*Review paper*

# Microalgal Carotenoids: Biosynthesis and green extraction

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**Abstract:** Microalgae produce a variety of photosynthetic pigments that include chlorophyll, carotenoids, xanthophylls etc. These compounds are used in different industries like pharmaceuticals, food, and cosmetics. Among these compounds, carotenoids have been proposed as health promoters due to their antioxidant and coloring properties. Due to the harmful effects of synthetic color, the use of microalgae as an alternative natural source becomes an attractive option. However, the extraction method is an important factor to produce carotenoids.

**Keywords:** Microalgae; Heavy metals; Chlorophyll; Carotenoids; Green solvent extraction

## 1. INTRODUCTION

Carotenoids are isoprenoid molecules, natural organic pigments that give color to most red, yellow, and orange vegetables and fruits. They are highly distributed pigments in nature and are found in cells and tissues of all representatives of wildlife (Zia-Ul-Haq et al., 2021). These pigments belong to a huge class of terpenes, most of which are tetraterpenes (C40). Carotenoids have an important ecological role, as well as a crucial one in various physiological processes. Often, they are carriers of energy and electrons (Higdon et al., 2007). An extensive range of preclinical and clinical studies shows that dietary carotenoids have a considerable health-enhancing outcome. There is significant evidence of their ability to reduce the risk of serious chronic diseases. In algae and higher plants, they also support the configuration and tasks of the photosynthetic complex, quench the triplet states of chlorophyll, purify ROS, dissipate excess energy, and help in light absorption (Miyashita et al., 2011). One of the most significant carotenoids is beta-carotene. Beta-carotene serves as a precursor of vitamin A (retinol) and can be revealed as a powerful antioxidant. Also, it has an immunostimulating and adoptogenic effect. The unsaturated structure of beta-carotene allows its molecules to absorb light and prevent the accumulation of free radicals and ROS (Reactive Oxygen Species). Beta-carotene suppresses the production of free radicals. It is assumed that in this way it protects the cells of the immune system from damage by free radicals and can improve the state of immunity (Santos et. al., 1997). Also, beta-carotene is widely known as a colorant in a generous number of industries. Plants, bacteria, fungi, and algae act as a source of beta-carotene.

**Citation:**.

Praveen et al. 2022. Microalgal Carotenoids: Biosynthesis and green extraction. Octa J. Biosci. Vol. 10 (1):6-12

Received: 12/2/2021
Revised: 30/5/2022
Accepted: 15/6/2022
Published:30/6/2022

## 2. SYNTHESIS OF CAROTENOID PRECURSORS (IPP & DMAPP):

Chloroplast is the site for photosynthesis, it also biosynthesized and accumulates carotenoids. Carotenoids are grouped into two categories, Primary and secondary carotenoids having a crucial role in photosynthesis and stress conditions. Under different stress conditions such as biotic and abiotic factors increase the carotenoid content of plants. Carotenoids are obtained from two isoprene isomers, which are as follows; IPP (isopentenyldiphosphate) and DMAPP (dimethylallyldiphosphate). Mostly, two types of pathways exist for the biosynthesis of IPP molecules in plants: the cytosolic mevalonic acid (MVA) pathway, which was discovered in the 1950s and the plastidic MEP pathway (Redriguez et al., 2004).
Cytosolic MVA pathway mostly occurred in fungi, archaebacteria and animals (Lichtenthaler et al., 1999), while the MEP pathway occurred in photosynthetic bacteria, eubacteria, cyanobacteria, microalgae and malarial parasites.

### Mevalonic acid pathway (MVA Pathway):

MVA is a special medium for IPP biosynthesis. By the presence of the enzyme HMG-CoA synthase, the synthesis of β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) occurs from two moles of acetyl-CoA. Mevalonic acid forms by the reduction of NADPH and HMG-CoA reductase (Alcaino et al., 2016). Additionally, decarboxylation of diphosphomevalonate decarboxylase occurs to form IPP (Lichtenthaler et al., 2004). After that, IPP isomerizes for the synthesis of DMAPP molecules (Iriti et al., 2009). Condensation of both IPP and DMAPP molecules forms geranylgeranyldiphosphate (GGPP, C20) which is a precursor of carotenoids.

### Methylerythritol phosphate (MEP Pathway) or non-mevalonate pathway:

In the presence of DXP synthase (DXS), deoxy-D-xylulose 5-phosphate (DXP) is formed with the help of two substrates, glyceraldehyde 3-phosphate and pyruvate. DXP (deoxy-d-xylulose 5-phosphate) is reduced to MEP in the presence of the enzyme DXP reductoisomerase (DXR). The enzymes DXS and DXR show a crucial role in the flux regulation of carotenoids. After several steps of sequential condensation, IPP and DMAPP are formed to yield the precursor of carotenoid biosynthesis, geranylgeranyldiphosphate (GGPP) (Wanke et al., 2001).

### Synthesis of Carotenoids (Fig1) (Swapnil et al., 2021):

GGPP is an instant metabolic substrate for the carotenoid biosynthesis pathway. Carotenoid biosynthesis starts in the presence of an enzyme phytoene synthase (PSY) by which condensation of two GGPP molecules into phytoene (Nisar et al., 2015). The enzyme PSY decided the pool size of the carotenoid, it is an important rate-limiting and key flux controlling enzyme. Phytoene occurs as a colorless carotene because the central atom of the hydrocarbon chain contains a triple bond. PDS and ZDS are two phylogenetically related enzymes, which proposed the four double bonds into phytoene. The phytoene was converted into tetra-cis-lycopene by two symmetrical dehydrogenations, which catalyzed these two enzymes. As per the previous data reported, the genes for PDS, ZDS and CRTISO are present in all groups of algae and the gene for Z-ISO is absent in red algae.

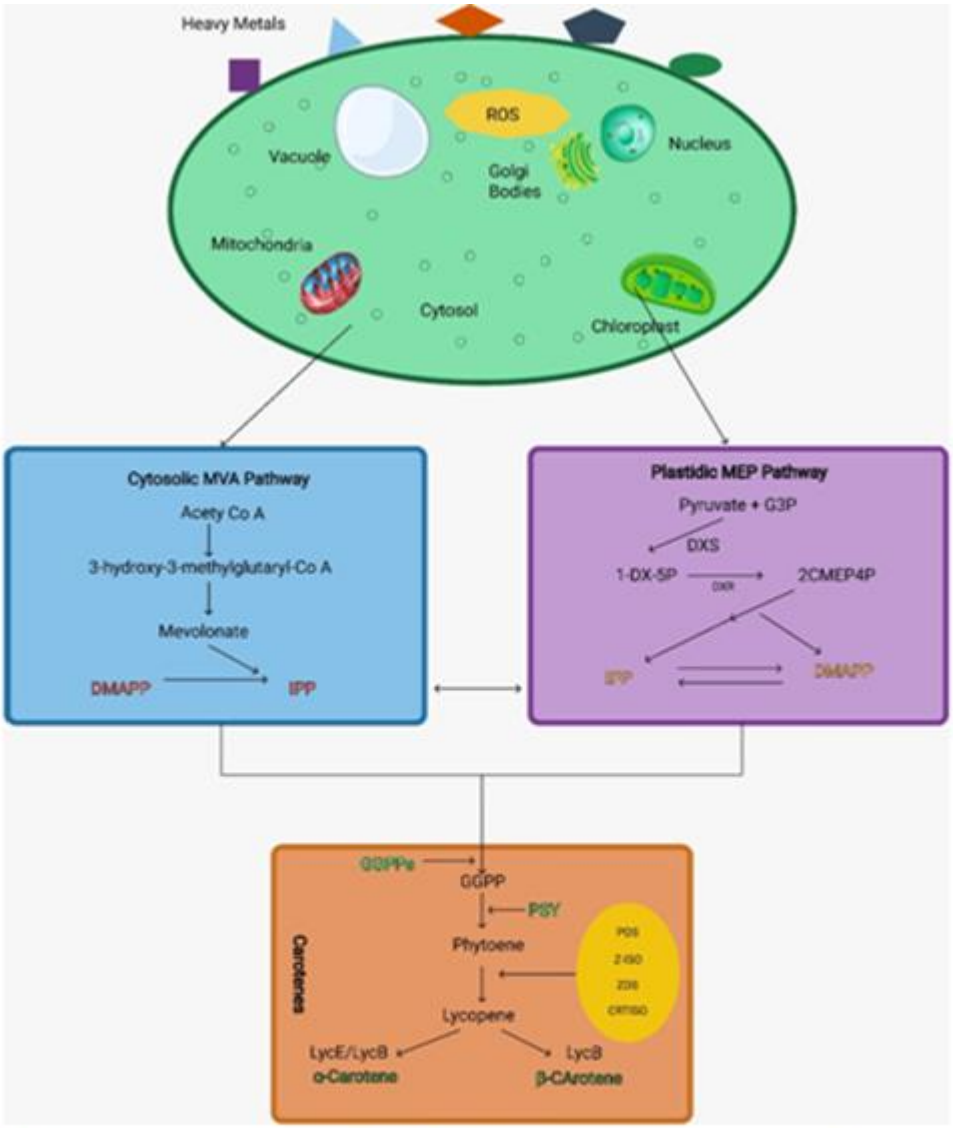


Fig.1: Biosynthesis of carotenoids; MEP(Methylerythritol 4-phosphate),GGPP (geranylgeranyldiphosphate),PSY(phytoene Synthase),PDS(phytoenedesaturase),Z-ISO (15-cis-f-carotene isomerase),ZDS(f-carotene desaturase),CRTISO(carotenoid pro-lycopene isomerase),LcyB(lycopene β-cyclase), LcyE(lycopene ε-cyclase) [11].

Lycopene is the important branching point, where the biosynthesis of both the carotenoids occurs and the ratio of $\alpha$-carotenoid and β-carotenoid is to be decided. The manufacture of $\alpha$-carotene and β-carotene is a two-step process catalyzed by the two different enzymes LycE and LycB. In the first step of the $\alpha$-carotene synthesis, cyclization at the one open end takes place by the enzyme LcyE which is converted into δ-carotene and the second step involves the $\alpha$-ring which is converted to lutein. B-carotene is also synthesized by two steps, but both of these steps are catalyzed by the enzyme LycB (Deng et al., 2020). Some groups of algae such as Bacillariophyceae, Crysophyceae, Phaeophyceae, Xanthophyceae lack the synthesis of δ-carotene and the β-carotene occur in all groups of algae and other photosynthetic organisms (Takaichi et al., 2011).

**3. EXTRACTION OF CAROTENOIDS:**

Various kinds of food products contain carotenoids; there is not a standard method for the extraction of carotenoids in the laboratory. There are various factors such as different polarities of carotenoids structure of the matrix and component shows a vital role in the selection of solvent for the extraction of carotenoids. Hexane is mostly used for non-polar and esterified carotenoids, while ethanol and acetone are used for polar carotenoids (xanthophylls) (Amorim-Carrilho et al., 2014). Plants and microalgal cells have a selectively permeable cell wall that blocks the entry of solvents into the cells. So, in the first step of the extraction cell wall are disrupted by physical, chemical and biological methods.

There are various mechanical methods which include grinding, bead milling, high-pressure homogenizer (HPH) and autoclave. The most used and easiest microalgal cell wall disruption is grinding, bead milling and HPH (Gogate et al., 2020). These methods have some limitations such as high energy requirements and excessive heat generated during the process which results in the degradation of carotenoids.

These are the Electrotechnologies include MAE, UAE, HVED and PEF worked as non-thermal extraction techniques which attacks intracellular compounds (Golberg et al., 2016). For the induction of cell wall disintegration and extraction of intracellular compounds, these Electrotechnologies passed an electric current through the cell or suspension. The higher pigment collection was reported by the quick extraction techniques UAE and MAE. In the PEF technique, the solvent requirement is reduced or decreased. The major disadvantages of these Electrotechnologies are an increase in the temperature during the process (Jacob-Lopes et al., 2020).

Green solvents are environment-friendly solvents or bio-solvent, which are derived from the processing of crops. The major advantage of GS is that they are produced from raw-material and eco-friendly crude oil solvents that are non-toxic and biodegradable. Bio-solvent like D-limonene was used as an alternative to dichloromethane for pigment extraction from tomato (Chemat-Djenni et al., 2010).

Plant dry matter or lignocellulosic biomass is used in the preparation of 2-Methyltetrahydrofuran (MTHF) which is known as green solvent and has the advantage of being biodegradable and easily recyclable. Due to its distinctive properties, MTHF is used in the extraction of carotenoids, as an alternative to n-hexane (Sicaire et al., 2014). In GSE, wet biomass was used for extraction, it is an eco-friendlier technique in which green solvent or biodegradable solvent are used. The microalgal biomass was harvested, obtained 20% dry weight biosolid and then stored at -20°C. Microalgae biomass was freeze-dried before extraction and lyophilized at -70°C at low pressure. With the help of pestle mortar, the lyophilized algal biomass was crushed into a fine powder, and then 80% acetone was added and followed by centrifugation. After centrifugation, the supernatant was collected, and the content of chlorophyll and carotenoids was measured based on absorbance in the spectrophotometer.

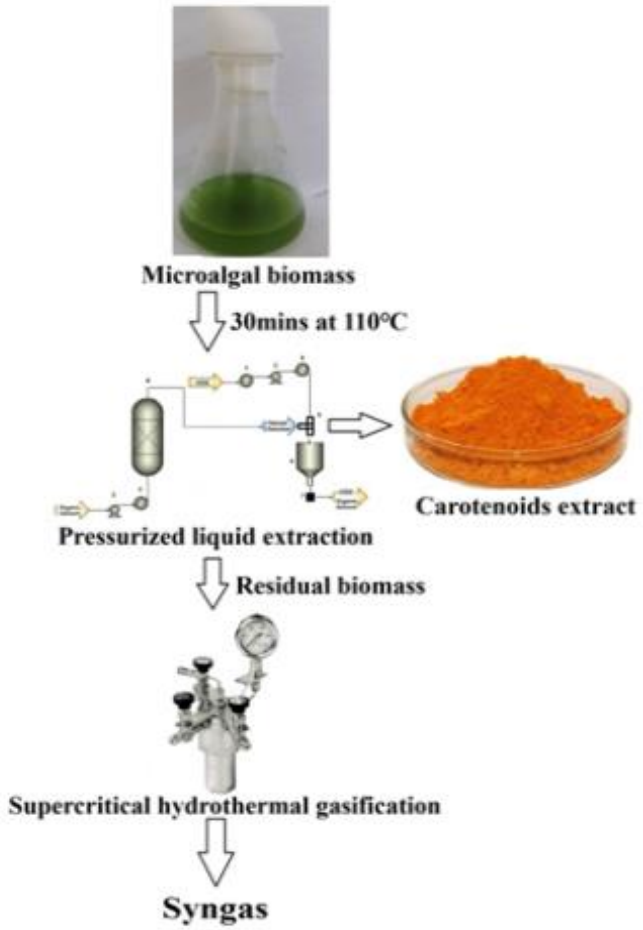


**Fig.2. Green extraction of carotenoids** (Damergi et al., 2017)

Extraction was performed by using two solvents mixtures, MTHF (100%) and an MTHF+ethanol (1:1) mixture at two temperatures (40° and 110°C) for a constant time (15 and 30 min) and constant pressure (1500psi and 103bars). When the temperature reached 40°C and 110°C, 2 and 6 minutes heating was used. After the extraction, the solvent was purified with N2 gas and depressurization occurs. The liquid extraction evaporated with a continuous stream of N2 gas until solvent evaporated completely. The extract was stored in dark at -20°C. After evaporation, the remaining part was resuspended in an acetone:hexane (2:3, v/v) mixture. Before, the mixture was injected into the high-performance liquid chromatography (HPLC), it was filtered with the help of a 0.2µm hydrophobic PTFE filter. The constituents of the carotenoids extract were measured by the HPLC system.

**4. Conclusion and future scope**

In this review the entire process of carotenoid synthesis and extraction is discussed. Because the health benefits of carotenoids are now better understood, natural carotenoid from biosynthesis is gaining market preference. Microalgae are good hosts for large scale production of carotenoids due to their high carotenoid content, rapid development, and many different advantages. Furthermore, additional efforts must be directed toward the development of technology for large-scale, cost-effective upstreaming and downstreaming techniques. There is an urgent need for more cost-effective, practical, and efficient harvesting technology that may be used in various microalgae industrial operations.

**5. Acknowledgment**

The author thanks the Department of Lifesciences, Graphic Era Deemed to be university, Dehradun, 248002, India

**Author Contributions:** Afreen: writing—original draft preparation, Irena: writing—review and editing, Vinod: writing—review and editing and Pankaj and Anna: writing—review and editing funding acquisition, M.T. All authors have read and agreed to the published version of the manuscript.

**Conflicts of Interest:** The authors declare no conflict of interest.

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