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Factors Affecting the Production of Astaxanthin in the Microalgae *Haematococcus pluvialis*: A Review

Muhsinin Soni[1*], Aligita Widhya[1], Rostinawati Tina[2], Levita Jutti[3]

[1]Faculty of Phramacy, Bhakti Kencana University, Jl. Soekarno Hatta No.754, Bandung, West Java, Indonesia 40286, India; [2]Department of Biology Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Jl. Raya Bandung-Sumedang km.21, Sumedang, West Java, Indonesia 45363, India; [3]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Jl. Raya Bandung-Sumedang km.21, Sumedang, West Java, Indonesia 45363, India.

ABSTRACT

Astaxanthin, a natural red pigment that belongs to the carotenoid group, has been known as a super antioxidant due to its very strong antioxidant activity (65 times higher than vitamin C, 54 times more potent than -carotene, and 14 times higher than vitamin E). *Haematococcus pluvialis* is known as microalgae with a high astaxanthin content. The benefit of astaxanthin in health issues is mainly its potential as the treatment for degenerative diseases caused by reactive oxygen or nitrogen species. Thus, it is important to developing *Haematococcus pluvialis* microalgae as a rich source of natural astaxanthin in the health and pharmaceutical industries.

Key Words: Astaxanthin, Antioxidants, Haematococcus pluvialis, Carotenoids, Microalgae, Anticancer

INTRODUCTION

Astaxanthin (3,3'-dihydroxy-β-carotene-4,4'dione) is a secondary metabolite belonging to the carotenoid group.[1–3] Astaxanthin has a high value in the pharmaceutical, nutraceutical, and cosmetic fields because of its potent antioxidant potential with an IC_{50} value of 39.1 ± 1.14 ppm.[4] The antioxidant activity produced by astaxanthin is 65 times higher than that of vitamin C, 54 times more powerful than β-carotene, 14 times higher than vitamin E, and 20 times stronger than its synthetic form.[5] Due to its potent antioxidant activity, astaxanthin can be used to treat several degenerative diseases caused by free radicals.[6]

Various sources of astaxanthin in nature can be obtained from several microorganisms such as the fungus *Phaffia rhodozyma*, microalgae *Chlorella zofingiensis,* and *Haematococcus pluvialis*.[7–9] However, of these microorganisms, *H. pluvialis* is known to show the highest astaxanthin accumulation capacity of up to 4% dry weight under stress conditions.[10,11]

The market price of astaxanthin also varies, ranging from $2,500 to 7,000/kg. In 2014, the global market potential of astaxanthin was approximately 280 tonnes for $400 million. However, more than 95% of the market is synthetic astaxanthin types that are sourced from petrochemicals. This happens because the production cost of synthetic astaxanthin is relatively cheaper than natural astaxanthin obtained from microalgae.[12] This synthetic type of astaxanthin has 20 times lower antioxidant power than the natural type.[5] In addition, related to safety issues, synthetic astaxanthin types are still not allowed to be consumed by humans due to differences in stereochemical form with natural type. Therefore, its use is only permitted as feed and dye for aquaculture organisms.[10]

Astaxanthin production can be done by various methods, including culture, chemical synthesis, and genetic engineering. The culture method can be done by adding stress induction to microalgae because it is known that *H. pluvialis* is a microalgae that can accumulate astaxanthin under stress. These stress conditions can be caused by several factors, including light stress,[13,14] nutritional deficiency,[15] salinity stress,[16,17] the addition of Fe^{2+},[18,19] and so on. In addition, another method is chemical synthesis using asta-C_{15}-triarylphosphonium salt and C_{10}-dialdehyde with the Wittig reaction,[20] which produces synthetic astaxanthin with antioxidant activity 20 times lower than natural astaxanthin. Then another method, genetic engineering, in several research journals has been widely reported overproduction of astaxanthin in several microorganisms such as fungi and bacteria.[21] This review article contains

Corresponding Author:
Muhsinin Soni, Jl. Soekarno Hatta N0.754, Bandung, West Java, Indonesia 40286, India.
Mobile: +6281320742590; E-mail: soni.muhsinin@bku.ac.id

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biological and physiological conditions, biochemical content, and methods of producing astaxanthin from *H. pluvialis* by culture and genetic engineering.

Biology of H. pluvialis

a. Taxonomy

*H. pluvialis*is a biflagellate unicellular microalgae that lives in freshwater. According to Lorenz (1999),[22] the classification of *H. pluvialis* microalgae is as follows:

Kingdom	Plantae
Divisio	Chlorophyta
Classis	Chlorophyceae
Ordo	Volvocales
Familia	Haematococcaceae
Genus	Haematococcus
Species	*Haematococcus pluvialis*

b. Habitat

The habitat of *H. pluvialis*is spread evenly in the world, especially in temperate areas. This microalgae has been isolated in Europe, Africa, North America, and Himachal Pradeslv India.[23,24] *H. pluvialis* is also found in various environmental conditions with extreme climates, which may be lethal to other types of microalgae. This is because *H. pluvialis* can defend itself by forming encysts (cells become closed with a thick membrane) quickly when under stress and extreme conditions.[25]

c. Morphology

The cell structure of *H. pluvialis* is similar to that of some groups of volvocalean green microalgae. The life cycle of *H. pluvialis* consists of four phases with different cellular morphology, namely macrozooid (zoospore), microzooid, palmella, and hematocyst (aplanospore).[10,26] The following is the morphology of *H. pluvialis* microalgae with descriptions (A) Motile macrozoid cells (zoospores) with a size <10 m or 20 m, (B) Microzoid cells, (C) Palmella cells with accumulation of astaxanthin, (D) Hematocyst cells with accumulation of astaxanthin with size > 50 m.

The macrozoid, microzoid, and palmella phases are also known as the green vegetative phase. The microzoid phase (zoospore) is when the cell has a spherical, elliptical or pear-shaped shape with two flagella of the same length and appears anteriorly and has cup-shaped chloroplasts (Figure 1A). In this phase, with the optimum environment, flagellated cells undergo rapid division and growth, producing 2-8 daughter cells.

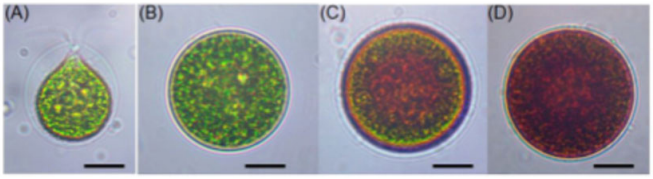

Figure 1: Morphology of *H. pluvialis*.
Figure adapted from Shah *et al*. (2016),[27] which is licensed under the Creative Commons Attribution License.

However, suppose the environmental conditions are unfavorable (stress). In that case, the cell will remove the flagella and begin to expand in size by forming an amorphous structure layered on the inside of the extracellular matrix and develops into non-motile cells called palmella (Figure 1B).[28] In this phase, the *H. pluvialis* cell wall thickens and consists of three layers. The first layer is a trilaminar layer containing materials such as sporopollenin, an algaenan that is resistant to acetolysis.[29] According to Kim *et al*. (2016),[30] the content of algaenans in the cell walls of *H. pluvialis* microalgae will inhibit the extraction process using several solvents such as acetone, methanol, dichloromethane. The second and third layers contain mannose and cellulose.[28,31,32]

The hematocyst phase was also referred to as the non-motile phase with astaxanthin accumulation (Fig. 1C and 1D). This phase occurs when the state of stress continues. This stress state can be in the form of nutritional deficiency, light stress with a certain intensity, salinity stress, and the addition of certain chemicals that can induce stress. Under these conditions, the palmella will turn into an asexual form or hematocyst (aplanospore). Mature hematocysts accumulate large amounts of carotenoids, especially astaxanthin, stored in lipid droplets in the cytoplasm.[28]

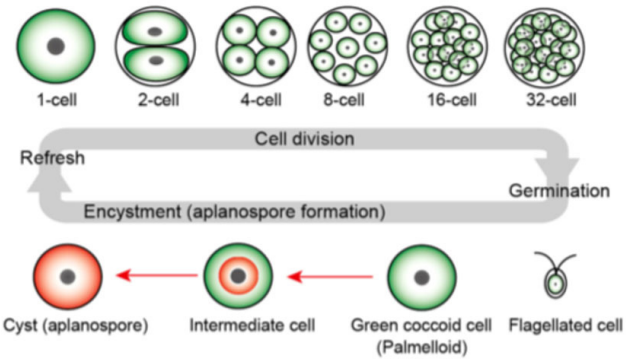

Figure 2: Illustration of the life cycle of *H. pluvialis*.
Figure adapted from Wayama *et al*. (2013),[33] which is licensed under the Creative Commons Attribution License.

After the environmental conditions return to normal and optimal, the hematocyst (aplanospore) will germinate again to form a microzoid (zoospore) which will re-initiate the start of a new vegetative growth cycle (Figure 2).[33]

Biochemical Content

The cellular content of the *H. pluvialis* microalgae varies between the green phase and the red phase due to its unique life cycle. The biochemical range of *H. pluvialis* in the green phase and red phase according to [34] is listed in Table 1.

Table 1: Biochemical content of *H. pluvialis*

Content (% Dry Weight)	Green Phase	Red Phase
Protein	29-45	17-25
Lipids (% total)	20-25	32-37
• Neutral Lipids	59	51.9-53.5
• Phospholipids	23.7	20.6-21.1
• Glycolipids	11.5	25.7-26.5
Carbohydrate	15-17	36-40
Carotenoids (% total)	0.5	2-5
• Neoxanthin	8.3	-
• Violaxanthin	12.5	-
• β-carotene	16.7	1.0
• Lutein	56.3	0.5
• Zeaxanthin	6.3	-
• Astaxanthin (including ester)	-	81.2
• Adonixanthin	-	0.4
• Canthaxanthin	-	5.1
• Echinenone	-	0.2
• Chlorophyll	1.5-2	0

Description (-): no data reported

According to Table 1, *H. pluvialis* produced 81.2% Astaxanthin (including ester) in the red phase. This amount is the highest compared to primary metabolites (Proteins, Lipids, Carbohydrates) and other carotenoid compounds. The green phase does not produce astaxanthin. *H. pluvialis* enters a logarithmic phase (growth phase) and produces more primary metabolites during this phase.

Astaxanthin

a. Sources of Astaxanthin

Natural sources of astaxanthin are found in several organisms, including algae, bacteria, fungi, salmon, shrimp, lobster.[35] But for the mass production of astaxanthin, microorganisms such as fungi and microalgae are more widely used because of their rapid growth. Some of the natural astaxanthin-producing microorganisms are listed in Table 2.

Table 2: Sources of Astaxanthin

Sources	Astaxanthin (% Dry Weight)	Reference
Chlorophyceae		
Haematococcus pluvialis	3.8	36
Botryococcus braunii	0.4	36
Neochloris wimmeri	0.6	37
Chlorella zofingiensis	0.001	38
Ulvophyceae		
Enteromorpha intestinalis	0.02	39
Ulva lactuca	0.01	39
Florideophyceae		
Catenella repens	0.02	39
Alphaproteobacteria		
Paracoccus carotinifaciens (NITE SD 00017)	2.2	40
Tremellomycetes		
Xanthophyllomyces dendrorhous (JH)	0.5	41
Labyrinthulomycetes		
Thraustochytrium sp. CHN-3 (FERM P-18556)	0.2	42

According to Table 2, *H. pluvialis* is the microalgae that produce the most significant amount of astaxanthin (up to 3.8%) (excluding esters). HPLC and LC-MS methods for analyzing astaxanthin compounds. The biomass of *H. pluvialis* was homogenized and extracted with acetone several times. The extracts were combined, evaporated with a rotavopar, and then redissolved in acetone.

b. Astaxanthin Biosynthesis

Astaxanthin biosynthesis in *H. pluvialis* is a complex series of processes that occur under stress conditions along with triacylglycerol (TAG) accumulation. Both compounds are deposited in lipid droplets in the cytosol during the red phase. The formation of astaxanthin begins with the glycolysis process, which produces pyruvate and glyceraldehyde-3-phosphate (G3P). Furthermore, pyruvate, together with glyceraldehyde-3-phosphate (G3P), will form the compound Isopentenyl Pyrophosphate (IPP) as the primary precursor in the synthesis of carotenoids.

Astaxanthin belongs to the carotenoid group, is one of the C_{40} tetraterpenes synthesized from the isoprene unit Isopentenyl Pyrophosphate (IPP). In principle, IPP synthesis can originate from two different pathways: the mevalonate pathway (MVA) occurring in the cytosol and the non-mevalonate pathway (MEP) or the 1-deoxy-D-xylulose-5-phosphate (DOXP) pathway occurring in chloroplasts.[43–45]

In *H. pluvialis*, IPP is synthesized from the non-mevalonate pathway. Furthermore, IPP undergoes isomerization to dimethylallyl diphosphate (DMAPP). Some research results

indicate that the conversion is catalyzed by the enzyme isopentenyl pyrophosphate isomerase (IPI) encoded by the ipi1 and ipi2 genes during astaxanthin accumulation.[2] However, the results of another study also stated that neither of the ipi1 and ipi2 genes was increased as long as *H. pluvialis* cells accumulated astaxanthin.[46] Another study reported that another enzyme with similar activity, namely 4-hydroxy-3-methylbut-2-enyl diphosphate reductase (HDR), was more likely to be responsible for catalyzing the intermediate conversion of IPP to DMAPP.[46–48]

Elongation of the isoprene chain begins with a DMAPP molecule, and the addition of three IPP molecules is catalyzed by the enzyme geranyl-geranyl pyrophosphate synthase (GGPS).[49,50] The next step of this process is the formation of the compound C_{20} geranyl-geranyl pyrophosphate (GGPP). GGPP is converted to C_{40}-phytoene as a precursor of astaxanthin and other carotenoids with the help of the phytoene synthase (PSY) enzyme encoded by the psy gene coupled with the head-to-tail condensation of two GGPP molecules.[50]

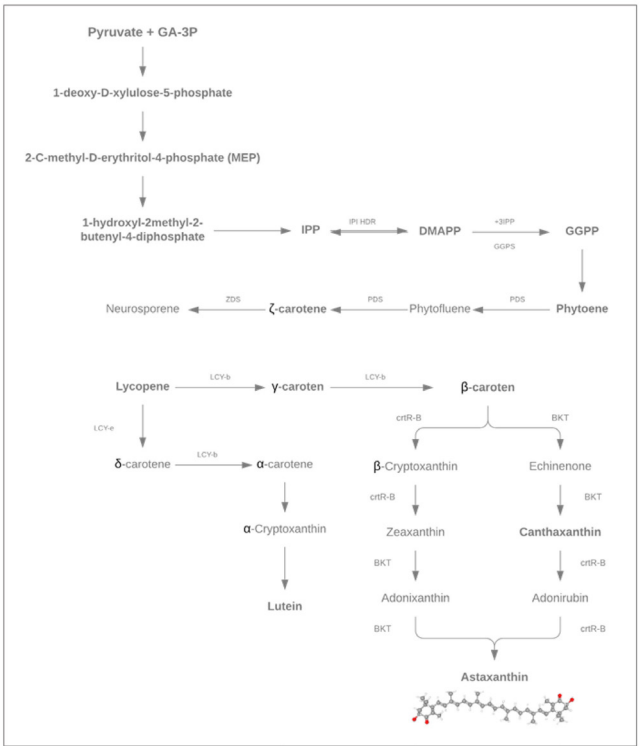

Figure 3: Biosynthesis of astaxanthin in *H. pluvialis*[27]

The formation of lycopene takes place through four desaturation steps catalyzed by two enzymes, namely the enzyme phytoene desaturase (PDS), which is encoded by the pds gene and z-carotene desaturase (ZDS), which is encoded by the zds gene.[51,52] The desaturation reaction will increase the number of conjugated double bonds in the carbon chain to form chromophore groups in carotenoids, change the colorless molecule of phytoene to the lycopene, and produce a red color.[50]

Lycopene undergoes cyclization catalyzed by the enzyme lycopene cyclase (LCY-e and LCY b), which is encoded by the lcy gene. Cyclization of carotenoid biosynthesis in most organisms produces α-carotene (a precursor to lutein) and β-carotene (a precursor to carotenoids including astaxanthin). The last two oxygenation processes are catalyzed by the β -carotene ketolase (BKT) enzyme encoded by the bkt gene, and the β-carotene hydroxylase (CrtR-b or BKH) enzymes encoded by the bkh or crtR-b genes are the final stages of astaxanthin synthesis.[53–55]

c. Pharmacological Activity of Astaxanthin

Astaxanthin as a nutraceutical has a variety of pharmacological activities, including those in Table 3.

Table 3: Pharmacological Activity of Astaxanthin

Activity	Description	Results	Reference
Antioxidant	Astaxanthin is a carotenoid that exhibits significant antioxidant activity. The purpose of this study was to determine the level of oxidative stress caused in human cells by a Fatty Acids mixture and the potential protective effect of Astaxanthin.	Astaxanthin can protect human lymphocytes from oxidative stress caused by a fatty acid mixture, most likely by blenching/quenching free radical production.	56
Anticancer	AGS, KATO-III, MKN-45, and SNU-1 human gastric cancer cell lines were treated with different doses of astaxanthin. Immunoblotting, cell viability testing, and cell cycle analysis were all conducted.	*In vitro* studies on KATO-III and SNU-1, gastric cancer cells showed inhibition of cancer cell proliferation	29
Antidiabetic	The effect of astaxanthin on insulin-stimulated glucose transporter 4 (GLUT4) translocation, glucose uptake, and insulin signaling was investigated utilizing a plasma membrane lawn test, 2-deoxyglucose uptake, and Western blot analysis in cultured rat L6 muscle cells.	*In vitro* studies on L6 muscle cells demonstrated an increase in glucose uptake by increasing the translocation of glucose transporter 4 (GLUT4).	57

Antiaging	The study enrolled 31 volunteers over the age of 40 (17 males and 14 females). RSSC samples were taken from the surface of the facial skin at the study's inception (day 0) and conclusion (day 29). Additionally, blood samples were obtained on days 0, 15, and 29 to determine systemic oxidative stress by detecting plasma malondialdehyde (MDA) levels.	The results support the theory by indicating that sustained astaxanthin ingestion has a high antioxidant impact, resulting in face skin regeneration that is notably noticeable in obese participants.	58
Immunomodulator	After 48 hours, culture supernatants were taken to determine cytokine production in cultured lymphocytes. The concentrations of IL-2 and INF-γ in the supernatants were determined using mouse IFN-γ and IL-2 assays.	*In vivo* studies in mice show an increase in INF-γ, IL-2	59
Antihypertensive	The effects of dietary astaxanthin (ASX-O) on oxidative parameters in spontaneously hypertensive rats (SHR) were studied by measuring the levels of nitric oxide (NO) end products nitrite/nitrate (NO_2 /NO_3) and lipid peroxidation in ASX-O-treated SHR.	*In vivo* studies in rats with hypertension showed a decrease in blood pressure	60
Hepatoprotective (Liver Fibrosis)	CCL_4 was used to induce liver fibrosis in a mouse model (intraperitoneal injection three times a week for eight weeks), and astaxanthin was given every day in three doses (20, 40, and 80 mg/kg).	*In vivo* studies in mice have shown a decrease in ALT and AST, thereby reducing lesions in liver fibrosis	61

Description: **ROS**: Reactive Oxygen Species; **KATO-III**: Human gastric carcinoma cell line; **SNU-1**: Human gastric carcinoma cell line; **GLUT4**: Glucose transporter type 4; **IRS-1**: Insulin receptor substrate-1; **INF-γ**: Interferon -γ; **IL-2:** Interleukin-2; **ALT**: alanine aminotransferase; **AST**: aspartate aminotransferase.

Astaxanthin Production Method

a. Culture Method

Culture System

In general, microalgae culture systems are divided into three: photoautotrophic, heterotrophic, and mixotrophic systems. *H. pluvialis* allows cultivation using all three methods, either with open or closed systems.[27] In the phototropic system, microalgae are very dependent on light as an energy source and CO_2 as a carbon source, both light from lamps or the sun. In heterotrophic systems, microalgae growth requires organic carbon as an energy source. Commonly used organic carbon substrate sources include glucose, acetate, and glycerol.[62] In this condition, the microalgae cell density achieved was higher than the phototropic condition, so that the cost required for harvesting was lower. The mixotrophic system is a combination of phototropic and heterotropic methods. The microalgae that grow in this system can assimilate sunlight and organic carbon as energy sources simultaneously or alternately.

Culture systems, especially those that require light (photoautotrophic), are divided into 2: closed and open culture systems. Advantages and disadvantages of culture with closed and open systems can be seen in Table 4.

Table 4: Advantages and Disadvantages of Open and Closed Culture Systems

Culture System	Advantages	Disadvantages
Outdoor Pool	• Lower costs • Easy to clean • Easy to maintain • More effective for large-scale production	• Low biomass productivity • Requires large flat land • Easily contaminated • Difficult to control culture conditions • The deeper the pool, the lower the sunlight intensity
Tubular Photobioreactor	• Has a large lighting surface area • Have good biomass productivity • Easy to control culture conditions • Minimize contamination	• Requires a large area • There is a change in pH, dissolved oxygen and CO_2 along the pipe.

Table 4: *(Continued)*

Culture System	Advantages	Disadvantages
Flat Plat Photobioreactor	• High biomass productivity • Easy to sterilize • Has a large lighting surface area • Suitable for outdoor culture	• Very difficult to do in large sizes • Very difficult to regulate the temperature
Column Photobioreactor	• High mass transfer • Low energy consumption • Easy to sterilize	• Small lighting area • Relatively more expensive

Cultural Conditions

The success of microalgae culture is strongly influenced by several important factors, including nutritional and environmental factors. *H. pluvialis* culture conditions can be seen in Table 5.

Table 5: Condition of Culture of *H. pluvialis*

Parameter	Description	Reference
Water type	Sterile mineral water	63
Media	Rudic's Medium	64,65
	BG-11	66
	OHM	67
	Bold Basal Medium	10
pH	7 – 7.85	68
	6 – 9	69
Temperature	25 - 28° C (temperature for microalgae growth phase)	69
Light intensity	40-50 µmol $m^{-2}S^{-1}$	15
	3000 lux	69
Photoperiod (light:dark)	16 hours: 8 hours 12 hours: 12 hours	66

Stress Induction

H. pluvialis can accumulate astaxanthin under stress. The accumulation of astaxanthin is a response of microalgae to protect themselves from oxidative stress conditions.[70] Several studies of stress induction, either physically or chemically, are listed in Table 6.

Table 6: Types of Stress Induction

Types of Stress Induction	Description	Results	Reference
Salinity stress (addition of NaCl)	Variation of concentration of NaCl added: • Control (-) without NaCl and Sodium Acetate • Sodium acetate 2.2 mM • NaCl 0.25% + Sodium Acetate 2.2 mM • NaCl 0.5% + Sodium Acetate 2.2 mM • 1% NaCl + Sodium Acetate 2.2 mM	The highest astaxanthin accumulation was found in the addition of 0.25% and 0.5% NaCl, an increase of 2.5 to 4 times compared to the control.	68
light stress	Variation of light intensity exposed: • 445 µmol photon m^{-2} s^{-1} • 546 µmol photon m^{-2} s^{-1}	The highest astaxanthin accumulation was found in the light exposure treatment with an intensity of 546 µmol photon m^{-2} s^{-1}.	64
Nutritional restriction (nitrogen deficiency)	At eight days of culture, microalgae were transferred to new media with the addition of different $NaNO_3$: • Without $NaNO_3$ • 75 mg/L • 150 mg/L • 225 mg/L • 300 mg/L • 375 mg/L	The highest astaxanthin accumulation was found in the treatment with the addition of $NaNO_3$ 75 mg/L and without the addition of $NaNO_3$.	69
Butylated Hydroxyanisole	Variation of added BHA concentration: • 0 mg/L • 2 mg/L • 4 mg/L • 8 mg/L	The highest astaxanthin accumulation was found in the treatment with the addition of BHA 2 mg/L, reaching 29.03 mg/g (% dry weight); this value was 2.03 times higher than the control.	71
Butylated Hydroxytoluene	Variation of added BHT concentration: • 0 mg/L • 1 mg/L • 2 mg/L • 3 mg/L	The highest astaxanthin accumulation was found in the treatment with the addition of 2 mg/L BHT.	72

Melatonin	Variation of added melatonin concentration: • 0 µM • 5 µM • 10 µM • 15 µM	The highest astaxanthin accumulation was 16 found in the treatment with the addition of 10 µM melatonin. The full astaxanthin content achieved was 31.32 mg/g.
Fe^{2+}	Variations in the concentration of Fe_2SO_4 added: • 0 µM • 90 µM • 180 µM • 270 µM • 360 µM • 450 µM	The highest astaxanthin accumulation was 65 found in the treatment with the addition of 450 µM Fe_2SO_4.

Harvesting

An efficient harvesting technique is an important step that must be done to get a high concentration of harvested biomass. Several harvesting methods commonly used for *H. pluvialis* are flotation and centrifugation methods.[27,73]

- **Centrifugation Method**

Centrifugation is a method of harvesting microalgae based on the application of rotary power to precipitate microalgae cells so that they are separated from the liquid growth medium. The separation was supported by the difference in density between the microalgae cells and the liquid medium in which the cells grew. The centrifugation method can produce microalgae in a paste with a solid content of up to 15%. Several studies also show that the faster the centrifugation cycle, the microalgae biomass obtained can reach up to 95%.

- **Centrifugation Method**

This method is a separation process based on gravity where the microalgae cells attach to air or gas bubbles so that the cells float on the surface. Under these conditions, microalgae cells can be harvested easily. In certain types of microalgae, cells can flow naturally if the lipid content in the cells increases. In the flotation method, the need for operational costs will be even greater if it involves flocculants.

Extraction

Extraction methods commonly used include maceration and percolation.[74] Astaxanthin is a lipophilic compound and is soluble in organic solvents and oils. Organic solvents such as acetone, DMSO, methanol, n-hexane, and vegetable oils such as olive oil, soybean oil, and corn oil have been used for astaxanthin extraction.[35,74]

b. Genetic Engineering

The development of biotechnology today also supports the use of microalgae as a producer of bioactive compounds. Most of the bioactive compounds produced by microalgae are secondary metabolites, which have low cellular production. So that the mass production of bioactive compounds from microalgae culture (without modification and engineering) is still not efficient; on the other hand, the synthesis of bioactive compounds with chemicals, especially astaxanthin compounds, will produce products that are stereochemically different from the natural products so that they are not allowed to be consumed by humans.[5] However, with the advancement of biotechnology, the "factory" of microalgae biomass can be made more optimal. The use of science and methods of mutagenesis and genetic engineering is a solution that must continue to be developed. Several studies on the production of carotenoid compounds such as astaxanthin by genetic engineering are listed in table 7.

Table 7: Production of Astaxanthin by Genetic Engineering

Host	Clone [21] Description	Result
Saccharomyces cerevisiae	• Inserting gene constructs encoding carotene biosynthesis in the host. • The carotene biosynthetic genes crtE (GGP synthase), crtYB (encodes phytoene synthase and lycopene synthase), crtI (phytoene desaturase) were amplified from *Xanthophyllomyces dendrorhous* cDNA. • The gene is inserted into the pMRI plasmids pMRI-21, pMRI-31 and pMRI-32. • Plasmids were transformed into S. cerevisiae using 1.5 kV electroporation. • Selection of recombinant colonies using GAL10-GAL1 bidirectional promoters. • Recombinant colonies were cultured in Erlenmeyer glass with YPD liquid medium, incubated at 30°C.	Obtained as much as 11 mg/g total carotenoids (72.57mg/L) and 7.41mg/g β-carotene.

Table 7: ***(Continued)***

Clone [21]		
Host	**Description**	**Result**
Combination of Chemical Mutations and Engineering of Biosynthetic Pathways[75-77]		
Microorganisms	Description	Result
Xanthophyllomyces dendrorhous	• The gene construct encoding carotene (astaxanthin) biosynthesis is inserted in *X. Dendrorhous.* • The gene encoding carotene and astaxanthin biosynthesis, crtYB, was inserted into the pPR13F plasmid and inserted into the pPR2TN2BPAT modified plasmid containing the asy gene. • The mutagen was nitrosoguanidine (200 g/ml), exposed to *X. dendrorhous* cells for 30 minutes. • Large, red colonies are removed. The AXG-13 mutant colonies were selected after repeated transfer on plates containing 200 g/ml pyrne dedocanoic acid, and the AXJ-20 mutant was chosen with 200 g/ml triazine.	The AXJ-20/crtYB mutant produced astaxanthin up to 3.9 mg/g and the AXJ-20/crtYB transformant up to 9.7 mg/g, while the wild type was only 438 g/g dry weight.

CONCLUSION

This study provides valuable pieces of information on astaxanthin, particularly regarding its pharmacology activities, biosynthesis pathway, various methods of its production in microalgae, harvesting, and extracting techniques, that will add insight to uncover the critical area of astaxanthin from microalgae.

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CONFLICT OF INTEREST

None declared.

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AUTHOR'S CONTRIBUTIONS

Soni Muhsinin (SM) is responsible for the conception of the study. SM and Widhya Aligita (WA) collected the articles, drafted, and wrote the manuscript. Tina Rostinawati (TR) and Jutti Levita (JL) supervised, reviewed, and finalized the manuscript. All authors have read and approved the final manuscript to be published.

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