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Chapter

Organic Culture Media for Sustainable Carotenoid Production from Microalgae

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Abstract

Antioxidants, particularly those carotenoid produced in microalgae, can be increased by induced stress, including light, nutrients, and salinity. Nutrient stress can be achieved by using imbalanced nutrients to boost antioxidant production without compromising the growth, that is, biomass. Culture media is an important factor in microalgae production because it is affecting growth and biomass production, as well as the biochemical content of microalgae. Synthetic or conventional culture media is considerably expensive for mass culture and the supply is limited, especially for developing countries. Therefore, there is a need for cheap and easily available culture media. This chapter discusses the use of organic media to culture several species of microalgae and cyanobacteria (*Arthrospira platensis*) for antioxidant production, particularly of those total carotenoids and beta carotene. The antioxidant content data mainly comes from our research with several organic culture media, such as fermented water hyacinth biomass, soybean processing waste, and Acadian Marine Plant Extract Powder (AMPEP). Carotenoid can be used for pharmaceuticals, including for anticancer, anti-inflammatory, and anti-obesity. Also, they can be used in aquaculture to increase cultured animals' health and immunity. Using organic media that may also serve as waste stream microalgae, which is aiding in a sustainable microalgae culture. Additional data presented in this chapter come from literature reviews of similar research topics.

Keywords: carotenoid, microalgae, organic, culture media

1. Introduction

Microalgae are microscopic organisms that are known to have very efficient photosynthesis capabilities. These organisms in nature are generally phytoplankton that acts as constituents of secondary metabolites in the form of natural pigments [1]. These natural pigments play an important role in microalgae photosynthesis and growth both for light harvesting and cell protection from stress, thus as an

antioxidant. Antioxidant compounds contained in microalgae can be pigments, such as chlorophyll, phycobilin protein, and carotenoids [2]. Microalgae produce different types of carotenoids, more than 40 carotene and xanthophylls have been isolated and characterized. Carotenoid compounds are natural pigments found in bacteria, algae, fungi, and plants but are not produced by animals.

Carotenoids are formed from eight isoprene molecules, so that they have 40 carbon atoms. In general, carotenoids are grouped into carotene (pure hydrocarbon carotenoids, having no oxygen atoms) and xanthophyll (oxygen atom-carrying carotenoids) [3]. Carotenoids have several types, including α-carotene, β-carotene, astaxanthin, lycopene, lutein, zeaxanthin, β-cryptoxanthin, and fucoxanthin [4–6]. Carotenoids also have perishable or degradable properties caused by light, heat, and oxygen, and prolonged exposure to those factors decreases the content of carotenoids in the biomaterial [7]. Carotenoids are organic pigments found in chloroplasts and chromoplasts of plants and other groups of organisms. Carotenoid compounds provide several health functions for the body, especially as antioxidants, that may protect the body from free radicals. Because of these functions, carotenoids are also applied to nutraceutical products [8]. These pigments are found in almost all classes of microalgae and can be used as pharmaceutical or health products because they can reduce the risk of developing cancer, vitamin A precursor for good vision and eye health, a strong immune system, and the health of the skin and mucus membranes. In the food industry, β-carotene is used as a pigment in food, in the pharmaceutical industry, β-carotene acts as a tablet coloring agent, and in the cosmetic industry, it is used as a bioactive ingredient in creams, which protects the skin from exposure to UV radiation [2–4].

Microalgae can be propagated by culture or cultivation in controlled closed photobioreactors or open ponds and raceways. Algae culture activities are one of the efforts to develop and meet the needs of carotenoid-producing microalgae. Microalgae in the process of their growth require macroelements of N and P and various other microelements to increase the growth rate and produce maximal nutrient content. The complete nutrient composition and proper concentration of nutrients determine biomass production and nutritional content of microalgae [9, 10]. Synthetic culture media commonly used for microalgae culture include Walne, Bold Basalt Medium (BBM), Conway, and F/2 media [9]. Meanwhile, organic media that have been used as microalgae culture media include seaweed waste [11], fermented water hyacinth [12], chicken manure [13], and brown seaweed extract [14]. Conventional culture media tend to be expensive and limited in availability for mass culture of microalgae, especially in developing countries. Using organic media is considered more sustainable and cheaper, particularly for the mass culture of microalgae. Also, culturing microalgae in organic media can be used as a bioremediation strategy and waste stream microalgae. Therefore, alternative culture media continues to be researched and developed. More importantly, organic media is considered as nutrient imbalances media, particularly of those N and P, and therefore may induce nutrient stress in microalgae that may lead to high production of carotenoid. This chapter discussed the use of several organic culture media, such as Acadian Marine Plant Extract Powder (AMPEP), fermented water hyacinth biomass, and soybean processing waste from tempeh production. These media were used to culture several species of microalgae in our lab, such as *Chlorella vulgaris, Dunaliella salina, Nannochloropsis* sp., *Tetraselmis* sp., and *Arthrospira (Spirulina) platensis,* that has been experimented in our lab for carotenoids, including β-carotene production.

2. Carotenoid biosynthesis in microalgae

Microalgae produce a variety of beneficial compounds, such as anticancer, anti-inflammatory compounds, antioxidants, vitamins, minerals, omega-3 fatty acids, and pigments [15]. One of the antioxidant compounds in microalgae is carotenoids, including β-carotene. Carotenoids exhibit biological activity as antioxidants, influencing cell growth regulation and modulating gene expression and immune responses. Carotenoids are natural pigments found in plant chloroplasts together with chlorophyll. Carotenoids act as additional pigments that help chlorophyll in absorbing light energy. The formation of carotenoids in microalgae increases in physiological conditions that are not balanced in cells caused by various environmental pressures, including nutrient content in nonoptimal media. This response is modulated by the phytoene synthase (PSY), an enzyme responsible for carotenoid biosynthesis in the photosynthetic organism. It is suggested that different PSY genes family is responsible for microalgae development and cell defense under environmental stress [3, 16]. Also, the composition and combination of nutrient content in the medium (C:N:P ratio) can affect the content of carotenoids in microalgae [17].

Carotenoids are synthesized in plastids through phytoene to lycopene synthesis and resulting in α- and β-carotene. The most common carotenoid used in several industries is β-carotene, which is a yellow, orange, or red organic pigment that occurs naturally in photosynthetic plants. β-Carotene can be fat-soluble, insoluble in water, and easily damaged by oxidizing at high temperatures. β-Carotene can be useful as a natural food coloring, antioxidant, and pro-vitamin A source for humans and can be beneficial in treating and preventing tumors and cancer [2–4]. β-Carotene can be commercially synthesized from natural source extraction. β-Carotene was found to accumulate in oil globules in thylakoids present in chloroplasts and consisted of two isomers, all-trans, and 9-cis β-carotene [18] (**Figure 1**).

β-Carotene biosynthesis begins with head-tail condensation on two molecules of C20 geranylgeranyl pyrophosphate (GGPP), resulting in C40 phytoene catalyzed by phytoene synthase (PSY). Phytoene is further modified gradually into β-carotene, neurosporent, lycopene by phytoene desaturase (PDS), β-carotene desaturase (ZDS), and carotenoid isomerase (CRTISO). There is an increase in the number of conjugated double bonds at each stage. The terminal structure of isoprene on lycopene molecules is further cyclized by lycopene β-cyclase (LCYB) and forms β-carotene (**Figure 2**). GGPP, PDS, ZDS, CRTISO, and LCYB coding genes are found in all terrestrial plants and algae. The path of carotenoid biosynthesis to the β-carotene stage has been conserved in these organisms [4, 19, 20]. Many other important carotenoid types are β-carotene derivatives, such as astaxanthin, zeaxanthin, and dinoxanthin (**Figure 2**).

Almost all types of carotenoids (**Figure 3**) are found in microalgae, but distribution is varied among classes and species. Carotenoids in algae contain allene

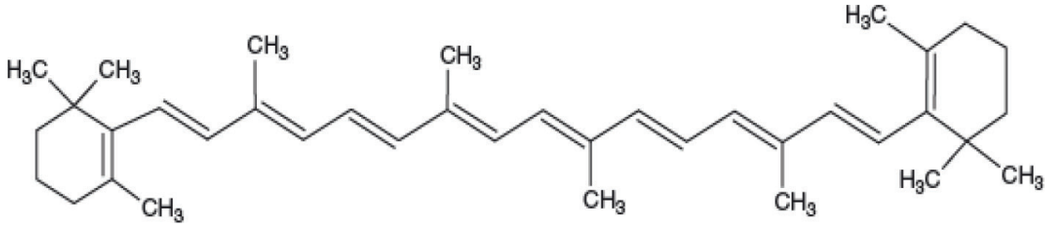

Figure 1.
Chemical structure of β-carotene [19].

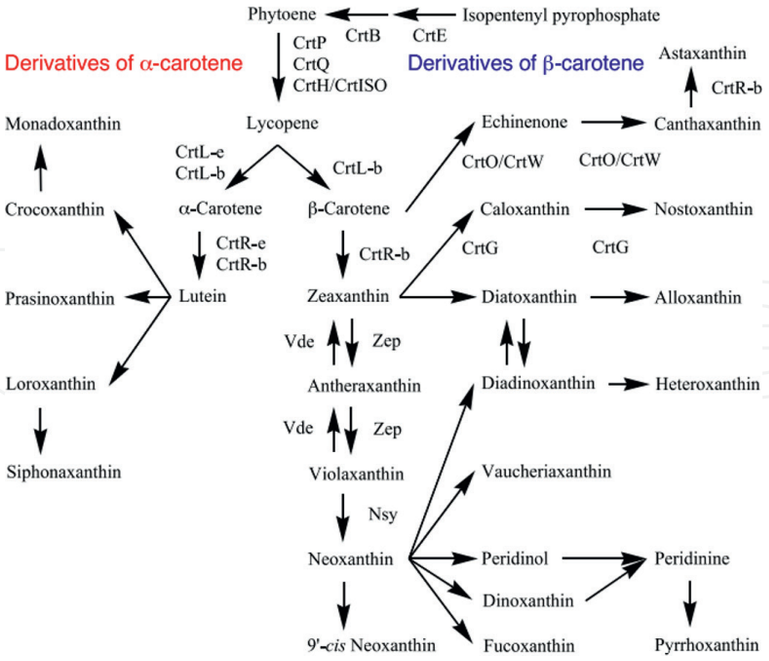

Figure 2.
Carotenoid biosynthesis in microalgae [4].

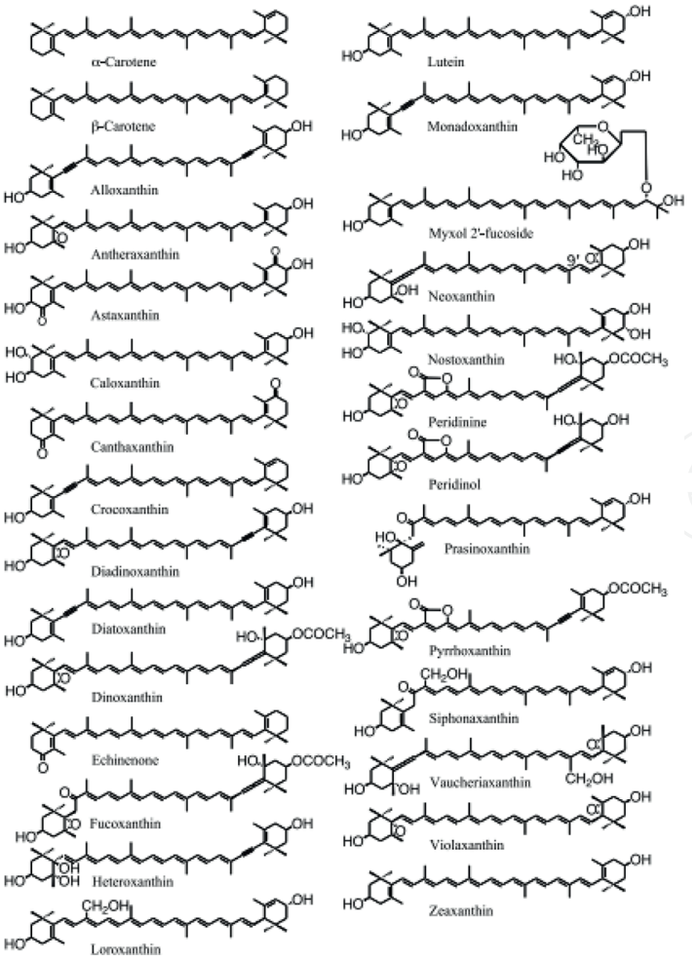

Figure 3.
Carotenoid structure found in microalgae [4].

(C═C═C) and acetylene (C≡C) bounds. Allenic carotenoids found in algae include fucoxanthin in brown algae and diatoms, 19′-acyloxyfucoxanthin in Haptophyta and Dinophyta, peridinin only in dinoflagellates, and 9′-cis neoxanthin in green algae. Whereas acetylenic carotenoids, such as alloxanthin, crocoxanthin, and monadoxanthin, are found in Cryptophyta, and diadinoxanthin and diatoxanthin in Heterokontophyta, Haptophyta, Dinophyta, and Euglenophyta. Acetylated carotenoids (-O-CO-CH3), such as fucoxanthin, peridinin, and dinoxanthin, are also mainly found in algae, such as Heterokontophyta, Haptophyta, and Dinophyta. Many cyanobacteria contain β-carotene, zeaxanthin, echinenone, and myxol pentosides (myxoxanthophyll), while some species lack part of these and some contain additional carotenoids, such as nostoxanthin, canthaxanthin, and oscillol dipentoside [4]. Several microalgae species are known to produce abundant carotenoid, and therefore commercially cultured include *Haematococcus pluvialis*, *Dunaliella salina*, *Chlorella vulgaris, Nannochloropsis* sp., and *Arthrospira (Spirulina)* sp.

3. Effect of organic culture media on carotenoid production in microalgae

The growth and carotenoid production in microalgae are affected by culture media through the availability of nutrients. The nutrient ratio of C/N/P is an important regulator in carotenoid production. Different concentrations of N and P are found in organic media as shown in our studies. This concentration was higher compared to Walne, a commercially available culture media commonly used for culturing microalgae and cyanobacteria (**Table 1**).

Optimal utilization of nutrients produces maximum growth indicated by high cell number and biomass one culture cycle, thus may affect carotenoid content. Media composition plays a role in nutrient utilization and pigment production of microalgae [25], including carotenoids [26]. A growth medium depleted in phosphorus content has a positive effect on the synthesis of β-carotene. The P element has a role in the process of energy metabolism, but the response to P stress in culture media is different for each microalgae species. Depleted P content in the microalgae growth medium of *Tetraselmis marina* increases its carotenoid content [27]. Conversely, the β-carotene content in *Oocystis* sp. can be improved by giving excess P nutrients. The highest β-carotene content in the microalgae *Oocystis* sp. was detected in induction treatment with a fivefold addition of KH_2PO_4 [28].

Some organic media that have been successfully used as microalgae culture media for carotenoid production are green bean sprouts extract for growth and carotenoid content of *D. salina* [29], lamtoro leaf extract medium for growth and contains carotenoids *Dunaliella* sp. [30] and fermented water hyacinth for the growth and carotenoids of *C. vulgaris*. We found that organic media from fermented hyacinths with a concentration of 0.1% was able to produce maximum *C. vulgaris* growth on the sixth day with a culture volume of 150 mL with a density value of 66.7×10^4 sel mL^{-1} with the highest carotenoid content of *C. vulgaris* obtained at a 1% organic media concentration of 0.545 μg mL^{-1} [31].

Other organic culture media that was experimented in our lab is brown seaweed extract or commercially sold as AMPEP. This is derived from extracts of brown algae (*Ascophyllum nodosum*), which have been used to increase the productivity of agricultural crops and have the potential to be used as a microalgae culture medium for the production of carotenoids. The experiment of microalgae grown for 7 days without the addition of *A. nodosum* extract was able to increase the cell density of *C. vulgaris*

Media	N (mg L^{-1})	P (mg L^{-1})	Reference
1	*2*	*3*	*4*
AMPEP	1.040	0.930	Our lab
Walne	0.00001	0.0002	Our lab
F/2	12.353	1.125	[21]
Fermented water hyacinth	0.67	190,143	[22]
Liquid hotel waste	13.35	0.43	[23]
Soybean processing waste (tofu)	8.74	1.06	[24]
Soybean processing waste (tempeh)	1.95	1.07	Our lab

Table 1.
N and P concentration in organic and synthetic culture media.

and *Scenedesmus* sp., whereas the addition of *A. nodosum* extract at concentrations of 3 and 4% inhibited the growth and antioxidant activity of *C. vulgaris* and *Scenedesmus sp*., although it was able to improve protein synthesis. Conversely, the addition of *A. nodosum* extract at low concentrations (1 and 2%) was able to increase the growth and antioxidant activity of *C. vulgaris* and *Scenedesmus* sp. [14]. Therefore, low doses of *A. nodosum* extract can be applied for the acceleration of microalgae cultivation and the production of antioxidants, particularly of those carotenoids. The use of AMPEP in low concentrations will be very profitable in terms of the cost and productivity of microalgae cultures for carotenoid production.

Our studies with 10 ppm AMPEP concentration for culturing *D. salina,* resulting in high biomass and *β-carotene* production at 418.1 × 10^4 cells mL^{-1} and 0.3545 μg mL^{-1}, respectively. Similar trends were found in our experiment when *Spirulina* sp. was cultured in the same AMPEP concentration, although the growth and carotenoid content were lower compared to *D. salina*. The growth and carotenoid content of *Arthrospira* (*Spirulina*) was also the highest in 0.1% of tempeh processing waste and 25% of moringa leaf extract [32]. Conversely, the lowest growth of *C. vulgaris* was found at 10 ppm AMPEP culture media but with the highest carotenoid content at 0.267 μg mL^{-1}. Our study indicated that different species responded differently in terms of growth and carotenoid content when using the same concentration of AMPEP (**Tables 2** and **3**). It seems that cyanobacteria *Arthrospira* sp. adapted well in different organic culture media except for AMPEP with good growth and considerably high carotenoid content (**Table 4**).

No	Culture media	S	L	T	D (10^4)	C	References
1.	Green bean sprout extract	30–35	16.2	20–23	477	0.97	[29]
2.	Lamtoro leaf extract	30	20.25	26	470	1.07	[30]
3.	Moringa leaf extract	30	27	25	1073	1.39	[32]
4.	AMPEP	30	16.2	29–30	418	0.36	Our Lab
5.	*Walne*	30	16.2	29–30	347	0.17	Our Lab

Notes: S = salinity (ppt), L = light intensity (μmoles m^{-2} s^{-1}), T = temperature (°C), D = cells density (cells mL^{-1}), and C = β-carotene (carotenoid) = μg mL^{-1}.

Table 2.
β-Carotene and carotenoid content of D. salina cultured in various organic culture media.

No.	Media	Culture condition					Carotenoid (μg mL^{-1})	Reference
		V	P	S	pH	I		
1.	AMPEP	1.000	12:12	32	8	19	0.27	Our lab
2.	Walne	18.000	12:12	30	8	1.49	0.24	[33]
3.	F/2	150	12:12	30	7	10	0.52	
4.	Fermented water hyacinth	149.85	12:12	30	7	10	0.54	Our Lab
5.	BG-11	18.000	12:12	30	8	1.49	0.33	[33]
6.	Beneck	18.000	12:12	30	8	1.49	0.31	[33]

Notes: V = culture volume (mL), P = photoperiod (dark: light), S = salinity (psu), and I = light intensity (μmol photon $m^{-1} s^{-2}$).

Table 3.
Carotenoid content of C. vulgaris cultured in various organic media.

No.	Culture media	P	V	B	L	S	Sh	C	References
1.	*Walne*	12:12	2	Glass beaker	40.48	30	20–30	0.00183	Hanani et al., [34]
2.	Hearing waste	12:12	1	Glass beaker	25.64	30	25–29	0.5459	Pramusinta et al. [26]
3.	*Zarrouk*	12:12	0.4	Erlenmeyer	53.97	15	29	5.346	Fakhri et al. [35]
4.	*Walne*	12:12	0.2	Plastic bottle	13.49	29–30	28–30	0.98	
5.	Tempeh processing waste	12:12	0.2	Plastic bottle	13.49	29–30	28–30	0.80–1.70	
6	AMPEP	12:12	0.3	Plastic bottle	13.49	29–30	28–30	0.0691	
7	*Walne*	12:12	0.3	Plastic bottle	13.49	29–30	28–30	0, 1354	

Note: *P = photoperiod (jam), V = culture volume (L), B = bioreactor, I = intensitas cahaya (μmol photon $s^{-1} m^2$), S = salinitas (psu/ppt), Sh = Suhu (°C), C = karotenoid (μg mL^{-1}).*

Table 4.
Carotenoid content of A. platensis *cultured in various media.*

The content of carotenoids in microalgae is highly dependent on the species cultured and the media used. In addition, cell density, culture volume, bioreactor, light intensity, salinity, and temperature also affect the carotenoid content (**Tables 3** and **4**). The difference in carotenoid content from each study is thought to be due to differences in nutrient content in each medium used as well as supporting factors that affect such as light, salinity, pH, and temperature. All such factors must be in optimum conditions for maximum microalgae cell growth and carotenoid content.

In our lab, the standard culture condition for carotenoid production in microalgae is 28–30°C, salinity of 29–30 psu, pH 7–8, and light intensity of 16.2 μmoles $m^{-2} s^{-1}$ [31]. Besides organic culture media, these conditions have been proven to induce stress during microalgae culture, thus the carotenoid content in cultured species.

4. Conclusion

Organic culture media, such as fermented water hyacinth, tempeh processing waste, and AMPEP, can be used to culture several microalgae species, including D. salina and C. vulgaris, and cyanobacteria *A. platensis*. However, AMPEP is not ideal for culture *A. platensis* for carotenoid production and may need some adjustment to its N and P content.

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