Purification and Analysis of Carotenoids from Micro Algae (Dunaliella salina) Growing under Stressed Conditions

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Abstract

Dunaliella Salina a dino flagellated green marine micro algae accumulates massive amounts of carotenoids (12.6%, d.w), for its survivelance. The carotenoids having high commercial value so, the present research work majorly focused on the efficient carotenoids extraction strategies using different compositions of organic solvents. Artificial sea water media is used for its cultivation and the carotenoids are extracted with the solvents acetone, petroleum ether, and n-hexane. The analysis of carotenoids by HPLC (high pressure liquid chromatography) and their purification by Poly ethylene glycol 400. Also dunaliella salina accumulates essential fatty acids like arachidonic acid, Eicosapentanoic acid and their analysis with the gas chromatography.

Key words: *Dunaliella salina, Carotenoids, Lutein, Zeaxantin, Beta Carotene, HPLC, GC*

Introduction

Dunaliella Salina, a single-cell dino flagellated , marine micro algae. The well known species of Dunaliella are Dunaliella salina, tertiolecta, primolecta, viridis, bioculata, acidophyla, parva and Dunaliella media etc. Dunaliella salina is a commercial source of glycerol and to survive, it accumulates high concentrations of Beta carotene. There about 1000 carotenoids found in nature. Carotenoids are chemical compounds with high significant value and commercially used as pharmaceuticals, neutraceuticals, and cosmetics. These compounds have antioxidant properties and have attracted attention as potential agents in chemo prevention of cancers. Dunaliella salina, accumulates massive amounts of Carotenoids (13%, d.w), including Beta carotene (50 %), Lutein (8.5%), Zeaxanthin (12%), Astaxanthine (18%) when cultivated under low nitrogen level and salted stress condition. Dunaliella salina has lipid content of between 45- 55% of its total weight. According to the applicability of stress parameters the specific compound accumulation varies.

Biological activities of the Carotenoids

Carotenoids are having multiple biological functions such as antioxidant, anticarcinogenic, anti-inflamnatory, and antimutagenic activities. Carotenoids are also acts as chemo preventive agents against the cancer disease in various organs like stomach, lung, breast and prostate. It is believed that Beta carotene protects immune cells from oxidative damage caused by free radicals AMD (Age related macular degeneration) successfully treated by the intake of lutein and zeaxanthin through diet.

Materials and Methods

The Dunaliella salina strain is purchased from the SAG University, Germany.

Culture media composition and growth conditions

The Artificial seawater medium (ASM) which was used, to cultivate the Dunaliella strain contained: 50mM NaCl, 4.5mM MgCl2.6H2O, 0.5mM MgSO4.7H2O, 3mM KCl, 3mM CaCl2.2 H2O, 5mM KNO3, 25mM NaHCO3 , 0.13mM K2HPO4, 0.02mM FeCl3, 0.02mM EDTA, 1mg /l of trace elements stock with 50mM H3BO3, 0.2mM CoCl2.6H2O, 10mM MnCl2.4H2O, 0.8mM ZnSO4.7H2O, CuSO4.5H2O. In order to avoid precipitation of certain compounds, all stock solutions were sterilized separately at 121°C and cooled aseptically. PH was adjusted to 7.5 by addition of 40 mM of NaOH kept at 30°C. The cultivated Dunaliella salina with the above media is shown in figure.1.

Figure.1: Dunaliella salina cultured under laboratory conditions

Growth Estimation

Cells were determined by direct cell counting, using a light microscope (magnification × 40) with a %1 mm deep counting chamber (Neubauer improved). Once in every 3 days the sample was collected and cell count is performed by the addition of PEG 400, on a haemocytometer. The regular growth is tabulated. The algal pellet was dried with the spray drier.

Determination of total Carotenoids

The total Carotenoids were Spectrophoto metrically estimated at 450 nm and ß-carotene served as a standard compound, was used for preparing the calibration curve.

Analysis of Carotenoids with HPLC

HPLC (High Pressure Liquid Chromatography) consisting of a spectra system (UV 2000) detector and eluted with Methanol: Hexane (60:40 v/v), at a flow rate of 1 ml min-1. After drying of sample with spray drier, the 100mg of biomass is mixed with 2 ml of organic solvents individually and kept at 50°C for about 20 minutes or orbital shaking for 30 minutes. Supernatant was spined at 2,500 rpm for 5-6 minutes. 20µl of the sample injected for HPLC analysis. The 99% pure standard carotenoids: lutein, zeaxanthin, and ß-carotene, purchased from (Sigma Chemical Co.) were injected individually under same conditions and the retention times are 2.3, 4.3, and 10.4 minutes respectively.

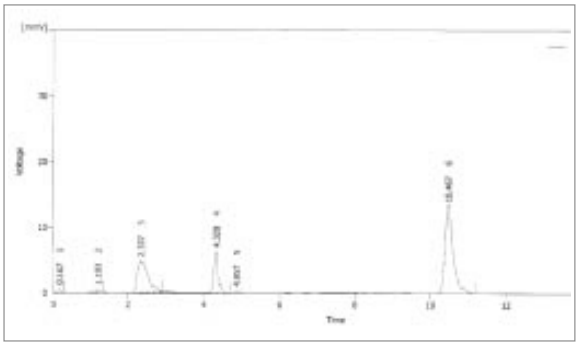
Purification of the Carotenoids

After mixing with the first phase of solvents, in case of Beta carotene purification, add PEG (poly ethylene glycol 400) in 4:1 ratios. The Beta carotene remains in the upper layer. All the impurities settled in poly ethylene glycol and are discarded (16). Now the 20µl of the sample is taken and insert for HPLC analysis and the purity is checked. The set of experiments performed for the different organic solvents and the purity of the carotenoids were observed and the highest purity giving solvents were tabulated.

Results and Discussion

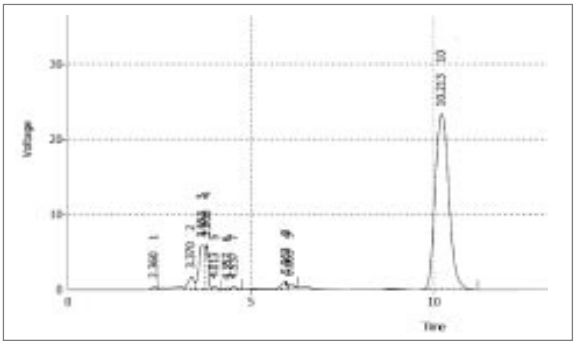
Because of bipolar nature, Acetone based solvent extraction yields both polar and non polar carotenoids into the solvent. The result is shown (see Graph.1).

Graph.1 : Dunaliella salina dried biomass (100mg/10 ml Acetone) HPLC analysis



The Beta carotene extracted with 89.4% purity by the mixture of organic solvents petroleum ether: n-hexane (6:4) ratios followed by PEG 400 purification (See graph. 2).

Graph.2
Purification of Beta carotene with PEG 400



Lutein extraction by Ethanol and evaporation with the nitrogen gas yielded 92.1% purity (see table.1). Zeaxanthin extraction with Acetone and purification with non-polar solvent n-Hexane yielded 75% purity (see table.1).

Table.1
Qualitative and Quantitative analysis of the Carotenoids

S. no	Carotenoid name	% of Quantity per (100mg)	% of Purity with solvent
1	Beta-Carotene	28 %	89.4% (Petroleum ether: n - Hexane(6:4))
2	Lutein	12 %	92.1% (Ethanol)
3	Zeaxanthin	15 %	75% (n-Hexane)

The optimization studies revealed dunaliella salina required a slightly alkaline pH 8, high light intensity 18,000 lux, low temperature 20°C and low NaCl concentration (0.5M) for the maximum production of the valuable carotenoids.

Conclusion

The carotenoids can be extracted from the plant source. But, the purification of specific carotenoid is highly difficult because, plant cells accumulate variety of carotenoids as well as proteins. In case of microalgae carotenoids accumulation is highly specific and purificationalso easy when compared to other systems. Because carotenoids are having high commercial value, the cultivation of microalgae and purification with organic solvents is economically cheap.

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