

Extraction of Astaxanthin from Hyperproducing *Haematococcus Pluvialis* Using Different Oils

Nisha Tiwari*, Surendra Singh

Algal Biotechnology Laboratory, Department of Post Graduate Studies and Research in Biological Science,
Rani Durgawati University, Jabalpur-482001, Madhya Pradesh, India

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ABSTRACT

Astaxanthin is a high antioxidant which is red carotenoid pigment obtained from many microalgae species. It is an important preservative of the immune system and it is used in the treatment of cancer, skin and heart diseases. The most efficient source of astaxanthin is *Haematococcus pluvialis* microalgae. The aim of the present investigation was to extract the astaxanthin, a food, a feed and pharmaceutical valuable secondary carotenoid from the *Haematococcus pluvialis* with common vegetable oils. Without a cell harvest process step, the culture was directly mixed with the vegetable oils; the astaxanthin inside the cell was extracted into the vegetable oil phase by hydrophobic interaction. We have found that the maximum extractability of astaxanthin 98.28 % in soybean oil from mutant strain.

Keywords:- Astaxanthin, microalgae *Haematococcus pluvialis* vegetable oil, extractability.

I. INTRODUCTION

Algae are one of the most useful natural resources that can be used to produce different bioactive compounds such as vitamins, proteins, unsaturated fatty acids, antioxidants and carotenoids, including astaxanthin. Currently, research findings mentioned that the green microalga *Haematococcus pluvialis* (*H. pluvialis*) was recognized in synthesizing and accumulating the largest amount of natural astaxanthin (Shah *et al.*, 2016). *Haematococcus pluvialis* is a green, biflagellate and freshwater microalga which has been subject to much interest due to its ability to produce the

bioactive compound astaxanthin in high yields. Astaxanthin, a red keto-carotenoid pigment, is produced in its free, mono-, and diester forms, as a secondary metabolite of this microorganism. The valuable antioxidant capacity of astaxanthin - especially in its polyunsaturated fatty acid (PUFA) ester forms- is due to its unique molecular structure, and is 10-fold greater than other carotenoids such as lutein, zeaxanthin, canthaxanthin and β -carotene, all of which are also strong antioxidants (Nagaraj *et al.*, 2012).

Astaxanthin, one of the main xanthophyll carotenoid pigments, possesses 500-fold and 38-fold times

stronger free radical antioxidant activity of vitamin E and β -carotene, respectively (Sarada *et al.*, 2006). Owing to its strong antioxidant properties, astaxanthin plays an important role in protection against inflammation, UV-light photooxidation, aging and age-related macular degeneration, and cancer and in enhancement of the immune response, liver function, heart health, and so forth (Guerin *et al.*, 2003). Hence, astaxanthin has widespread applications in the pharmaceutical, cosmetic, food, and feed industries (Holanda *et al.*, 2006). Nowadays, astaxanthin has two main commercially available formations: chemical synthesis and natural resources from microalgae, yeast, and crustacean byproducts. Though dominating the current world market, chemical synthetic astaxanthin has been banned into health food market by the U.S. Food and Drug Administration (FDA) due to its low bioavailability and security. Therefore, astaxanthin obtained from natural resources has triggered more and more attention in recent years (Seabra *et al.*, 2010).

II. MATERIAL AND METHODS:-

Algal culture and growth condition

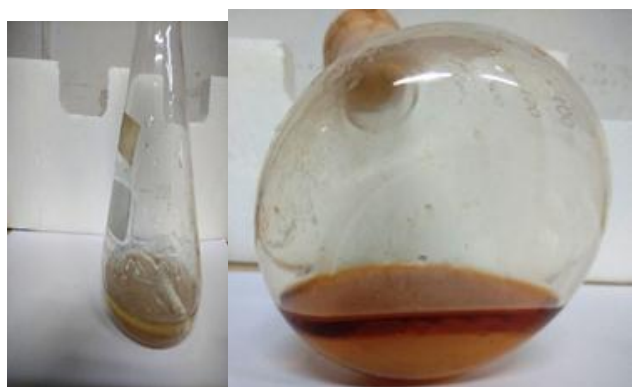
The wild type *Haematococcus pluvialis* (strain UTEX - 2505) was obtained from the Algal Biotechnology Lab., Department of P.G. Studies and Research in Biological Science, R. D. University, Jabalpur (M.P.). The different algal strain, namely EMS 12, EMS 24, EMS 36, EMS 48, UV 15 and UV 30 were obtained in own laboratory by treating the wild type strain with ethylmethanesulphonate and UV irradiation. All the cultures were inoculated into 250ml Erlenmeyer flasks and cultivated in Bold Basal Medium. The lighting was supplied by white fluorescent tubes in a air – conditioned culture room with $35 \mu\text{mol m}^{-2} \text{s}^{-1}$ in light/ dark cycle 16:8 hrs under $25 \pm 2^\circ \text{C}$. Subculture of the algal cells were made weekly.

Extraction of astaxanthin: - The extraction process was performed following (Kang and Sim 2008). A 30 ml of high growth mutated culture and wild culture

were mixed with 30ml of each edible oil (coconut oil and soybean oil). After vigorous stirring, on, magnetic stirrer at room temp for 48 hrs. The mixture was permitted to settle under gravity after stopped the stirring before collected the oil extracted supernatant. The absorbance of the oil extract (super layer) was scanned for total carotenoid content with UV spectrophotometer (400-700nm) using actual oil as blank and astaxanthin was quantified at 480 nm compared with an authentic standard (Kobayashi *et al.*, 1997).

III. RESULTS

Astaxanthin extraction from the red cyst cell culture was performed with each of the vegetable oils in the proposed process. The carotenoid pigment of astaxanthin was extracted into oil phase (upper layer) due to their hydrophobic property. There is a difference in polarity between the culture (water phase) and oils, which creates a phase separation, and allows the oils to separate from culture medium containing the cell debris by gravity. The extraction of astaxanthin, the redness color of the oil phase deepened in which astaxanthin has a specific absorbance peak at 480 nm. Almost all astaxanthin was extracted from the red encysted cells to each vegetable oil after extraction for 48 hrs. The absorbance peak at 480 nm of individual 2 oils extracts from wild and mutant strains were compared with the counterpart base oil and standard astaxanthin. The results indicate that highest amount of astaxanthin 98.28 % was found in soybean oil extract from mutant strain EMS 24 as compared with wild type.

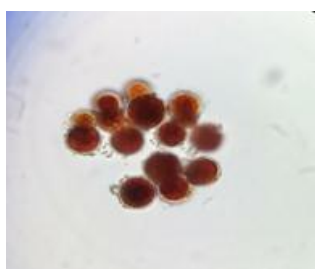


(A)

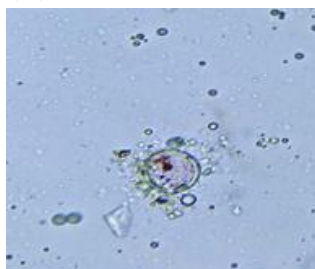


(B)

Figure 1:- Images of the oil extracts during extraction of astaxanthin from red encysted cells of *Haematococcus* cultures (A) separation layer of the oil and water phase (B) oil extracts with soybean and coconut oil

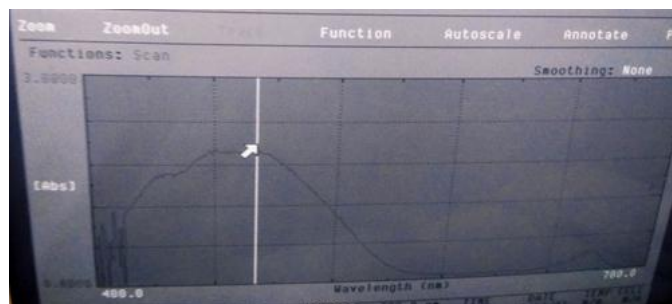


(A) before extraction

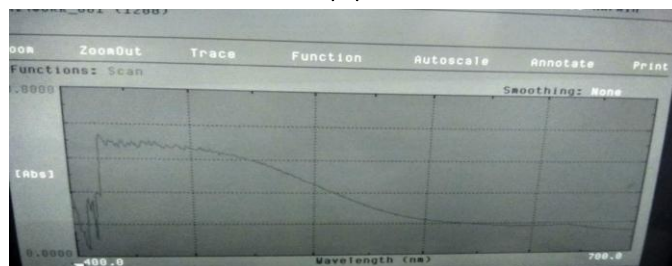


(B) after extraction

Figure 2:- Photomicrographs of *Haematococcus pluvialis* encysted cells



(A)



(B)

Figure 3:- Absorbance spectra (400-700) of oil extract from encysted cells of *Haematococcus pluvialis* (A) soybean oil extract (B) coconut oil extracts

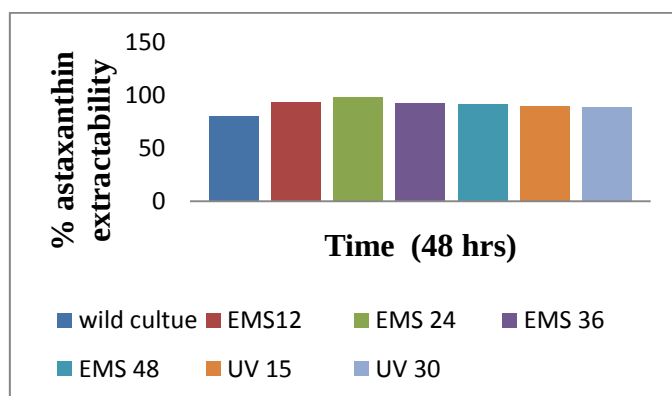


Figure 4:- extractability of astaxanthin at (48 h) from the encysted *Haematococcus* wild and mutant cultures with soybean oil

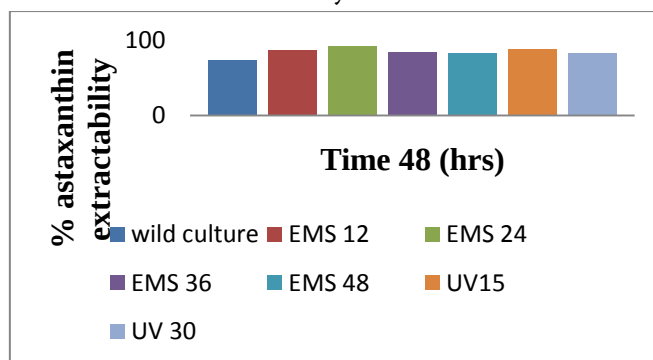


Figure 5:- extractability of astaxanthin at (48 h) from the encysted *Haematococcus* wild and mutant cultures with coconut oil

IV. DISCUSSION

The astaxanthin pool of red encysted *H. pluvialis* consists of approximately 70% monoesters 25% diesters, and 5% free forms, which leads to a very lipophilic property of *Haematococcus* cells astaxanthin (Lorenz and Cysewski 2000; Hussein *et al.*, 2006). Different types of enzymes were also used for the extraction of astaxanthin from cells of *Haematococcus pluvialis* cells with 40% (v/v) acetone for 2 min at 80°C, and the extractability of astaxanthin was achieved 70% (Kobayashi *et al.*, 1997; In *et al.*, 2008). The direct extraction of *Haematococcus* astaxanthin has been performed by using common vegetable oils such as soyabean oil; grapes seed oil, corn oil, and olive oil, with recovery yield of over 87.5%. The highest recovery yield of astaxanthin has been indicated in olive oil extract (Kong and Sim 2008). Therefore, present study, have some advantages such as being simple, quick, reproducible, free of corrosible material and without presenting significant losses.

V. CONCLUSION

In the present study, we examined the comparison of wild and mutant strains of *H. pluvialis* extracted with soybean oil and coconut oil vegetable oils extraction is a beneficial method to extract biological active compound without bring pollution to the environment with a short extraction time. Further investigation would help to apply vegetable extraction method in the food industry and cosmetic industry.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

ORCID

Nisha tiwari <https://orcid.org/0000-0002-0342-5333>

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