115-39.

Ruggeri. B. A., Gray R. J. H., Watkins, T. R. & Tomlins, R. I. (1985). Effects or low-tempe rature acclimation and oxygen stress on tocopherol production in *Euglena gracilis.* Z. *Appl. Environ. Microbial. ,* 50, 1404-8.

Soeder, C. J. (1980). The scope of microalgae for food and feed. In *Algae Biomass, Prodluction and Use.* Elsevier North Holland Biomedical Press, Amsterdam, pp. 9-20.

Walne, P. R. (1974). In *Culture a/Bivalve Mollusks. 50 years Experience at COIllvy.* Fishing News (Books) Ltd , Surrey, UK, p. 173.