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Economically Potential Pigments from Marine Blue-Green Algae for the Application in Food and Health

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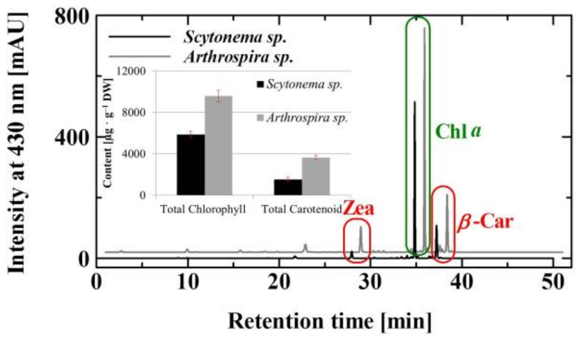
Abstract

Current efforts to explore the potency of marine resources have been made to promote economic development in Indonesia, especially in the industrial sector. Marine cyanobacteria such as *Arthospira* sp. and *Scytonema* sp. are two potential candidates of the blue-green algae that can be used in the application for food and health industries. This article focused on the identification of the composition of the dominant chlorophylls and carotenoids. The results showed the presence of zeaxanthin, chlorophyll *a*, and *β*-carotene. In addition, the economic potency of those dominant pigments will be discussed in this article.



Short Description

Arthospira sp. and *Scytonema* sp., marine cyanobacteria, contained zeaxanthin (Zea), chlorophyll *a* (Chl *a*), and *β*-carotene (*β*-Car) as the dominant pigments analyzed by HPLC. Total chlorophyll and total carotenoid of these species were in the range of 5.9 to 9.6 mg · g^{-1} DW and 1.4 to 3.7 mg · g^{-1} DW, respectively. Therefore, both cyanobacteria are the potential source of Zea, Chl *a*, and *β*-Car which have the economic potency.

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Keywords: *Arthospira* sp., blue-green algae, chlorophyll and carotenoid, economic potency of pigment, *Scytonema* sp.

INTRODUCTION

Geographically, Indonesia is being considered as the largest maritime countries in the world. Most of the islands in Indonesia are surrounded by ocean. Currently, the marine potential is used as an effort to increase Indonesia's economy. One of the marines potential that can be developed in the areas of food, health, and renewable energy is microalgae. Microalgae are photosynthetic microorganisms, which are capable to produce biomass. Researches on microalgae as a source of functional food, pharmaceutical compound, nutraceutical agent, and alternative bioenergy have been carried out [1]. Blue green algae is one of the microalgae which have high yield of the blue pigment-protein complex called phycocyanin. It is a water-soluble and adheres to the membrane. In addition to macronutrient and micronutrient components, it contains photosynthetic pigments that can be developed in the areas of food, health, and renewable energy [2-5]. There are three major classes of photosynthetic pigments, chlorophylls, carotenoids, and phycobilins which has been known to be non toxic and non carcinogenic [1]. This article focuses on photosynthetic pigments that exist within the membrane such as chlorophylls and carotenoids.

Chlorophyll is a green colour pigment which is classified into two main types, chlorophyll *a* (Chl *a*) and chlorophyll *b*

(Chl *b*). The difference between each type depends on ring substituents. Chl *a* is mostly found in blue-green algae and plays a role as natural food coloring and antioxidant [6]. Yellow-orange colour pigments called carotenoids are divided into two main classes, xanthophylls and carotenes. Zeaxanthin (Zea) is a predominant xanthophyll that has ability to maintain eye health, e.g. cataracts and age-related macular degeneration (AMD) [7]. Meanwhile, β-carotene (β-Car), one of carotenes, is a precursor of vitamin A and provides antioxidant activity which relates to eye health [8]. This article will give a short report on the composition of dominant pigments that occurs in blue green algae genus *Arthrospira* sp. and *Scytonema* sp. Both species has been abundantly cultivated in Indonesia. This article also discussed the economic perspective of microalgae in the field of food and health.

EXPERIMENTAL

General

The cells of *Arthrospira* sp. and *Syctonema* sp. were used as it was received in the form of pellets from Research Centre for Biotechnology, Indonesia Institute of Sciences, Cibinong Bogor, West Java, Indonesia.

Pigment extraction

Sample (0.1 g wet weight) was added by trace portion of sodium L-ascorbate and calcium carbonate to minimize oxidation and reduce acidification. Following this, the addition of 0.75 mL solvent mixture of acetone: methanol (7 : 3, v/v) was applied to extract pigments of *Arthrospira* sp. and *Scytonema* sp. Homogenisation on a Vortex for 3 min and centrifugation at 10 000 rpm for a minute was repeated six times until the residue became colorless. The extractions and measurements were carried out under dimmed lights at room temperature and under the stream of ultra-high purity (99 %) nitrogen gas (PT. Samator, Surabaya, Indonesia).

Concentration of total chlorophylls and carotenoids

The extracted pigments were diluted in a diethyl ether prior to determine the total chlorophylls and carotenoids using UV-1700 spectrophotometer (Shimadzu, Japan). The calculation of pigments concentration was on the basis of Lichtenthailler's method [9].

HPLC analysis for identification and quantification of pigment separation

Chromatographic analysis was carried out with HPLC (LC) 20AD equipped by photodiodearray detector, SPD–M20A and column oven CTO–20A (Shimadzu, Kyoto, Japan). The analytical column was a Symmetry C8 column (3.5 µm, 4.6 i.d. × 150 mm). Prior to injection, sample pigment was filtrated through a membrane filter (0.2 µm, nylon, Whatman, Maidstone, UK). The injection volume was 20 µL. HPLC analysis was performed using Zapata's method with a mobile phase consisting of eluent A is a mixture of methanol: acetonitrile: aqueous pyridine solution 0.25 M (50 : 25 : 25, v/v/v) and eluent B is a mixture of methanol: acetonitrile: acetone (20 : 60 : 20, v/v/v) [10]. The flow rate was set at 1 mL·min^{-1} at 30 °C and the pigments were detected at 430 nm.

Data analysis

The absorption spectra and chromatograms were recorded by LC solution version 1.24 SP1 (Shimadzu) and processed using Plotx32 version 1.35 (created by Akifumi Ikehata, NFRI, Tsukuba, Japan). Both numeric and graphic data represent an average from triplicate analyses with SE. Prior to identification the recorded spectra were compared to those in the literature.

RESULT AND DISCUSSION

Pigment composition of *Arthrospira* sp. and *Scytonema* sp. can be detected through analysis using HPLC. Figure 1 shows that the dominant pigments of the two microalgae are similar. These pigments were Zea (peak 1), Chl *a* (peak 2), and β-Car (peak 3). However, the peak intensity of the pigments between the two microalgae was different. In *Arthrospira* sp. the peak intensity of its pigments is higher than *Scytonema* sp. Table 1 shows the relative area of the peaks of these three dominant pigments in each microalgae. The highest peak area in *Arthrospira* sp. and *Scytonema* sp. chromatograms was provided by Chl *a* (6 574 ± 183; 4 060 ± 602), followed by β-Car (2 324 ± 92; 1 199 ± 145) and Zea (1 208 ± 28; 1 200 ± 146), respectively.

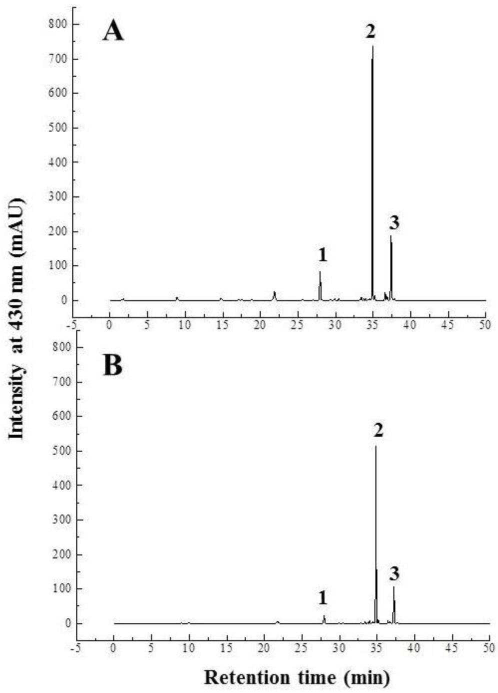

Figure 1. HPLC chromatograms of photosynthetic pigments from *Arthrospira* sp. (**A**) and *Scytonema* sp. (**B**).

Previous studies have identified that these pigments have positive value in food and health area. In human eyes, there is high concentration of Zea and lutein. Both functions as blue-light filters and reactive oxygen species scavengers in the retina to protect the cells from oxidative stress [11]. Butnariu *et al.* also showed a physico-chemical protection barrier, providing protection against damage triggered by free radicals to the DNA and to the lipids of the functional membranes [12]. In addition, it also has been reported that it could prevent atherosclerosis [1] and reduce AMD and cataracts related blindness [7]. Daily intake of Zea in some foods is generally low. Therefore, Zea-containing foods are required by humans. Blue green algae can be used widely as source of Zea [13].

Table 1.Identification and area quantification of pigments from *Arthrospira* sp. and *Scytonema* sp.

Sample	No Peak	Ret. Time (min)	Max absorption (nm)			Pigment	Peak Area ± SE
			I	II	III		
***Arthrospira* sp.**	1	27.9		453	480	Zea	1 208 ± 28
	2	34.9	431	617	663	Chl *a*	6 574 ± 183
	3	37.4		453	479	*β*-Car	2 324 ± 92
***Scytonema* sp.**	1	27.9		453	479	Zea	392 ± 28
	2	34.8	431	617	663	Chl *a*	4 060 ± 602
	3	37.2		453	479	*β*-Car	1 200 ± 146

The highest dominant pigment in this microalgae is Chl *a*. Some studies have provided antioxidant activity in chlorophylls [14, 15] in which through experiments using radical scavenging activity, DPPH and ABTS radicals. Chl *a* showed stronger antioxidant activity than Chl *b* [1]. Despite the application in health area, Chl *a* can also be used as natural food colouring agent [14]. Another carotenoid group, *β*-Car can also be employed to be an antioxidant therapy against anticancer and immunomodulator [8], AMD [16], cardiovascular disease and cataract [17]. The most common need of *β*-Car is related to its role as the precursor of Vitamin A [8].

In order to know the potency of these microalgae as alternative source pigments, total pigments concentration in each microalgae have been measured. Figure 2 shows that *Arthrospira* sp. contained higher amount of total chlorophyll and carotenoid, *i.e.* 9 594 ± 550 dry weight (DW) and 3 657 ± 203 μg · g^{-1} DW, respectively. Meanwhile, *Scytonema* sp. contained 5 889 ± 298 μg · g^{-1} DW of total chlorophyll and 1 540 ± 201 μg · g^{-1} DW of total carotenoid. These results indicate that these microalgae have economic potency for application in food and health. According to earlier studies about pigment content of blue green algae (*Spirulina platensis*), it contained a number of photosynthetic pigments with chlorophylls as the main pigments, contributing around 6 800 μg · g^{-1} to 15 100 μg · g^{-1} [18], while carotenoids as the second highest photosynthesis pigment presented at concentration of 3 400 μg · g^{-1} to 4 000 μg · g^{-1} [19]. Furthermore, the studies conducted by Sandeep *et al.* [20] revealed that *Spirulina platensis* contained about 4 950 μg · g^{-1} DW to 7 850 μg · g^{-1} DW of Chl *a*, and about 1 550 2 114 μg · g^{-1} DW to 2 114 μg · g^{-1} DW of carotenoid. This shows that the results obtained in this research was still in the range of the result of previous studies.

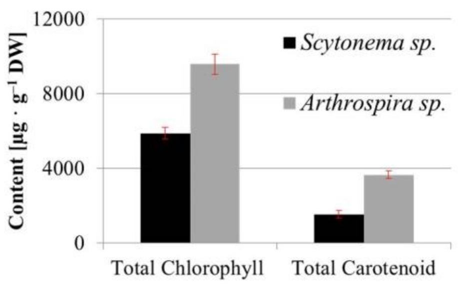

Figure 2. Total chlorophyll and carotenoid (μg · g^{-1} DW) from *Arthrospira* sp. and *Scytonema* sp.

Table 2 presents commercial products on the dominant pigments collected from different sources. It can be seen that the pigments has been used and processed into food colouring agent, nutraceutical and cosmeceutical. Chaitanya [21] has found that food colour influenced appetite of individual. This shows that food colourant plays an important role in flavor perception of food and beverage. According to FMI [22], food colour demand would increase by twice from 2010 to 2015. Furthermore, another research conducted by FMI stated that there will be enhancement from 54.9 % in 2014 to 60 % in 2020. In food industry, chlorophyll and *β*-Car have been used for natural pigment ingredient in processed food. The demand increases due to the strong colour and health benefit provided. However, due to the limitation of pigment sources, the production of synthetic colour has switched the presence of the natural pigments [16]. Nevertheless, FMI conducted the research and showed the opportunity of demand in natural food colour which was 2.8 times higher than synthetic colour. Moreover, demand of carotenoids has also been predicted to increase by 16.67 % from 2010 to 2018 [23].

Regarding to health concern, nutraceuticals have been commons in society especially in Japan, China, and India due to the intention to change the use of synthetic medicine. This report was supported by the research institution which focuses on nutraceuticals product market [24]. This research was in line with FMI which found that the rising will occur between 2015 and 2020, reaching Compound Annual Growth Rate (CAGR) by 5.3 % [22].

Concerning on beauty trends, cosmeceuticals have gained interest nowadays. RNCOS Business Service reported that global cosmetic markets contributed 13 % in 2012 and will increase by 16 % in 2017 [25], supported by Research and Market which predicted CAGR will grow 8.62 % from 2014 to 2019. Furthermore, these pigments have also opened opportunities for patents. It is shown in Table 3. Most of the patent concern on extraction method, application in food and health derived from higher plants such as vegetable and fruits. Therefore, this could be an opportunity to develop other innovations for optimizing the methods and use microalgae as the source of pigments. This is supported by result from FMI stating that Spirulina extract provides significant growth between 2015 and 2020 and it was expected to keep increasing year by year. This means that blue-green algae, *Arthrospira* sp. and *Scytonema* sp. have economic potential in application for food and health.

Table 2. List of commercial products derived from Zea, Chl *a*, and *β*-Car

Pigment	Product	Price	Formulation	Application	Source	Company
Zea	Ultra Zeaxanthin	$ 15.57	Capsules	Dietary supplements	*Tagetes erecta*	Solaray
	OmniXan	$ 9.99	Capsules and oil suspension	Dietary supplements	*Capsicum*	Swanson
	Lutein & Zeaxanthin	$ 14.25	Capsules and oil suspension	Dietary supplements	*Tagetes erecta*	Lutemax
	ZeaGold	unknown	Oil suspension and cold water soluble beadlet	Food colorant, dietary supplements and flavor masking	*Capsicum*	Kalsec
Chl *a*	Chlorophyll	$ 36.24	Liquid	Dietary supplements	*Medicago sativa*	Bernard
	Green Food	$ 25.76	Capsule	Dietary supplements	*Medicago sativa*	Swanson
	Chlorophyll extract (E140i)	unknown	Powder	Food colorant	*Fescue grass*	Holland ingredients
β-Car	CaroCare	unknown	Powder	Food colorant, food fortification, dietary supplements.	Fungus (*Blakeslea trispora*)	DSM
	Lyc-*O*-Beta	unknown	Liquid and oil suspension, powder	Food colorant, food fortification, dietary supplements.	Fungus (*Blakeslea trispora*)	LycoRed
	Natural Beta Carotene	unknown	Capsules and oil suspension	Dietary supplements	Fungus (*Blakeslea trispora*)	Aavalabs
	Natural Beta Carotene	$ 14.97	Capsules and oil suspension	Dietary supplements	*Dunaliella salina*	Now Foods
	Beta Carotene	$ 11.49	Capsules and oil suspension	Dietary supplements	*Dunaliella salina*	Nature's Way

Source: Bernard, DSM, Kalsec, Holland ingredients, and LycoRed in Berman *et al*. [23]; Aavalabs, Lutemax, Now Foods, Nature's Way, Solaray, and Swanson, in www.amazon.com [26].

Table 3. List of patents on the dominant pigments, Zea, Chl *a*, and *β*-Car, during 2010–2015

Pigmen	Patent	Topic	Publication date
Zea	US 8871984 B2	Preparing method for xanthophyll crystals with higher content of zeaxanthin from plant oleoresin	14 July 2011
	US 8425948 B2	Process for isolation of lutein and zeaxanthin crystals from plant sources	23April 2013
	WO 2013123618 A1	Process for manufacture of extract containing zeaxanthin and/or its esters	29 August 2013
	WO2014008851 A1	Method for preparing high level of zeaxanthin	16 January 2014
	US 20140086986 A1	Capsicum variety exhibiting a hyper-accumulation of zeaxanthin and products derived therefrom	27 March 2014
Chl *a*	US 20110287061 A1	Dietary composition and method for promoting healthy hair growth and melanogenesis	24 November 2011
	CN 102584838 A	Natural ferrous aluminum chlorophyll and preparation method thereof	18 July 2012
	CN 102796108 A	Natural chlorophyll iron-manganese salt and preparation method thereof	28 November 2012
	CN103653164 A	Plant chlorophyll health-care beverage and manufacturing method thereof	26 March 2014
	CN104026703 A	Chlorophyll cereal normal juice beverage and processing method thereof	10 September 2014
	US 20140308309 A1	Composition comprising chlorella extract for preventing or treating liver disease	16 October 2014
β-Car	CN 103204791 A	Method for supercritical CO_2 extraction of *β*-carotene in spirulina	17 July 2013
	EP 2627200 A1	Food supplement	21 August 2013
	EP 2658959 A1	Novel algae extraction methods	6 November 2013
	CN 103772255 A	Method for extracting *β*-carotene from algae by low-molecule organic matters/inorganic salt aqueous two-phase extraction	7 May 2014
	WO 2015052182 A1	*β*-carotene formulation and use thereof in coloring edible products	16 April 2015

CONCLUSION

Result of this research identified three dominant pigments, Zea, Chl *a*, and *β*-Car, in *Arthrospira* sp. and *Scytonema* sp. These pigments are potential to be applied in food, nutraceuticals, and cosmeceuticals industries. Research market has shown a tendency that the demand of the pigments in application mentioned will increase by years especially on food and health as the main concerns. Therefore, further research related to these pigments is encouraged to be conducted by the researchers which in turn would provide opportunity for patent and also give enough information for real application in human daily life.

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Abstrak

Upaya saat ini untuk mengeksplorasi potensi sumber daya laut telah dilakukan untuk mempromosikan pembangunan ekonomi di Indonesia, terutama di sektor industri. Sianobakteria laut, seperti *Arthospira* sp. dan *Scytonema* sp., merupakan dua kandidat potensial dari ganggang biru-hijau yang dapat digunakan dalam aplikasi untuk industri makanan dan kesehatan. Artikel ini berfokus pada identifikasi komposisi klorofil dan karotenoid dominan. Hasil penelitian menunjukkan adanya zeaksantin, klorofil *a*, dan *β*-karoten, selain itu potensi ekonomi dari pigmen dominan tersebut akan dibahas dalam artikel ini

Kata kunci: *Arthospira* sp., ganggang biru-hijau, klorofil dan karotenoid, potensi ekonomi pigmen, *Scytonema* sp.