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Composition of the Main Dominant Pigments from Potential Two Edible Seaweeds

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Two seaweed species – *Kappaphycus alvarezii* (Rhodophyta) and *Padina australis* (Phaeophyta) – have been commercially viable raw materials for the food industry. Despite their usefulness as sources of carrageenan and alginate, there is little information concerning their chlorophylls and carotenoids. Composition and quantification of the chlorophylls and carotenoids in *K. alvarezii* var. brown and *P. australis* were studied using reverse-phase high-performance liquid chromatography (RP-HPLC) with a recently developed 3D-multi-chromatogram analysis method. Identification of the most dominant pigments was confirmed by mass spectrometry using positive electron spray ionization. Samples were collected from three different locations in Indonesia (Jepara, Madura, and Maluku). A total of 39 pigments were found from the crude extracts of *K. alvarezii* and *P. australis*, and the four main dominant pigments (chlorophyll *a*, *β*-carotene, fucoxanthin, and zeaxanthin) were quantified by recently developed 3D-multi-chromatogram analysis method. Both seaweeds in three locations had almost similar pigment composition and only a small variation on minor pigments, except for the Maluku Island samples. The relationship between pigment concentration and environmental factor of solar irradiation was investigated using the pigment ratio between chlorophyll *a* and main carotenoids. The effect of solar irradiance on pigment formation is discussed.

Keywords: 3D-multi-chromatogram, carotenoid, chlorophyll, *Kappaphycus alvarezii*, *Padina australis*

INTRODUCTION

Kappaphycus alvarezii (Doty ex P.C. Silva 1996) is an introduced-species, which has been mono-cultivated as the largest seaweed commodity not only in Indonesia, but also in other tropical Asian countries. Indonesia is currently leading the production of *K. alvarezii* with nearly 1.5 x 10^6 tons in 2009, driven by the increasing demand for *κ*-carrageenan (Meinita et al. 2012). Another potential seaweed is *Padina australis* (Hauck 1887), a native species of brown seaweed that is increasingly being cultivated and studied as source of alginate (Widyastuti 2012). In addition to carrageenan and alginate, *K. alvarezii* and *P. australis* are also rich sources of natural pigments that have other additional biological functions (i.e., antioxidant, anti-obesity, anti-inflammation, and anti-hyper cholesterol) as well as natural colorants, depending on the chemical properties of the pigment. However,

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while great interest has been expressed in recent decade to characterize the distribution, biosynthesis, and functions as well as application of the photosynthetic pigments of microalgae, little effort has been dedicated to characterize other pigments in those seaweeds.

Separation and identification of photosynthetic pigments from a wide range of pigment polarities on seaweeds have been mainly performed by using reverse-phase high-performance liquid chromatography (RP-HPLC). Previously, pigment composition from three types of seaweed – red, brown, and green – has been reported (Hegazi et al. 1998). In addition to the type and species of seaweeds, different environmental factors found in their habitat may influence their pigmentation. In fact, seasonal changes were found on the content of pigments of brown seaweed (Gerasimenko et al. 2010). Different water depths of seaweed cultivation influenced pigment composition due to the chromatic adaptation of seaweed (Marinho-Soriano 2012). However, there is little information regarding pigment composition of macroalgae compared to those of phytoplankton (Zapata et al. 2000), in particular for quantification even in main pigments. Furthermore, reports are limited largely to edible species and pigments such as carotenoids (Andersson et al. 2006; Schubert et al. 2006), and there is no comparison of pigment variations even in the same species.

On the progress of developing fast and accurate method for the analysis of photosynthetic pigments, a 3D-multi-chromatogram method has also been developed for pigment quantification by calculating information recorded by diode array detection (Indrawati et al. 2012). In this study, a comparison of pigment distribution is presented among the commercially potent raw materials for food industry. These include the cultivated *K. alvarezii* and the naturally growing *P. australis*, both of which were collected from the three different locations (i.e., Jepara, Madura, and Maluku in Indonesia).

MATERIALS AND METHODS

Reagents and standards

All chemicals were of analytical grade from Merck (Darmstadt, Germany). The *β*-carotene was purchased from Sigma (Type I approx. 95 % UV, St. Louis, MO, USA), while other standard pigments – chlorophyll *a*, fucoxanthin, and zeaxanthin – were purchased from NATChrom (Malang, Indonesia).

Field sampling

Brown variety of *K. alvarezii* cultivated by floating method was harvested from three plantation areas in Indonesia: (i) Maluku (Wael village, West Seram) (3° 2' 52.6338" S, 128° 4' 23.181" E) in May 2011; (ii) Jepara (Teluk Awur village, Central Java) (6° 37' 30.7914" S, 110° 38' 8.6238" E) in Oct 2011; and (iii) Madura (Padike village, East Java) (7° 5' 18.0636" S, 113° 56' 20.1804" E) in May 2012. *P. australis* was naturally grown on the littoral bottom and collected from (i) Maluku (Ambon bay) (3° 39' 11.2932" S, 128° 13' 25.7664" E) in Oct 2011; (ii) Jepara (Teluk Awur village, Central Java) (6° 37' 30.7914" S, 110° 38' 8.6238" E) in Oct 2011; and (iii) Madura (Padike village, East Java) (7° 5' 21.321" S, 113° 56' 59.8086" E) in May 2012. Samples were cleansed from epiphytes and any associated debris by rinsing them with sea water, after which they were frozen and stored immediately at -20° C for further analyses.

Environmental parameters

Solar irradiation (pmol photon) and sea surface temperature (ºC, 50 km resolution) in the three sampling locations were obtained from Jan 2010 to Dec 2012 via two facilities. These are the NASA Langley Research Center POWER Project – funded through the NASA Earth Science Directorate Applied Science Program – for the solar irradiation, and Comprehensive Large Array-data Stewardship System (CLASS) SST50 for the surface temperature.

Pigment extraction

Seaweed sample was ground with a few amounts of sodium ascorbate and $CaCO_3$ to avoid pigment oxidation and acidification. The homogenate (1 g wet weight) of either *K. alvarezii* or *P. australis* was extracted with 20 mL of 100% methanol or 20 mL of acetone, respectively. This procedure was repeated three times until the residue became colourless. The crude pigment extract was then filtered and concentrated by the use of rotary evaporator, and finally N_2 (UHP grade) was applied to complete dryness of the extract. The dry extract was kept under argon and the sample was immediately kept at -20° C for further measurements. In order to minimize photo-degradation of the pigments, the extractions and measurements were carried out under green dim light at room temperature.

Preparation of standard pigments

Five different concentrations of standard pigments were prepared in acetone and calculated by measuring absorption spectrum of each pigment solution with specific extinction coefficients. The values reported by Jeffrey and colleagues (1997) are as follows: chlorophyll *a* (88.15 $L \cdot g^{-1} \cdot cm^{-1}$ at 662 nm), *trans*-fucoxanthin (166 $L \cdot g^{-1} \cdot cm^{-1}$ at 443 nm), zeaxanthin (234 $L \cdot g^{-1} \cdot cm^{-1}$ at 452 nm), and *β*-carotene (454 $L \cdot g^{-1} \cdot cm^{-1}$ at 454 nm).

HPLC analysis

Pigment compositions and concentrations were determined by HPLC using LC-20A equipped with SPD-20MA diode array detector (Shimadzu, Kyoto, Japan) and an RP column of Shimpack VP-ODS C-18 (4.6 I.D. × 250 mm length, 4.6 µm particle size) (Shimadzu). HPLC analysis was performed using a modified method of Hegazi and colleagues (1998) at a column temperature of 30° C and flow rate of 1 mL · min^{-1}. The pigments were detected in the range of 190-800 nm and post-analyzed. For purity check of the standards, water was used instead of ammonium acetate to avoid the formation of degradation products.

Prior to injection, dry pigment sample was dissolved in 5 mL acetone and filtrated through a membrane filter (0.2 µm, Nylon, Whatman, Kent, UK); 20 µL pigment solution was then subjected to analysis. Each separated pigment was identified based on its retention time and spectral characteristics in the HPLC eluent. In addition, to identify the pigments more precisely, spectroscopic parameters of each pigment isolated from HPLC analyses were compared in three different solvents (acetone, hexane, and ethanol for carotenoid group; acetone, diethyl ether, and ethanol for chlorophyll group) with references (Britton et al. 1995; Jeffrey et al. 1997; Hegazi et al. 1998).

ESI-MS/MS analysis

Seaweed pigment extract was solubilized in methanol and filtrated through a 0.2 µm nylon membrane filter (Whatman) prior to injection. The pigment separation was performed by HPLC through a column of 4.5 mm × 150 mm Cosmosil 5C18-MS-II (Nacalai Tesque, Kyoto, Japan) using an isocratic elution of acetonitrile with 0.1% formic acid and methanol with 0.1% formic acid as mobile phase at a column temperature of 30° C and flow rate of 1.0 mL · min^{-1}. The pigments were monitored at 450 nm and 660 nm using an SPD-M20A diode array detector (Shimadzu). Electron spray ionization (ESI) triple quadrupole mass spectrometer LCMS-8030 (Shimadzu) was operated with the following settings: interface voltage (4.5 kV), interface current (0.1 µA), nebulizing gas (N_2) flow (3.0 L · min^{-1}), drying gas (N_2) flow (15 L · min^{-1}), desolvation temperature (250° C), and heat block temperature (400° C). In the positive mode, the single ion monitoring (SIM) and full scan (450-950 m/z range) were simultaneously used to confirm the identity of the dominant pigments in the crude extract.

Data analysis by 3D-Multi-Chromatogram

Concentrations of the main pigments were calculated based on their standard pigment equations, which were created via 3D-multi-chromatogram method. In this method, a trapezoidal rule using MATLAB R2010a was employed to calculate the peak area at every single wavelength with resolution of 1 nm. The standard equations were then calculated by plotting a linear regression of five different concentrations of standard pigments as function of their averaged peak area in ranges of 330-600 nm and 300-720 nm for the carotenoid group and chlorophyll *a*, respectively. The analysis was repeated three times at each concentration. Concentrations of the main pigments in seaweeds were expressed in either mg or µg · g^{-1} of seaweed sample on wet weight basis.

RESULTS AND DISCUSSION

The isograms (spectrochromatograms) of the pigment extracts from *K. alvarezii* and *P. australis* are shown in Fig. 1. The x-axis represents wavelength and the y-axis represents retention times. Different contour colours are used to represent the intensity in absorbance unit. The red spots on the contour map indicate the apex of an absorption peak, while the blue region indicates the baseline. The top three panels (A, B, and C) show the isograms of the pigment extract from *K. alvarezii* obtained from three different locations. Following the retention time of 0-80 min on these isograms, four dominant peak-lines were clearly indicated. Those boldly apparent peak-lines were recorded at 5.29, 19.65, 37.65, and 60.24 min. The bottom three panels (D, E, and F) show the isograms of the pigment extract from *P. australis* harvested from three different locations. Eight peak-lines were clearly seen at retention time of 0-80 min on the isograms, and those were determined to be at 6.42, 10.13, 13.52, 17.52, 19.55, 39.44, 55.84, and 60.24 min.

In order to identify peak-lines from isogram of the pigment extracts from *K. alvarezii* and *P. australis*, the absorption spectrum of the isolated fractions from each peak-line was recorded in three different solvents (data not shown). The λ_{max} of these peak-line fractions were compared with those of the pigments in the references as listed in the Table 1. In *K. alvarezii*, the three dominant peak-lines were assigned as zeaxanthin, chlorophyll *a*, and *β*-carotene, respectively. Through more detailed analysis that includes minor peaks, seven species of chlorophylls and 14 pigments of carotenoids could be separated. However, only seven pigments showed clear chromatographic and spectroscopic properties of chlorophyllide *a*, violaxanthin, *α*-cryptoxanthin, anteraxanthin, zeaxanthin, chlorophyll *a*, chlorophyll *a*', and *β*-carotene. Chlorophyll *a*' could be seen apparently in the isogram despite its low concentration. The chlorophyllide *a* is usually undetectable, but it appears to be produced enzymatically by chlorophyllase (Jeffrey & Hallegraeff 1987). Accordingly, 18 pigments were

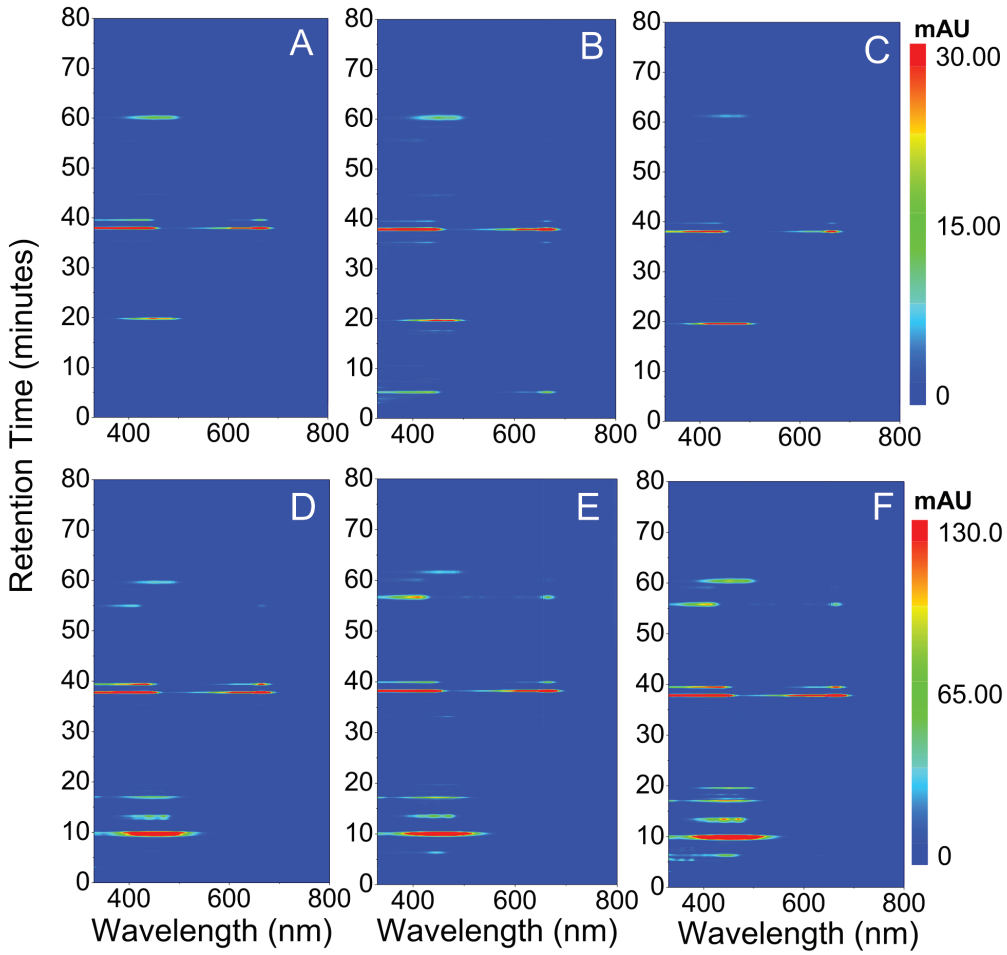

Figure 1. HPLC isograms of the pigment extracts from *K. alvarezii* (top) and *P. australis* (bottom), which were harvested from three different locations, Jepara beach (A and D), Madura Island (B and E), and Maluku Island (C and F).

Table 1. Identification and peak area of dominant pigments of *K. alvarezii* and *P. australis* from three localities in Indonesia.

t_R (min)	Identification	*K. alvarezii*[a]			*P. australis*[a]			λ_{max} (nm) in the eluent	Molecular Ion Species (m/z)	Ref.[b]
		Jp	Md	Ml	Jp	Md	Ml			
5.29	Chlorophylide *a*	+	+	−	−	−	−	432,610,665	n.d.	1,2
6.42	Chlorophyll c_1	−	−	−	+	+	+	444,684,635	n.d.	1,2
10.13	*trans*-fucoxanthin	−	−	−	+	+	+	(429),450,(468)	659.5 $[M+H]^+$, 641.5 $[M+H-H_2O]^+$, 581.4 $[M+H-H_2O-C_2H_4O_2]^+$	1,2
13.52	Violaxanthin	−	−	+	+	+	+	416,440,469		1,2
17.52	Antheraxanthin	+	+	+	+	+	+	(425),443,(464)	n.d.	1,2
19.55	Zeaxanthin	+	+	+	+	+	+	(421),473,446	568.8 $[M]^+$, 569.6 $[M+H]^+$, 551.4 $[M-OH]^+$	1,2
37.85	Chlorophyll *a*	+	+	+	+	+	+	430,617,664	893.5 $[M+H]^+$, 871.6 $[M+3H-Mg]^+$	1,2
39.47	Chlorophyll *a*'	+	+	+	+	+	+	432,615,665	n.d.	
55.84	Pheophytin *a*	+	+	+	+	+	+	408,608,665	n.d.	1,2
60.24	*β*-carotene	+	+	+	+	+	+	(428),451,477	536.7 $[M]^+$, 537.5 $[M+H]^+$	1,2

Note: [a]Jp = Jepara beach, Md = Madura Island, MI = Maluku Island, n.d. = not identified
[b]1 = Hegazi et al. (1998), 2 = Zapata et al. (2000)

found in *K. alvarezii* from Jepara beach and Madura Island, and 13 pigments were found from Maluku Island. Violaxanthin was recorded only from the Maluku sample, while zeaxanthin could be obviously detected in all of the samples. In *P. australis* (Fig. 1; D, E, F), the eight peaklines were assigned to be chlorophyll c_1, fucoxanthin, violaxanthin, antheraxanthin, zeaxanthin, chlorophyll *a*, pheophytin *a*, and *β*-carotene, respectively. Finally, 10 pigments from chlorophyll group and 16 pigments from carotenoid group were recorded through further detailed analysis.

The identification of the main dominant pigments was confirmed via ESI-MS/MS using simultaneous full scan and SIM modes (Table 1). The mass spectrum of fucoxanthin showed the $[M+H]^+$ at m/z 659.5 and the fragment ions at m/z 641.5 and m/z 581.4, corresponding to the loss of water and the loss of water and ester group, respectively. In the mass spectrum of the zeaxanthin fraction, the observed peak at m/z 568.8 and m/z 569.6 were assigned as $[M]^+$ and $[M+H]^+$. In the MS spectrum of chlorophyll *a*, the $[M+H]^+$ was observed at m/z 893.5 and there was additional peak at m/z 871.6, which corresponds to the degradation of chlorophyll *a* to become pheophytin *a* with the loss of magnesium in the presence of acidic mobile phase. The mass spectrum of *β*-carotene showed the $[M]^+$ and $[M+H]^+$ at m/z 536.7 and m/z 537.5, respectively.

The peak area of the standard pigments chlorophyll *a*, *β*-carotene, fucoxanthin, and zeaxanthin – which was generated by multi-chromatogram method from 3D data in specific wavelength ranges – is presented in histograms (Fig. 2, left). The average peak area of each standard pigment in five different concentrations was used to create a linear standard equation (Fig. 2, right). The R^2 values from linear equations of chlorophyll *a* (0.9987), *β*-carotene (0.9954), fucoxanthin (0.9989), and zeaxanthin (0.9985) were close to one another, indicating that the average peak area and concentration of standard pigments are significantly correlated.

The chlorophyll *a* and *β*-carotene concentrations of *K. alvarezii* were relatively close to the samples from Jepara and Madura, but not for Maluku (Table 2). On the other hand, the concentration of zeaxanthin in *K. alvarezii* was high in the sample from Maluku (3.22 μg · g^{-1} wet weight), and samples from Jepara and Madura had only 1.39 μg · g^{-1} and 1.86 μg · g^{-1} wet weight, respectively. In *P. australis*, the sample from Maluku had higher pigment concentrations in all four dominant pigments. The concentrations of chlorophyll *a* in Maluku were 276.96 μg · g^{-1} wet weight, as compared to those of Jepara (165.45 μg · g^{-1} wet weight) and Madura (192.84 μg · g^{-1} wet weight). The concentrations of *β*-carotene were almost similar between the Jepara and Madura samples, but high in Maluku. Variation in concentrations of a marker pigment (fucoxanthin) was observed. The Maluku sample had 1.5-fold higher fucoxanthin concentration (122.55 μg · g^{-1} wet weight), as compared to those from Jepara (81.96 μg · g^{-1} wet weight) and Madura (87.27 μg · g^{-1} wet weight). Other main carotenoids also had a similar tendency.

HPLC has been proven to be a reliable chromatography technique for separation and identification of photosynthetic pigments. It has been applied to separate pigments of a wide range of polarity – from polar to non-polar pigments such as chlorophyllide to pheophytin in chlorophyll group or xanthophyll to carotene in carotenoid group (Zapata et al. 2000). In this study, separated pigments could be identified according to their retention time and spectral characteristics obtained from 3D data, compared to the standards and data from references (Table 1). Moreover, concentrations of the main pigments were calculated based on their standard pigment equations, which were created by 3D-multi-chromatogram method (Fig. 2). A 3D quantification using multi-chromatogram approach has shown its excellence to minimize the experimental errors, particularly in the quantitative analysis of photosynthetic pigments, in which each pigment has its own typical absorption peaks (Indrawati et al. 2012). The collection of peak area of chlorophylls was focused on the wavelength range of 300-720 nm that covers the absorption of Soret, Q_x, and Q_y bands. On the other hand, the wavelength range of 330-600 nm was applied to accommodate the absorption of the fine structure of carotenoids. This procedure appears to be a reasonable alternative to the more conventional methods of 2D-chromatogram and peak height method.

It has been demonstrated that many photosynthetic pigments are limited to particular classes or even genera. It is shown that *K. alvarezii* collected from three different locations composed of 21 pigments (data not shown), of which composition slightly varied in minor pigments but the overall main pigments in three different locations were almost similar and consistent with those of Rhodophyta (Table 1). The composition of the carotenoids in *K. alvarezii* is also in agreement with other red algae (Schubert et al. 2006), except for lutein (Andersson et al. 2006). Minor differences in pigment composition appear to reflect the adaptation of *K. alvarezii* to their environments. It is known that the different pigment composition of red algae is influenced by solar irradiance (Dawes 1992).

Pigment compositions of *P. australis* collected from three different regions were also similar in each location. As mentioned above, fucoxanthin is dominant, specific, and unique from their functional aspects in brown seaweeds. Fucoxanthin concentrations (81.96 μg · g^{-1} to 122.55

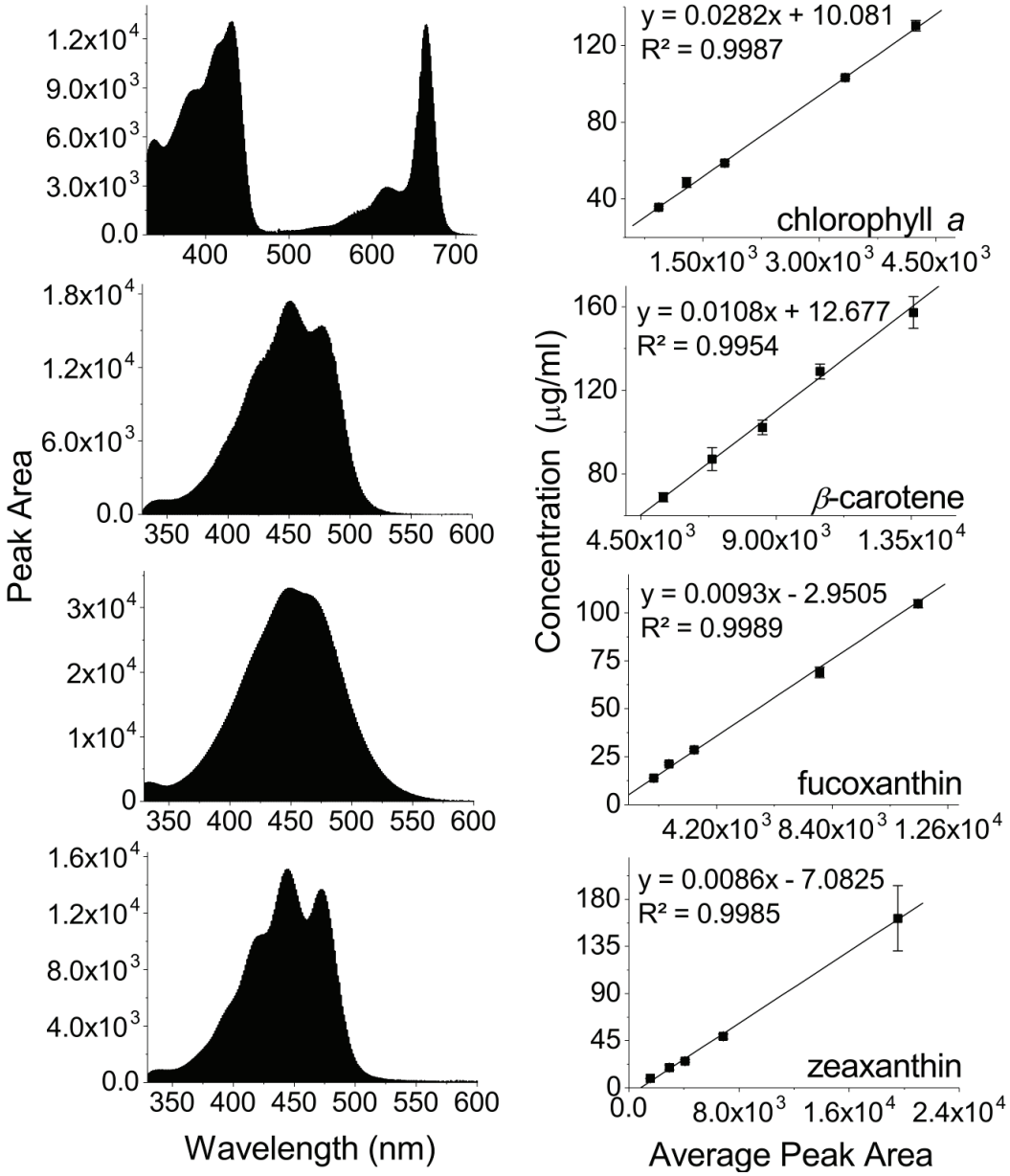

Figure 2. The histograms of the average peak area (left) and linear equation formula (right) of the standard pigments chlorophyll *a*, *β*-carotene, fucoxanthin, and zeaxanthin that were generated after multi-chromatogram calculation on the 3D HPLC data. Data were obtained from three repetitions and show average and SE.

μg · g^{-1} wet weight) in *P. australis* were much higher than the concentrations of *β*-carotene and zeaxanthin, indicating that fucoxanthin is the dominant carotenoid of this alga. However, quantification is not only related to the selection of algal species or growing place, but also to the necessary optimization of the pigment extraction. Such data give some practical suggestions on the mass production of certain pigments. In the present study, methanol and acetone were chosen on the basis of their ability to penetrate the algal cells and extract the species of chlorophylls and carotenoids that may be present in *K. alvarezii* and *P. australis*.

Solar irradiation and sea surface temperature, as environmental parameters in the three sampling locations of *K. alvarezii* and *P. australis*, are shown in Figure 3. As can be seen, both environmental factors measured in the three locations oscillated almost synchronously with 2 or 3 peaks a year, although their intensities were different. Therefore, average values of data from the sampling

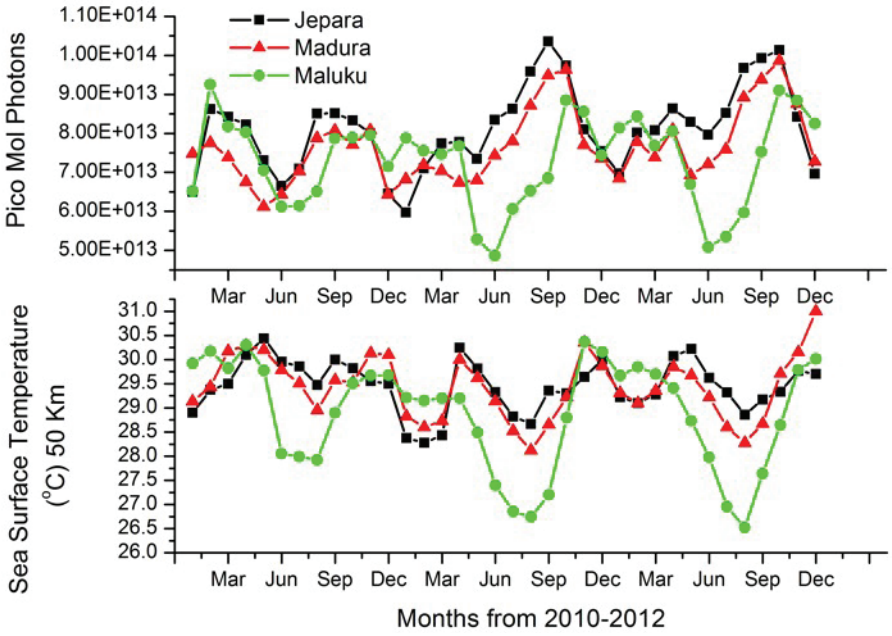

Figure 3. Data of NASA solar irradiation and NOAA sea surface temperature (50 km resolution) in three sampling locations from January 2010 to December 2012. Original sources are described in the Materials and Methods section.

month and one month prior to the sampling were used in this study. The highest value of the averaged solar irradiation was measured in Jepara beach (10×10^{13} pmol photons) for both seaweeds, followed by Madura Island (7.5×10^{13} pmol photons) and Maluku (6.5×10^{13} pmol photons) for *K. alvarezii*, and Maluku (7.8×10^{13} pmol photons) and Madura (7.5×10^{13} pmol photons) for *P. australis*. The average values of sea surface temperature were varied from 28.9˚ C (Maluku) to 29.8˚ C (Madura) for *K. alvarezii* and from 28.0˚ C (Maluku) to 29.8 ˚C (Madura) for *P. australis*.

To reveal the relationship between pigment concentrations and environmental factors (e.g., solar irradiation), the concentration ratio between chlorophyll *a* and main carotenoids was applied to eliminate the influence of wet mass. The following carotenoids were used for calculation: *β*-carotene and zeaxanthin for *K. alvarezii*; and *β*-carotene, zeaxanthin, and fucoxanthin for *P. australis*. As shown in Table 2, the ratios were estimated to be from 1.79 of Jepara beach to 1.95 of Madura Island for *P. australis*, and from 2.0 of Maluku to 5.01 of Madura Island for *K. alvarezii* under relatively small variation of the sea surface temperature (28.0-29.8° C).

In *P. australis*, the highest ratio was obtained in Madura Island among all regions. In contrast, the solar irradiance of Madura Island ranked the lowest among three locations (Table 2). Thus, the pigment ratio increased as the solar irradiance decreased. Similar results were obtained in the ratios of *K. alvarezii*, except for Maluku Island. These findings are consistent with the fact that in low light conditions, a greater amount of pigment is required to enhance the absorbed photons by light-harvesting (LH) systems (Lüning 1990). In addition, results from the pigment ratios further suggest that chlorophyll *a* as the main pigment in LH systems is biosynthesized in higher amounts, as compared to carotenoids in low solar irradiation. However, the ratio of *K. alvarezii* from Maluku Island was fairly low compared to those obtained from other locations. Furthermore, the composition and quantity of the pigments from Maluku were also poor and low compared to those of other locations as described above. Taking into account a similar level of solar irradiance in Madura Island, the low ratio in Maluku Island appears to be derived from other environmental factors rather than solar irradiance. The cause of inferior pigment composition and concentration of the sample may be ascribed to aging of the seaweeds, although there are other possible factors such as nutrient and salinity. For *K. alvarezii* containing phycobilins, further investigation on the distribution of phycobilisomes with respect to the chlorophyll pigments that are bound in the integral membrane LH and reaction center may be necessary.

It has been demonstrated that the variation on the xanthophyll group pigments is closely related with

Table 2. Concentration and pigment ratio of the main dominant pigments from *K. alvarezii* and *P. australis* and solar irradiance in three sampling locations.

Seaweeds		***K. alvarezii***			***P. australis***		
Sampling locations		**Jepara**	**Madura**	**Maluku**	**Jepara**	**Madura**	**Maluku**
Pigments (μg·g^{-1} wet weight)	**Chlorophyll *a***	28.08	38.01	12.25	165.45	192.84	276.96
	***β*-Carotene**	4.91	5.72	2.92	10.24	11.21	22.27
	Fucoxanthin	–	–	–	81.96	87.27	122.55
	Zeaxanthin	1.39	1.86	3.21	0.04	0.49	4.92
Pigment ratio*		4.46	5.01	2.00	1.79	1.95	1.85
Solar irradiance (× 10^{13} pmol photons)**		10	7.5	6.5	10	7.5	7.8

K. alvarezii = chlorophyll *a*/ *β*-Carotene + zeaxanthin; *P. australis* = chlorophyll *a*/ *β*-Carotene + fucoxanthin + zeaxanthin.
**Two-month averaged values are shown.

the irradiation of solar light (Rmiki et al. 1996). In comparison with other phototropic organisms, many of the red algae exhibit a simple carotenoid pattern, dominated by a single xanthophyll cycle (Marquardt & Hanelt 2004). In the present study, several xanthophyll group pigments (e.g., zeaxanthin, antheraxanthin, and violaxanthin) as well as *β*-carotene can be found in both *P. australis* and *K. alvarezii*. Furthermore, zexanthin was highly accumulated in *K. alvarezii*, while violaxanthin was found in high amounts in *P. australis* as an intermediate of the violaxanthin cycle. This variation can be understood from the point of view of an adaptation of these seaweeds against the environmental conditions, especially on solar irradiation. Although light is essential for photosynthesis, an excess amount of light can lead to photoinhibition or even depression in photosynthetic efficiency (Powles 1984). Therefore, formation of de-epoxidated xanthophylls pigments is needed to protect from photoinhibition.

CONCLUSIONS

A total of 39 pigments were found from the extracts of *K. alvarezii* and *P. australis*, and the four main dominant pigments (chlorophyll *a*, *β*-carotene, fucoxanthin, and zeaxanthin) were quantified by recently developed 3D-multi-chromatogram analysis method. Chlorophyll *a* was found to be the primary photosynthetic pigment in both algae, and numerous carotenoids as accessory pigments appeared after chromatographic separation. Fucoxanthin is found to be the most dominant carotenoid in *P. australis* and particularly produced by brown seaweeds. Each seaweed in three locations had almost similar pigment composition and only a small variation on minor pigments, except for Maluku Island. The relationship between pigment concentration and environmental factors (e.g., solar irradiation) was investigated through the pigment ratio of chlorophyll *a* and main carotenoids. The results indicate that the pigment is produced in response to solar irradiation in three sampling locations and that in low light condition, a high amount of chlorophyll *a* is synthesized rather than those of carotenoids.

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