Iranian Journal of Fisheries Sciences 19(6) 2901-2908 2020
DOI: 10.22092/ijfs.2020.122837

Research Article

Optimization of production and antioxidant activity of fucoxanthin from marine haptophyte algae, *Isochrysis galbana*

Mousavi Nadushan R.[1]*; Hosseinzade I.[1]

Received: July 2017 Accepted: December 2017

Abstract

This study compared the biomass production, fucoxanthin production and antioxidant capacity of the fucoxanthin from the marine haptophyte algae, *Isochrysis galbana* under different nitrogen concentrations. At first step in the salinity test, *I. galbana* could grow in 20‰ and 35‰ but the 35‰ salinity was optimal. At second step, five different nitrogen concentrations (N-NO_3) of 2, 4, 8, 12 12 mM, at the salinity of 35‰, were investigated. Algal cell density increased as nitrogen concentrations increased, but a low growth rate occurred in the culture with the highest nitrogen concentration (12 mM). The maximum cell density of 72×10^5 and the maximum amount of fucoxanthin (18.1 mg g^{-1}) was obtained in *I. galbana* cultured in media containing four mM nitrogen (N-NO_3). The purified fucoxanthin exhibited strong antioxidant properties, with the effective concentration for 50% scavenging (EC50) of 1, 1-dihpenyl-2-picrylhydrazyl (DPPH) radical, being 0.2 mg/ml. This study suggests that the production and fucoxanthin concentration of *I. galbana* can be improved using nitrogen-replete culture in 35‰ salinity. Also under this condition this microalgaecan be a commercial source of fucoxanthin for human health and nutrition.

Keywords: *Isochrysis galbana*, Culture, Nitrogen replete, Salinity, Fucoxanthin.

1- Department of Marine Biology, Tehran North Branch, Islamic Azad University, Tehran, Iran.
*Corresponding author's Email: Mousavi.nadushan@gmail.com

Introduction

Fucoxanthin, a major carotenoid present in the chloroplasts of brown seaweeds, contributes to more than 10% of the estimated total production of carotenoids in nature (Miyashita *et al*., 2011; Peng *et al*., 2011). This pigment, along with chlorophylls and β-carotene, widely distributed in brown algae like *Eisenia bicyclis*, *Laminaria japonica*, and *Undaria pinnatifida*, and diatoms such as *Phaeodactylum tricornutum* and *Odontella aurita* (Peng *et al*., 2011; Takaichi, 2011). Diatoms are unicellular planktonic microalgae and exhibit a characteristic golden-brown color due to a high amount of fucoxanthin that plays a major role in their light-harvesting complex of photosystems (Saudi-Helis *et al*., 1994; Tezovsnis *et al*., 1997; Bertrand, 2010).

Fucoxanthin has a unique structure with an unusual allenic bond and a 5, 6-monoepoxide in its molecular structure, and ~~is~~ will metabolized into fucoxanthinol, amarouciaxanthin A, and halocynthiaxanthin after absorption into the human body (Sangeetha *et al*., 2010).

It provides protective effects on liver, blood vessels of the brain, bones, skin and eyes. It has anti-obesity, anti-diabetic properties, and anti-inflammatory, anti-malarian and anti-angiogenic activities. Moreover it is very effective in inhibiting cell growth and inducing apoptosis in human cancer cells. Particularly, fucoxanthin is distinctly more potent than β-carotene and astaxanthin for anti-obesity activity and the induction of apoptosis in human leukemia (D'Orazio *et al*., 2012; Kim *et al*., 2011; Peng *et al*., 2011). However, although fucoxanthin is clearly a valuable pigment with various biological activities, its use has been limited due to the low extraction efficiency from marine sources and the difficulty of chemical synthesis (Yamano *et al*., 1995; Kanazawa *et al*., 2008; Kajikawa *et al*., 2012). Several studies have been conducted to extract fucoxanthin from brown macroalgae (Kanazawa *et al*., 2008; Kim *et al*., 2010), but because these macroalgae are traditional foods in South-East Asia and some European countries, and they contain very low concentrations of fucoxanthin, the production of fucoxanthin from brown macroalgae is not commercially feasible (Kim *et al*., 2012). Consequently, searching for alternative sources of fucoxanthin is necessary.

Isochrysis galbana is a small unicellular (~5–7 μm) phytoplankton without a cell wall (Wikfors and Patterson, 1994), from which fucoxanthin could be extracted easily. This microalga contains high concentrations of carotenoid, the long chain polyunsaturated fatty acid, eicosapentaenoic acid (EPA, 20:5ω3) commonly grown for the commercial production of live food and lipids (Spolaore *et al*., 2006, Domer et al, 2014).

Therefore, in the present study; we assumed that *I. galbana* can accumulate high concentration of fucoxanthin in routine culture condition. Thereafter we investigated the effect of nitrogen concentration and salinity on fucoxanthin production. Also the

antioxidant activities of crude fucoxanthin produced were evaluated.

Materials and methods

Culture conditions

The marine microalga *I. galbana* was provided from the Iran Shrimp Research Center. Basal culture media was made with the f/2-Si formula at two different salinity, 20 and 35‰ (T_1 and T_2). Also five different nitrogen concentrations 2, 4, 8, 12mM (T_3, T_4, T_5, and T_6) were investigated (all cultures were run in 3 replicate). The microalgae were cultivated in 1.5-l plastic bottles at 20^{oC} using f/2-Si medium (Guillard and Ryther, 1962), prepared from sterilized distilled water and sea salt, and air was continuously supplied at 5 l/min by an air-lift pump. Light was provided by 60 W fluorescent lamps at an intensity of 2,500 lx. The culture was continuously active and lasted for 10 days after onset of the process. The cells were filtered through a 0.45µm Millipore membrane filter on Büchner funnel (Schott brand) and were freezed at -20^{oC}.

Determination of fucoxanthin contents of I. galbana

Afterward, filter papers were solved in methanol at room temperature for 1 h to extract fucoxanthin from algal samples and then centrifuged at 12000 rpm for 10 minutes twice. Fucoxanthin was quantified using a HPLC system. The mobile phase of methanol and water was eluted with a 1 ml min^{-1} flow rate by increasing the methanol from 90 to 100% over 30 min and holding for the following 10 min and the chromatogram was recorded at 445 nm. Each concentration was analyzed by HPLC as described above.

The scavenging activity of DPPH radical was determined as describe by Sachindra *et al*.(2007) briefly, 2 ml methanolic fucoxanthin solution (0.05–0.3 mg ml^{-1}) was mixed with 2 ml 0.16 mM methanolic solution of DPPH. The mixture was shaken vigorously and incubated for 30 min at room temperature in the dark. The absorbance was measured at 517 nm. Ascorbic acid was taken as a positive control. The scavenging ability was calculated as:

DPPH radical scavenging activity (%) = $[1-(A_1-A_2)/A_0]\times100$,

Where A_0 is the absorbance in the lack of fucoxanthin (using distilled water instead of fucoxanthin), A_1 is the absorbance in the presence of fucoxanthin, and A_2 is the absorbance of methanolic fucoxanthin solution (using methanol instead of DPPH).

Statistical analysis

All data were determined from three replicate of each experiment. Mean values and standard deviations were calculated with Microsoft Excel software. For additional exploration of the differences, the results of one-way ANOVA were compared with Fisher's Least Significant Difference (LSD) in SPSS 19.

Results

The maximum density of the cultures occurred on day 10, in all 6 treatments but a low growth rate occurred in the culture with the highest nitrogen concentration, 12 mM nitrogen supply,

and the 20‰ salinity, containing a low density even after 10 days. Therefore the biomass didn't harvest from T_1 and T_7.

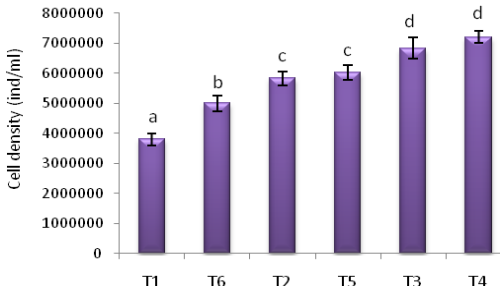

Figure 1: Effect of salinity and nitrogen concentrations on algal biomass (cell density).
Data are shown as Mean ± STDEV (*n*=3).

The fucoxanthin contents and even color of *I .galbana* in 5 treatments changed during 10-day cultivation in the high nitrogen cultures (Fig. 2).

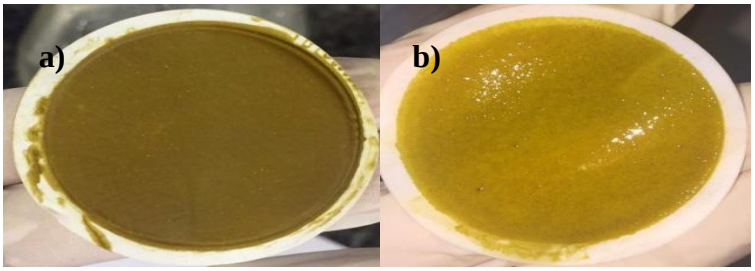

Figure 2: Effect of nitrogen concentration on color of *Isochrysis galbana* . Filter paper with algal sample harvested from a) basal culture medium and, b) culture medium containing four mM.

The fucoxanthin extracted from the 5 treatments increased from 12.1 mg g^{-1} to 18.1 mg g^{-1} during 10-day cultivation in the high nitrogen cultures.

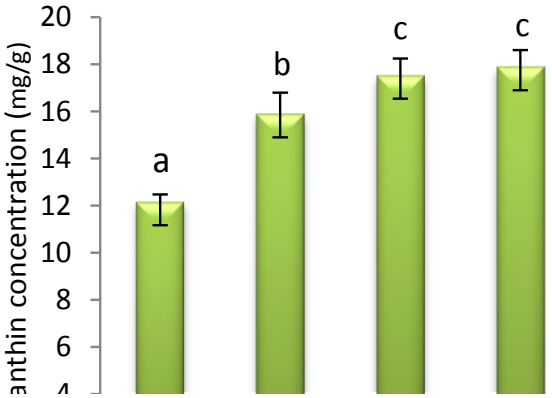

Figure 3: Fucoxanthin concentration of *Isochrysis galbana* cultivated in two different salinities (T_1 and T_2) and 3 different nitrogen supplys (T3, T4 and T5). Each value is expressed as mean±STDEV (*n* = 3).

Fucoxanthin extracted from *I. galbana* exhibited strong antioxidant properties, with the effective concentration for 50% scavenging (EC_{50}) of 1,1-dihpenyl-2-picrylhydrazyl (DPPH) radical (Fig. 4). Also the DPPH radical scavenging activity was linearly dependent on the fucoxanthin concentration; the effective concentration for 50% scavenging (EC50) was 0.2 mg ml^{-1}(Fig. 4).

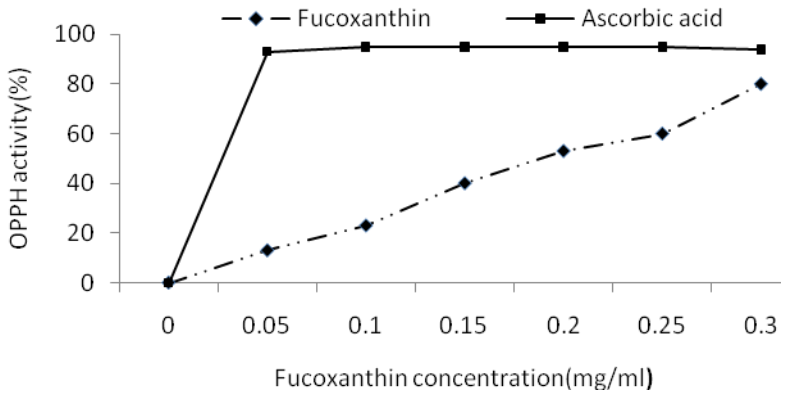

Figure 4: Antioxidant assay for the purified fucoxanthin from *Isochrysis galbana*, scavenging of DPPH radical.

Discussion

Nitrogen concentration and salinity are two major factors affecting growth and pigment biosynthesis of microalgae (Hu, 2004; De la Peña., 2007). A common trend of cellular response to stress conditions, such as high or low salinity and nitrogen depletion, appears to increase secondary carotenoids (e.g., β-carotene, astaxanthin, lutein), which serve as photoprotective agents (Hu, 2004).

In this research, we evaluated the potential of fucoxanthin production in *I. galbana* in different growth experiments. At first step *I. galbana*, were cultivated in basal culture media with 2 different salinities (T_1 and T_2) and different levels of nitrogen (T_3, T_4 T_5 and T_6), then all harvested samples were extracted for fucoxanthin and were analyzed by HPLC at 445 nm.

The maximum density of the cultures of *I. galbana* occurred in 35‰ salinity and media containing four mM nitrogen, also Isochrysis harvested from medium containing four mM nitrogen had the highest amount of fucoxanthin (18.1 mg g^{-1}) (Fig. 3). Fucoxanthin produced by *O. aurita* cultivated in basal culture media was measured 4.28 but maximum fucoxanthin concentration of 18.47 mg g^{-1} was obtained in cultures grown in low light and high nitrogen supply (Xia *et al*., 2013). Harker *et al.*(1996) reported that the astaxanthin content increased when *H. pluvialis* was cultivated in media deficient in nitrogen. Conversely fucoxanthin concentration decreased in *O. aurita* cultured in the low nitrogen media. Carreto and Catoggio (1976) found that fucoxanthin act as a primary carotenoid, whereby transferring light energy to the

photosynthetic reaction centers for photosynthesis (Hu, 2004). Under stress conditions, changes in the organization of the photosynthetic apparatus (e.g., chloroplast fragmentation, degradation of thylakoid membrane) occur, chlorophyll *a* and other pigments involved in photosynthesis decrease, while the secondary carotenoids increase. These variations in pigment content might be as a quotient between photosynthetically active pigments and other functional pigments.

I. galbana cultured in basal media containing 35‰ salt had the lowest amount of fucoxanthin (12.1 mg g^{-1}), increasing nitrogen (N-NO3) to four mM, led to a considerable increase in cell density and the fucoxanthin concentration in the cells (18.1 mg g^{-1}) (Figs. 1 and 3). Herzig and Falkowski (1989) also proved that the carotenoid and chlorophyll content are in accordance with nitrogen deficiency. Moreover they demonstrated the nitrogen is a limiting factor for the carotenoid production. Thereafter De la Pena (2007) confirmed that under nutrient repletion chla and photosynthetic pigments decrease, while secondary carotenoids such as fucoxanthin increase.

Whereas in other studies on macroalgae, the fucoxanthin concentration was too low, for example (0.01 mg g^{-1}), in *Sargassum fusiforme*, (1.01 mg g^{-1}) in *S. duplicatum,* (0.73 mg g^{-1}) in *Undaria pinnatifida* for fresh sample (Kim *et al*., 2012), and 1.01 mg g^{-1} in dried sample of *S. duplicatum.* In contrast, the reported fucoxanthin concentration in microalgae ranges from 2.24 in *Chaetoceros gracilis* to 18.47 mg g^{-1} in dry biomass of *O. aurita,* which is higher than that found in macroalgae, indicative of the great potential of diatoms as a promising source of fucoxanthin for various commercial applications.

Fucoxanthin extracted from .*I. galbana* exhibited strong antioxidant properties. The DPPH radical scavenging activity was linearly dependent on the fucoxanthin concentration; the effective concentration for 50% scavenging (EC50) was 0.2 mg ml^{-1}. It was reported that the extracts of brown seaweed *Cystoseira hakodatens* is exhibited a strong DPPH radical scavenging activity, due largely to the presence of fucoxanthin (Airanthi *et al*., 2011). Sachindra *et al.*(2007) assessed the radical scavenging abilities of macroalgae-derived fucoxanthin and its two metabolites, fuxoxanthinol and halocynthiaxanthin, against DPPH, and suggested that fucoxanthin and fucoxanthinol exhibited antioxidant activity higher than or similar activities to α-tocopherol.

The production of fucoxanthin from .*I. galbana* was very attractive and promising, with the maximum yield of 18.1 mg g^{-1} achieved in the culture medium containing four order nitrogen. Also the purified fucoxanthin showed strong antioxidant properties. Finally, our results may aid the commercial development of this microalgae for large-scale fucoxanthin production as a natural bioresource for human health.

References

Airanthi, M.W.A., Hosokawa, M. and Miyashita, K., 2011. Comparative antioxidant activity of edible Japanese brown seaweeds. *Journal of Food Science*, 76, 104–111.

Bertrand, M., 2010. Carotenoid biosynthesis in diatoms. *Photosynthesis Research*, 106, 89–102.

Carreto, J.I. and Catoggio, J.A., 1976. Variations in pigment contents of the diatom *Phaeodactylum tricornutum* during growth. *Journal of Marine Biology*, 36, 105–112.

D'Orazio, N., Gemello, E., Gammone, M.A., de Girolamo, M., Ficoneri, C. and Riccioni, G., 2012, Fucoxantin: A treasure from the sea. *Marine Drugs*, 10, 604–616.

De la Peña, M.R., 2007. Cell growth and nutritive value of the tropical benthic diatom, *Amphora* sp., at varying levels of nutrients and light intensity, and different culture locations. *Journal of Applied Phycology*, 19, 647–655.

Dörner, J., Carbonell, P., Pino, S. and Farías, A., 2014. Variation of fatty acids in Isochrysis galbana (T-Iso) and Tetraselmis suecica, cultured under different nitrate availabilities. *Fisheries and Aquaculture Journal*, 5(**3**), 1-3.

Harker, M., Tsavalos, A.J. and Young, A.J., 1996. Factors responsible for astaxanthin formation in the Chlorophyte *Haematococcus pluvialis*. *Bioresource Technology*, 55, 207–214.

Herzig, R. and Falkowski, P.G., 1989. Nitrogen limitation in *Isochrysis galbana* (Haptophyceae). I-Photosynthetic energy conversion and growth efficiences. *Journal of Phycology*, 25, 462-471.

Hu, Q., 2004. Environmental effects on cell composition. In Handbook of Microalgal Culture: Biotechnology and Applied Phycology; Richmond, A., Ed.; Blackwell Science Ltd.: Oxford, UK, pp. 83–93.

Kajikawa, T., Okumura, S., Iwashita, T., Kosumi, D., Hashimoto, H. and Katsumura, S., 2012. Stereocontrolled total synthesis of fucoxanthin andits polyene chain-modified derivative. *Organic Letters*, 14, 808–11.

Kanazawa, K., Ozaki, Y., Hashimoto, T., Das, S.K., Matsushita, S., Hirano, M., Okada, T., Komoto, A., Mori, N. and Nakatsuka, M., 2008. Commercial-scale preparation of biofunctional fucoxanthin from waste parts of brown sea algae *Laminalia japonica*. *Food Science and Technology Research Journal*, 14, 573–582.

Kim, S.M., Shang, Y.F. and Um, B.H., 2010. A preparative method for isolation of fucoxanthin from *Eisenia bicyclis* by centrifugal partition chromatography. *Phytochemical analysis*, 22, 322–329.

Kim, S.K. and Pangestuti, R., 2011. Biological activities and potential health benefits of fucoxanthin derived from marine brown algae. *Advances in Food and Nutrition Research*, 64, 111–128.

Kim, S.M., Jung, Y.J., Kwon, O.N., Cha, K.H., Um, B.H., Chung, D.

and Pan, C.H., 2012. A potential commercial source of fucoxanthin extracted from the microalga *Phaeodactylum tricornutum*. *Applied Biochemistry and Biotechnology*, 166, 1843–1855.

Miyashita, K., Nishikawa, S., Beppu, F., Tsukui, T., Abe, M. and Hosokawa, M., 2011, The allenic carotenoid fucoxanthin, a novel marine netraceutical from brown seaweeds. *Journal of the Science of Food and Agriculture*, 91, 1166–74.

Peng, J., Yuan, J.P., Wu, C.F. and Wang, J.H., 2011. Fucoxanthin, a marine carotenoid present in brown seaweeds and diatoms: Metabolism and bioactivities relevant to human health. *Marine Drugs*, 9, 1806–1828.

Saoudi-Helis, L., Dubacq, J.P., Marty, Y., Samain, J.F. and Gudin, C., 1994. Influence of growth rate on pigment and lipid composition of the microalga *Isochrysis aff*. galbana clone T. iso. *Journal of Applied Phycology*, 6, 315–22.

Sachindra, N.M., Sato, E., Maeda, H., Hosokawa, M., Niwano, Y., Kohno, M. and Miyashita, K., 2007. Radical scavenging and singlet oxygen quenching activity of marine carotenoid fucoxanthin and its metabolites. *Journal of Agricultural and Food Chemistry*, 55, 8516–8522.

Sangeetha, R.K., Bhaskar, N., Divakar, S. and Baskaran, V., 2010. Bioavailabilityand metabolism of fucoxanthin in rats: structural characterization ofmetabolites by LC-MS (APCI). *Molecular and Cellular Biochemistry*, 333, 299–310.

Spolaore, P., Joannis-Cassan, C., Duran, E. and Isambert, A., 2006. Commercial applications of microalgae. *Journal of Bioscience and Bioengineering*, 101, 87–96.

Takaichi, S., 2011. Carotenoids in algae: Distributions, biosyntheses and functions. *Marine Drugs*, 9, 1101–1118.

Wikfors, G.H. and Patterson, G.W., 1994. Differences in strains of Isochrysis of importance to mariculture. *Aquaculture*, 123, 127–35.

Xia, S., Li, A.F., Wan, L.L., Sang, M. and Zhang, C.W., 2013, Effects of nutrients and light on the growth and biochemical compositions of a marine microalgae *Odontella aurita*. Chin. *Journal of Oceanology and Limnology*, **31,** 1163–1173 DOI:10.1007/s00343-013-2092-4.

Yamano, Y., Tode, C. and Ito, M., 1995. Carotenoids and related polyenes. Part3. First total synthesis of fucoxanthin and halocynthiaxanthin using oxometallic catalyst. *Journal of the Chemical Society. Perkin Transactions*, 1, 1895–904.