Water and Lipid-Soluble Component Profile of Sargassum cristaefolium from Different Coastal Areas in Indonesia with Potential for Developing Functional Ingredient

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Abstract: Sargassum brown seaweed is known to have many health benefits and therapeutic effects. Preliminary chemical characterization of this seaweed is important as a bioprospecting strategy for seaweed industry development. This study aimed to evaluate chemical composition differences, both water and lipid-soluble component, of Sargassum cristaefolium from four different coastal areas in Indonesia, namely Pari Island/PI, Awur Bay/AB, Ujung Genteng Beach/UGB, and Sayang Heulang Beach/SHB. Principal component analysis (PCA) on water-soluble component made samples from different origins to be clearly distinguished (variance: 80.37%). SHB and UGB samples were characterized by a high content of ash, alginate, fucose-containing sulfated polysaccharides (FCSPs), and fucose content of FCSPs, while samples of AB and PI had a high amount of total sugar and crude fiber. PCA result on lipid-soluble components showed a different tendency that SHB and AB samples were located at close proximity and characterized by larger blade size, higher content of chlorophyll, fucoxanthin, carotenoid, PUFA, total n-3 fatty acids, total n-6 fatty acids, and also a lower ratio of n-6 to n-3 (variance: 75.42%). The overview of each samples’ chemical characteristics can be valuable knowledge for further development, especially for developing functional ingredients.

Key words: coastal area, fatty acids, FCSPs, pigment, Sargassum cristaefolium

1 Introduction

Sargassum is known as one of the brown seaweed popular genus, which is ecologically abundant and has important economic and cultural values. This type of brown seaweeds can grow well in both temperate and tropical area¹. Besides its valuable content of vitamins, minerals, dietary fiber, carbohydrates, and protein², Sargassum also contains a variety of bioactive components having various functional effects such as meroterpenoid, fucoidan(sulfated polysaccharides), and phlorotannin(polyphenols)³. Moreover, Sargassum has a typical functional lipid content that promotes human health, such as omega-3 and omega-6 PUFA, fucoxanthin, and fucosterol⁴. Some reported biological activities of Sargassum extract or purified compounds include anticancer, antibacterial, antifungal, anti-inflammatory, anticoagulant, antioxidant, hypoglycemic, hypolipidemic, and hepatoprotective⁵. Sargassum genus is widely spread in various water areas in Indonesia. Also, it has promising biomass potential because of the good growth in a tropical area. Since the chemical composition of seaweeds is strongly influenced by their environmental conditions⁶−¹⁰, the evaluation of different marine habitats on seaweed chemical content is important to be conducted. This is related to the bioprospecting strategy for seaweed industry development, especially for future functional ingredient sources. This
2 Experimental Procedures

2.1 Materials and apparatus

Brown seaweed *Sargassum cristaefolium* was obtained from four different coastal areas in Java Island, Indonesia. Other materials used for chemical profiling were chloroform, methanol, BF3 in methanol, HCl, and ethanol (Merck KGaA, Germany), dichloromethane (Chemiz Sdn Bhd, Malaysia), acetone (Mallinkrodt Baker Inc, USA), CaCl2 dihydrate and Na2CO3 from Cica Kanto - Japan, FAME Mix C4-24 (SUPELCO, USA), monosaccharides standards (Sigma Aldrich, USA), silicic acid 60 (Friendemann Schmidt, USA) and some other chemicals for samples characterization. Types of equipment used were gas chromatography (GC) instrument with a DB-23 column and flame ionization detector (SHIMADZU GC-2010, Japan), spectrophotometer UV-Vis (SHIMADZU UV2450, Japan), sonicator (BRANSON 3510, USA), rotary vacuum evaporator (Buchi R-300, Switzerland), centrifuge (Hermle Labortechnik Z 383 K, Germany), high-performance liquid chromatography (HPLC) system with ion exclusion column (BIO-RAD Aminex HPX-87H (300 × 7.8 mm), USA) and refractive index detector (Agilent Tech 1200 series, USA), and glassware.

2.2 Sampling and species identification

Brown seaweed sampling was conducted in the same monsoon season, March-April 2017. Samples were taken from the northern waters of Java Island, i.e. Awur Bay/AB, Jepara, Central Java (6° 36’ 54” S, 110° 38’ 55” E) and Pari Island/PI, Seribu Islands, DKI Jakarta (5° 51’ 48” S, 106° 36’ 29” E), and the southern parts of Java Island, i.e. Sayang Heulang Beach/SHB, Garut, West Java (7° 40’ 12” S, 107° 41’ 50” E) and Ujung Genteng Beach/UGB, Sukabumi, West Java (7° 21’ 39” S, 106° 24’ 10” E). Samples obtained from each location were then brought to the Marine Hydrobiology Division, Department of Marine Science and Technology, Bogor Agricultural University for identification.

2.3 Sample preparation

Fresh brown seaweeds from each area were cleaned first using tap water to remove physical impurities, such as gravel and sand. They were then dried using an oven dryer at 50°C until its water content reached <10%. The dried seaweed was subsequently ground using pin disc mill and filtered using an 18 mesh sieve to obtain seaweed powder. Brown seaweed powder was then stored at −20°C for further analysis. Morphological information of brown seaweed is shown in Table 1.

2.4 Chemical characterization

Some chemical characteristics observed in this study were proximate content, acid insoluble ash (AIA), crude fiber, total sugar, total phenolic content (TPC), alginate and fucose-containing sulfated polysaccharides (FCSPs) content, FCSPs sugar profile, pigment profile, lipid class composition, and fatty acid profile.

2.4.1 Proximate and water-soluble components analysis

Proximate content, crude fiber, and acid insoluble ash were measured according to AOAC method. Total sugar was measured by the anthrone reagent method and glucose was used as a sugar standard. Total phenolic content

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**Table 1** Morphological information of *S. cristaefolium* from different coastal areas.

| Parameter | Ujung Genteng Beach | Sayang Heulang Beach | Awur Bay | Pari Island |
|-----------|---------------------|----------------------|----------|-------------|
| Time of collection | March 2017 | March 2017 | April 2017 | March 2017 |
| Fresh thallus colour | Green | Brownish green | Dark brown | Brownish green |
| Thallus length (cm) | 10-20 | 10-20 | 22-51 | 30-50 |
| Blade width (cm) | 0.7 ± 0.2 | 1.4 ± 0.1 | 1.8 ± 0.2 | 1.3 ± 0.05 |
| Blade length (cm) | 1.6 ± 0.3 | 3.8 ± 0.2 | 4.5 ± 0.3 | 3.2 ± 0.4 |
| Seaweed powder colour (chromameter) | L: 31.99 ± 0.06; a: +1.60 ± 0.03; b: +8.31 ± 0.01 | L: 27.88 ± 0.09; a: +0.15 ± 0.03; b: +5.87 ± 0.02 | L: 29.79 ± 0.02; a: +0.50 ± 0.04; b: +7.14 ± 0.01 | L: 28.42 ± 0.06; a: +1.63 ± 0.02; b: +6.15 ± 0.01 |

Note: Data of blade width, blade length, and seaweed powder colour are provided in Mean ± SD.
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measurement was performed referring to Chandini et al.\textsuperscript{13}. Gallic acid was used as a standard of phenolic component. Determination of alginate content, FCSPs content, and sugar profile of FCSPs fraction was begun by preparation of sugar extract. The sample was defatted before extracted. The defatting procedure was based on Bligh & Dyer\textsuperscript{14} as described in the lipid-soluble component analysis session. The defatted samples were immersed in 0.03 M HCl with a ratio of 1:20 (w/v) and sonicated for 30 minutes. The extraction was continued using a water bath for 4 hours at 81°C. Thereafter, the suspension was filtered by filter paper to separate the filtrate (supernatant 1) with the sample residue. The sample residue was then washed with distilled water (1:5 b/v) and filtered to obtain supernatant 2. Supernatant 1 and supernatant 2 were combined into extract A. Extract A was added by 30 mL of 1 M CaCl$_2$, then stored for one day at 10°C to precipitate alginate. Precipitated alginate in extract A was separated by a vacuum filter with Whatman filter paper No. 42. The filtrate was subsequently added by a volume of ethanol (1:3, v/v) and stored for 1 day at 10°C to precipitate FCSPs. FCSPs was separated from the filtrate by centrifugation at 3,500 rpm for 30 minutes. The crude alginate precipitate and the obtained FCSPs were then dried by oven at 50°C overnight. FCSPs was stored at 4°C for further sugar profile analysis\textsuperscript{15}. Before HPLC analysis of FCSPs fraction, this fraction was firstly hydrolyzed using 0.5 mL of 2 N trifluoroacetic acid (TFA) at 121°C for 2 hours in a glass tube. The filtrate was then neutralized using a solution of NaOH 2 M. Sample was filtered by cellulose acetate membranes before injected to the HPLC system. The column temperature was set to be in the room temperature range. The mobile phase used was 5 mM H$_2$SO$_4$ and acetonitrile (95:5). Fucose, glucose, galactose (Gal), mannose (Man), rhamnose (Rham), and xylose (Xyl) were used as standard. Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined by injecting the lowest standard concentration for 5 repetitions. The lowest concentration of glucose and fucose standard was respectively 100 ppm, while the lowest concentration of Gal/Rham/Xyl/Man standard was 25 ppm. A refractive index detector was used for compound detection.

2.4.2 Lipid-soluble component analysis

Qualitative pigment analysis was conducted using the spectrophotometric method to scan the pigment spectrum at a visible wavelength (350-750 nm) as described by Hary-utfrelini et al.\textsuperscript{16}. Dried seaweed powder (1 g) was macerated by 10 mL cold acetone 90% (1:10, v/v) for 20 min in the darkroom, CaCO$_3$ was added to avoid acidification. Dilution of pigment extract by cold acetone 90% (1:10, v/v) was done before absorbance scanning using double beam spectrophotometer UV-Vis.

The semi-quantitative pigment profile was separately calculated by the spectrophotometric equation as explained by Connan\textsuperscript{17}. Brown seaweed pigment was extracted through the two-step extraction process using DMSO (X1) and acetone (X2). The acetone extract was then partitioned to yield hexane phase (X2A) and aqueous acetone-methanol phase (X2B). The diluted hexane phase (X2A) absorbance was measured at 661, 480, and 750 nm. The DMSO extract (X1) was measured at 665, 631, 582, 480 nm, and 750 nm, while X2B was measured at 664, 631, 581, 470, and 750 nm. Absorbance measurement at 750 nm represented sample turbidity and was removed from all other absorbance measurements. Equations for pigment determination in each fraction are as follows:

X1: ca. 4:1 DMSO:water
Chlorophyll a (µg/ml) = 13.74 × [(A$_{665}$ − A$_{750}$)]
Chlorophyll c (µg/ml) = 16.18 × [(A$_{631}$ − A$_{750}$)] + [(A$_{682}$ − A$_{750}$)] − 4.81 × [(A$_{665}$ − A$_{750}$)]
Fucoxanthin (µg/ml) = 7.69 × [(A$_{665}$ − A$_{750}$)] − 5.55 × [(A$_{631}$ − A$_{750}$)] + [(A$_{682}$ − A$_{750}$)] − 0.297 × [(A$_{665}$ − A$_{750}$)] − 0.377 × [(A$_{665}$ − A$_{750}$)]

X2A: ca. 10:1 acetone:hexane
Chlorophyll a (µg/ml) = 12.00 × [(A$_{665}$ − A$_{750}$)]
Carotene (µg/ml) = 5.18 × [(A$_{665}$ − A$_{750}$)] − 0.171 × [(A$_{665}$ − A$_{750}$)]

X2B: ca. 3:1:1 acetone: methanol: water
Chlorophyll a (µg/ml) = 13.59 × [(A$_{664}$ − A$_{750}$)]
Chlorophyll c (µg/ml) = 16.08 × [(A$_{631}$ − A$_{750}$)] + [(A$_{682}$ − A$_{750}$)] − 4.82 × [(A$_{664}$ − A$_{750}$)]
Fucoxanthin (µg/ml) = 7.09 × [(A$_{670}$ − A$_{750}$)] − 8.79 × [(A$_{631}$ − A$_{750}$)] + [(A$_{682}$ − A$_{750}$)] − 0.300 × [(A$_{664}$ − A$_{750}$)] − 0.195 × [(A$_{664}$ − A$_{750}$)]

* Determination of the total chlorophyll a, chlorophyll c, fucoxanthin, and carotene of the brown seaweed was done by summing each corresponding pigment content from three different fractions.

Before analyzing the lipid class composition and fatty acid profile, lipid was firstly prepared according to the method of Bligh and Dyer\textsuperscript{14} with a slight modification. Brown seaweed powder was soaked in water with a ratio of 1:9 (w/v) for one hour. After one hour, the rehydrated brown seaweed was soaked for two hours using chloroform and methanol (1:2, v/v) to produce a ratio of chloroform:methanol:water at 1:2:0.8 (v/v/v). Seaweed extract was filtered and residue on filter paper was washed using a mixture of chloroform:methanol (1:2 v/v). The brown seaweed residue was extracted once again to produce a maximum recovery rate. The filtrate was then added by chloroform and distilled water to get the final ratio of chloroform:methanol:water at 1:1:9.9 (v/v/v). The separated organic layer was flashly evaporated using a rotary vacuum evaporator (30°C) and dried by nitrogen gas.

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flushing to yield dried lipid extract. A total lipid extraction procedure was performed in the dim light.

Lipid class composition analysis was conducted using column chromatography according to Abomohra et al.\textsuperscript{18} with a slight modification of lipid to silicic acid (particle size 0.04-0.06 mm) ratio (1:75, w/w). Brown seaweed lipid (approximately 100 mg) was dissolved in 2 mL chloroform, and fractionated by using a glass column (20 mm × 300 mm) containing 7.5 g activated silicic acid. Activation of silicic acid was done by heating overnight at 80°C. Successive applications of dichloromethane 100 mL, acetone 100 mL, and methanol 100 mL produced fractions comprising of neutral lipid, polar lipid, and phospholipid, respectively. Each collected fraction was then evaporated, and lipid class composition was determined gravimetrically. Fatty acid profile analysis was conducted by gas chromatography with a flame ionization detector. A lipid extract was firstly converted to fatty acid methyl ester (FAME) form through a specific methylation process as described by the Official Method of AOCS with heptadecanoic acid as an internal standard. FAME was then injected into the GC instrument for separation. Quantification of fatty acid was based on a comparison between sample and external standard (FAME Mix C4-24) chromatogram. The detailed chromatography condition can be seen as below:

- **Column type**: Capillary column DB-23 (30 m × 0.25 mm i.d., 0.25 µm film thickness, J & W Scientific, Folsom, CA)
- **Mobile phase**: Helium with flow rate at 11.07 mL/min and nitrogen as make up gas with a flow rate of 31.25 mL/min, so it results in total final flow at 31.3 mL/min
- **Injector temperature**: 250°C
- **Split ratio**: 30
- **Running temperature**: Initial temperature is 120°C and maintained for 6 min. Temperature is gradually raised (4°C/min) until 230°C and maintained for 25 min.
- **Detector**: FID (Flame Ionization Detector) with the temperature at 260°C, H₂ (40 mL/min) and compressed air (400 mL/min) directly flow to the detector.

### 2.5 Statistical analysis

The effect of different sample origin on brown seaweed chemical characteristics was tested by analysis of variance (ANOVA). Tukey posthoc test was used to see the differences between samples. Principal component analysis (PCA) was used to show sample discrimination based on observed chemical characteristics. Statistical analysis was performed by IBM SPSS 20 and XLSTAT 2018 application.

### 3 Results and Discussion

#### 3.1 Proximate and water-soluble components

Proximate and water-soluble components profile of brown seaweed *Sargassum cristaefolium* can be seen in Table 2. Ash content of UGB and SHB samples tended to be higher than in the AB and PI samples. Different ash levels can be affected by different water salinity conditions. The study of Sinurat et al.\textsuperscript{9} showed that the ash content of *S. polycystum* had a positive correlation with the salinity of the seaweed habitat. Southern waters of Java are reported to have a relatively higher salinity compared to the northern waters. The average salinity of the northern waters of Java is in the range 32.5 to 33‰, whereas the salinity of the southern waters can reach 33 to 34‰.\textsuperscript{20} Moreover, Shohirin et al.\textsuperscript{21} observed that the salinity range of Sayang Heulang waters reached 34-38‰.

Samples of AB and PI had higher total sugar and crude fiber than UGB and SHB. Factors that may affect these outcomes are the presence of nutrients as well as water conditions that determine nutrient transport during the growth process of the brown algae.\textsuperscript{22, 23} As reported by several studies, the current velocity of the southern water of Java is in the range of 15-45 cm/s,\textsuperscript{21, 24} while the northern water of Java has a lower current velocity ranging from 1.62 to 10 cm/s.\textsuperscript{25, 26} UGB and SHB contact directly with the Indian ocean as open sea, while PI and AB are located in the Java Sea which is recognized as closed water area. Thus, the northern waters of Java have a lower mean significant wave height and lower wind speed than the southern waters of Java.\textsuperscript{27} The condition of high current velocity and wave height can be a decreasing factor for growth and biomass production of seaweed.\textsuperscript{20}

Samples of UGB and SHB had higher total alginate and FCSPs than the AB and PI samples. Alginate and FCSPs are important constituents of the algae cell wall. Alginate fine structure together with polyphenol cross-linking are responsible to form cell wall rigidity. In addition, FCSPs strengthens the cell wall by cross-linking matrix cellulose microfibrils. Seaweed tends to accommodate the high wave and water current velocity with the tough cell wall structure. Study of Bruhn et al.\textsuperscript{20} showed that mechanical stress in the form of increased exposure (high wave and high wind speed) could increase FCSPs content in brown seaweed *Saccharina latissima* and *Laminaria digitata* growing around Aarhus water area (Denmark), although this linear relationship between FCSPs and exposure could not be generalized to all environmental condition. Besides its function in forming cell wall integrity, FCSPs is known to play an important role in regulating the cell osmotic pressure under extreme salinity conditions.\textsuperscript{28, 30} Other factors such as reproduction, season, part of the thallus, and age of plant will determine FCSPs content in algae tissue.\textsuperscript{25, 31, 32} The resulted FCSPs were analyzed for its sugar profile using HPLC-RI. Sugar profiling results on
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Table 2: Proximate and water-soluble components of S. cristaefolium from different coastal areas.

| Parameter                          | Southern Java | Northern Java | Sample origin |
|------------------------------------|---------------|---------------|---------------|
| Proximate (% db)                   |               |               |               |
| Ash                                | 33.13 ± 0.23c | 27.13 ± 0.37b | 21.02 ± 1.79a | 25.12 ± 1.32b |
| Acid insoluble ash (AIA)           | 2.58 ± 0.27c  | 0.26 ± 0.11a  | 1.81 ± 0.46b  | 1.22 ± 0.62b  |
| Acid soluble ash (ASA)             | 30.55 ± 0.39d | 26.88 ± 0.27c | 19.21 ± 1.38a | 23.90 ± 0.70b |
| Protein                            | 8.18 ± 0.36b  | 6.63 ± 0.29a  | 7.53 ± 0.70a  | 9.10 ± 0.85a  |
| Fat                                | 0.82 ± 0.19bc | 1.09 ± 0.34a  | 1.12 ± 0.03b  | 0.63 ± 0.15a  |
| Carbohydrate                       | 57.97 ± 0.32c | 64.94 ± 0.57b | 70.54 ± 1.43a | 65.08 ± 1.17b |
| Crude fiber                         | 19.67 ± 0.27c | 21.72 ± 0.07b | 24.30 ± 0.43c | 21.49 ± 0.78b |
| Total sugar (mg/100 g sample db)   | 3.81 ± 0.26a  | 4.24 ± 0.02b  | 6.00 ± 0.09b  | 4.55 ± 0.16b  |
| Total crude alginate (% db)         | 4.64 ± 0.31b  | 4.01 ± 0.28a  | 2.52 ± 0.63a  | 2.39 ± 0.52a  |
| Total FCSPs (% db)                  | 2.39 ± 0.18b  | 3.09 ± 0.54a  | 1.43 ± 0.59a  | 1.02 ± 0.29a  |
| FCSPs sugar profile (mg/g FCSPs fraction) |           |               |               |
| Glucose                            | 0.69 ± 0.49e  | 2.87 ± 0.67b  | 0.72 ± 0.69a  | 1.89 ± 0.92b  |
| Gal/Rham/Man/Xyl                   | 1.29 ± 0.16e  | 0.77 ± 0.08c  | 1.59 ± 0.20b  | 1.12 ± 0.29c  |
| Fucose                             | 15.56 ± 0.90d | 20.13 ± 1.08c | 10.21 ± 1.51c | 11.16 ± 1.08e |
| Total phenolic (mg GAE/g sample db) | 1.33 ± 0.02c  | 2.07 ± 0.08d  | 1.85 ± 0.02c  | 1.43 ± 0.05c  |

Note: Values are given as mean ± SD. Means in the same row with different superscript differ significantly (p < 0.05), n = 4.

Gal/Rham/Man/Xyl is the peak of sugar mixture containing galactose/rhamnose/mannose/xyllose.

FCSPs fraction showed that southern waters samples had a higher content of fucose in FCSPs fraction than northern waters samples.

FCSPs is reported to have many biological activities including antiviral, antithrombotic and antioxidant, antitumor, immunomodulatory, anti-inflammatory, antioxidant, hepatoprotective effect, etc. FCSPs functionality will depend on monosaccharide sequences, sulfation levels, and connectivity of sulfate groups. Alginate, which is commonly used as thickener, gelling agent, and stabilizer in the food and pharmaceutical industry, also exhibits several biological activities, such as anti-inflammatory, tissue regeneration, antibacterial, antifungal, antiviral, anticoagulant, antioxidant, prebiotic activity, etc.

S. cristaefolium of this study had a higher total phenolic content (TPC) than S. vulgare (0.512 ± 0.05 mg gallic acid equivalent (GAE)/g) and S. binderi (0.369 ± 0.007 mg GAE/g). Budhiyanti et al. showed that TPC of Sargassum sp. from Gunung Kidul - Yogyakarta (southern waters of Java) was higher than Jepara sample (northern waters of Java). Several factors that may affect the seaweed phenolic compounds are seaweed maturity, seawater chemistry, differences in hydrodynamic or radiation conditions, different grazing pressure, and variations in energy allocations between the life stage. Sargassum phenolic compound, primarily phlorotannin, is reported to have diverse biological activities, such as anti-inflammatory, anti-allergic, antiviral, anticancer, antibacterial, antioxidant, anti-diabetic activities, and also radioprotective effects.

Principal component analysis (PCA) was actually carried out on the combined data of water and lipid-soluble components (data are not shown) and the separated chemical component groups. Since the discrimination patterns could be better defined in the separate analysis results, so the discussions of PCA results were divided into two different sessions based on their solubility (water and lipid-soluble component profile). PCA biplot result on the water-soluble component (Fig. 1) shows that samples from four different coastal areas are clearly distinguished each other (variance: 80.37%). The major contributors of principal component I/F1 were ash, carbohydrate, ASA, crude fiber, total sugar, total alginate, total FCSPs, and fucose content of FCSPs fraction, while the major contributor of F2 was AIA, TPC, glucose, and Gal/Rham/Man/Xyl mixture of FCSPs fraction. Determination of major contributor was based on a percentage of variable contribution (6.57-14.98%) and squared cosine value (0.421-0.777). AB and PI samples originated from northern water of Java are located at negative F1 factor score range, while the others are located at the positive F1 factor score range. SHB and UGB samples were characterized by a higher content of ash, alginate, FCSPs, and fucose content of FCSPs fraction, whilst AB and PI...
samples were characterized by a higher content of carbohydrate, crude fiber, and total sugar. UGB and SHB are located at different F2 axis range, because SHB had a higher content of glucose and TPC than UGB, while UGB had a higher content of AIA.

3.2 Lipid-soluble components

The pigment spectrum (350-750 nm) of *S. cristaefolium* from different water areas can be seen in Fig. 2. All observed samples had almost similar peak patterns distributed at some wavelength, i.e. 412-414 nm, 533.5-535.5 nm, 582-583.5 nm, 610.5-616.5 nm, and 664.5-665 nm. As an exception, SHB sample had an obvious form of the shoulder at 452 nm, while samples of AB, UGB, and PI had the peak/shoulder form at around 430-433 nm. All samples are shown to have a narrow peak at a wavelength of 664.5-665 nm in acetone 90%. This peak represents chlorophyll a and chlorophyllide a. Other peaks formed in the spectrum curve represent chlorophyll b and chlorophyllide b at around 412-414 nm and 430-433 nm, chlorophyll a, pheo-
Table 3  Lipid-soluble components of *S. cristaefolium* from different coastal areas.

| Parameter                          | Southern Java | Northern Java | Sample origin |
|------------------------------------|---------------|---------------|---------------|
|                                   | Ujung genteng | Sayang Heulang | Awur Bay  | Pari Island |
| Chlorophyll a                      | 152.03 ± 9.64\(^a\) | 169.76 ± 0.76\(^b\) | 322.81 ± 10.36\(^c\) | 146.19 ± 5.41\(^a\) |
| Chlorophyll c                      | 35.89 ± 0.89\(^b\) | 48.77 ± 0.66\(^b\) | 80.76 ± 0.91\(^d\) | 34.02 ± 0.50\(^b\) |
| Fucoxanthin                        | 42.97 ± 0.82\(^a\) | 68.96 ± 1.51\(^b\) | 98.92 ± 0.53\(^c\) | 44.26 ± 0.99\(^a\) |
| β-carotene                         | 1.80 ± 0.08\(^a\) | 4.56 ± 0.02\(^b\) | 3.64 ± 0.16\(^c\) | 2.22 ± 0.11\(^b\) |
| Total lipid (% w/w sample db)      | 2.76 ± 0.28\(^a\) | 3.55 ± 0.29\(^b\) | 4.32 ± 0.09\(^c\) | 2.73 ± 0.19\(^a\) |
| Lipid class composition (% w/w total lipid) |              |               |               |               |
| Neutral lipid                      | 11.67 ± 1.01\(^ab\) | 9.70 ± 1.33\(^a\) | 13.37 ± 1.12\(^b\) | 12.45 ± 0.83\(^b\) |
| Glycolipid                         | 54.15 ± 1.91\(^a\) | 55.52 ± 2.94\(^a\) | 54.83 ± 0.67\(^a\) | 53.31 ± 0.82\(^a\) |
| Phospholipid                       | 34.17 ± 1.42\(^a\) | 34.78 ± 2.79\(^b\) | 31.80 ± 1.76\(^b\) | 34.25 ± 0.40\(^a\) |
| Total fatty acids (mg/g extracted lipid) | 462.75 ± 149.8\(^b\) | 438.58 ± 18.64\(^b\) | 506.26 ± 36.47\(^c\) | 398.38 ± 26.07\(^a\) |
| Major fatty acids with n-6 and n-3 fatty acids content (% w/w total fatty acids) |              |               |               |               |
| C14:0                              | 3.78 ± 0.15\(^b\) | 2.72 ± 0.11\(^b\) | 2.94 ± 0.12\(^c\) | 3.96 ± 0.08\(^b\) |
| C16:0                              | 31.32 ± 0.43\(^c\) | 25.99 ± 1.59\(^b\) | 28.52 ± 0.90\(^a\) | 35.37 ± 0.49\(^d\) |
| C16:1                              | 6.43 ± 0.19\(^b\) | 5.10 ± 0.22\(^a\) | 6.12 ± 0.16\(^b\) | 6.97 ± 0.16\(^a\) |
| C18:1n9c                           | 11.10 ± 0.34\(^c\) | 9.74 ± 0.64\(^b\) | 8.50 ± 0.08\(^c\) | 10.25 ± 0.09\(^b\) |
| C18:2n6c                           | 5.42 ± 0.07\(^b\) | 4.74 ± 0.23\(^c\) | 4.78 ± 0.02\(^b\) | 4.65 ± 0.10\(^b\) |
| C18:3n6                            | 1.07 ± 0.04\(^b\) | 0.70 ± 0.03\(^a\) | 0.72 ± 0.02\(^b\) | 0.70 ± 0.06\(^a\) |
| C18:3n3                            | 3.40 ± 0.15\(^a\) | 7.17 ± 0.25\(^b\) | 5.15 ± 0.05\(^b\) | 3.24 ± 0.02\(^a\) |
| C20:2n6                            | 0.31 ± 0.01\(^c\) | 0.23 ± 0.02\(^c\) | 0.23 ± 0.01\(^b\) | 0.28 ± 0.01\(^b\) |
| C20:3n6                            | 0.42 ± 0.01\(^a\) | 0.54 ± 0.04\(^b\) | 0.50 ± 0.02\(^b\) | 0.49 ± 0.01\(^b\) |
| C20:4n6                            | 15.20 ± 0.41\(^b\) | 16.73 ± 0.99\(^b\) | 17.23 ± 0.56\(^a\) | 13.43 ± 0.20\(^a\) |
| C20:3n3                            | 0.64 ± 0.02\(^a\) | 0.82 ± 0.04\(^b\) | 0.81 ± 0.03\(^b\) | 0.05 ± 0.06\(^b\) |
| C20:5n3                            | 6.93 ± 0.25\(^b\) | 7.51 ± 0.28\(^c\) | 7.26 ± 0.26\(^c\) | 4.61 ± 0.07\(^a\) |
| C22:6n3                            | 0.42 ± 0.04\(^b\) | 0.22 ± 0.02\(^b\) | 0.26 ± 0.02\(^b\) | 0.43 ± 0.04\(^b\) |
| Unidentified FA                    | 8.97 ± 0.55\(^a\) | 14.68 ± 4.74\(^b\) | 13.14 ± 0.41\(^b\) | 11.82 ± 0.27\(^ab\) |
| Total SFA                           | 37.88 ± 0.90\(^c\) | 30.37 ± 1.94\(^b\) | 33.35 ± 0.91\(^b\) | 41.73 ± 0.53\(^d\) |
| Total MUFA                          | 19.35 ± 0.34\(^b\) | 16.31 ± 0.97\(^c\) | 16.57 ± 0.10\(^b\) | 18.58 ± 0.21\(^b\) |
| Total PUFA                          | 33.80 ± 0.84\(^a\) | 38.65 ± 1.88\(^b\) | 36.93 ± 0.93\(^c\) | 27.88 ± 0.40\(^a\) |
| n-6 PUFA                           | 22.42 ± 0.45\(^b\) | 22.93 ± 1.30\(^c\) | 23.46 ± 0.60\(^b\) | 19.54 ± 0.31\(^a\) |
| n-3 PUFA                           | 11.38 ± 0.40\(^b\) | 15.72 ± 0.58\(^d\) | 13.47 ± 0.35\(^c\) | 8.33 ± 0.14\(^a\) |
| Ratio n-6/n-3                       | 1.97 ± 0.03\(^c\) | 1.46 ± 0.03\(^b\) | 1.74 ± 0.02\(^b\) | 2.34 ± 0.04\(^d\) |

Note: Values are given as mean ± SD. Means in the same row with different superscript differ significantly (*p* < 0.05), n=4.

phytins a and pheophorbide a at around 533.5-535.5 nm, chlorophyll a, chlorophyllide a, and chlorophyll c1 + c2 at around 582-583.5 nm, and chlorophyll a, chlorophyllide a, pheophorbide a, and pheophytin a at around 610.5-616.5 nm\(^4\). The obvious form of shoulder found in the SHB sample at 452 nm may represent β-carotene, trans-fucoxanthin, or some xanthophyll such as zeaxanthin, flavoxanthin, and β-cryptoxanthin\(^4, 11, 42\).

According to visible spectrum observation of pigment extracts, pigment intensity could follow these trends: AB > SHB > P1 and UGB. This qualitative pigment analysis was in accordance with semi-quantitative analysis (Table 3),
showing that AB samples had the highest content of total chlorophyll (chlorophyll a + chlorophyll c) and fucoxanthin content. The shoulder form at 452 nm found in the SHB sample’s spectra became the most significant differentiator from other samples. Semi-quantitative pigment analysis showed that SHB samples had the highest content of β-carotene found in hexane fraction (4.56 µg pigment/sample db). Based on visible observation, lipid and pigment extract of SHB sample had a distinct brownish-green color, while other extracts had a solid green color.

The cause of inferior pigment concentration of the samples, especially UGB and PI, may be ascribed to the different blade size and morphological appearance that may also relate to different life stage/maturity, although there are other possible factors such as nutrient and salinity\(^\text{43}\). Gerasimenko et al.\(^\text{44}\) revealed that the content of photosynthetic pigments (carotenoids and chlorophylls) in brown seaweed Costaria costata increased with age. Pigment content variation of seaweed is affected by some factors, such as physiological status, season, and environmental parameters like temperature, pH, salinity, dissolved oxygen, NO\(_3\), NO\(_2\), NH\(_4\), total nitrogen, and total phosphorus\(^\text{45, 46}\). Some natural pigments derived from marine algae exhibit several biological activities, including antioxidant, anticancer, anti-inflammatory, anti-obesity, anti-angiogenic, neuro-protective activities, and etc\(^\text{47}\).

The total lipid (TL) content range observed of S. cristaefolium was 2.73-4.32% db. Glycolipid was found as a major lipid class (53.31-55.52% in TL) in all samples, followed by phospholipid (31.80-34.78% in TL) and neutral lipid (9.70-13.37% in TL). According to the fatty acid profile result, all samples had similar fatty acid distribution although they had marked morphological variations. The observed predominant fatty acids were palmitic (16:0), palmitoleic (16:1), oleic (18:1n9), linoleic (18:2n6), linolenic (18:3n3), arachidonic/ARA (20:4n6), and eicosapentaenoic acid/EPA (20:5n3). Palmitic acid was observed to be the most abundant in Sargassum lipids\(^\text{48, 49}\). A low amount of docosahexaenoic acid/DHA (22:6n3) was found in the present study, and it is also reported by other research groups\(^\text{45, 50, 51}\).

In this study, we could not determine stearidonic acid (18:4n3) content which is described as another predominant fatty acid in brown seaweed due to a different type of external standard used. Supelco FAME Mix C4-24 as reference in this study does not contain methyl ester form of C18:4n3. According to all samples’ chromatogram results, we found a typical peak having quite significant intensity (high %area) which emerged at a retention time around the 27\(^\text{th}\) minute. This peak was located between C18:3n-3 and C20:0. This peak might be predicted as C18:4n3, because this is related to the characteristic of seaweed lipid which typically contains stearidonic acid as reported by several studies\(^\text{5, 10, 52}\). Noviendri et al.\(^\text{53}\) reported C18:4n3 content of Sargassum duplicatum which was analyzed by GC-FID system with capillary column Omega- wax 320 (30 m × 0.32 mm i.d.; Supelco, Bellefonte, PA). They found that the peak of C18:4n3 was located between C18:3n-3 and C20:0. In addition, Lang et al.\(^\text{54}\) attributed the elution of C18:4n3-methyl ester after C18:3n3-methyl ester in the FAME mixture of marine microalgae, which was analyzed by GC-FID with a capillary DB-23 column (30 m × 0.25 mm, 0.25 µm coating thickness, J&W, Scientific, Agilent, Waldbronn).

The ratio of n-6 to n-3 of observed samples was in the range of 1.46-2.34. According to WHO\(^\text{55}\), the ratio of n-6 to n-3 should not exceed 10, as it can prevent the risk of inflammatory, cardiovascular disease (CVD), and neurological disorders. PUFA/SFA ratio of our samples was about 0.67 (PI) - 1.27 (SHB). British Department of Health & Social Care\(^\text{56}\) recommended minimum value of PUFA/SFA to be 0.45. The inclusion of brown seaweed lipids in dietary fat intake will be beneficial in getting proportional quality lipid, primarily lipid containing important eicosanoid precursors such as EPA and ARA. EPA-DHA and ARA are recognized as the active form of LNA (18:3n3) and LA (18:2n6). ARA plays an important role in the body’s immune response, thrombosis, proper muscle, and brain function\(^\text{6}\). EPA and DHA are important for fetal development and proper function of cardiovascular, immune, and cognitive system\(^\text{57}\). Because of human poor ability to convert LNA and LA to their active forms, the dietary intake of EPA, DHA, and ARA become necessary to promote human health.

PCA biplot result of lipid-soluble components (Fig. 3) shows that AB and SHB samples are clustered together in close proximity, whilst PI and UGB samples are located in the opposite F1 score range (positive factor score). This result was not in line with the previous findings on the trend of water-soluble component profile. Based on morphological observation (Table 1), AB and SHB had a larger blade size than PI and UGB. Referring to the squared cosine value and percentage of variable contribution, AB and SHB samples which have larger blade size were characterized by a higher content of total lipid, pigment, C18:3n-3, C20:4n6, C20:5n3, total PUFA, and lower n-6/n-3 ratio, while samples with smaller blade size (PI and UGB) were characterized by a higher content of saturated fatty acids (C14:0, C15:0, C16:0, C18:0, C20:0), some monounsaturated fatty acids (C16:1, C17:1, C18:1n9, C22:1n9), C20:2, and C22:6n3. Although PI and UGB samples are grouped together in the positive F1 factor score range, they are both clustered at a considerable distance with different range of F2 factor score. UGB samples were distinguished from PI samples because of their higher content of C12:0, C18:2n6, C18:3n6, C20:1n9, C20:5n3, and total PUFA.

From PCA results, we found a positive correlation between pigment content (chlorophyll a, chlorophyll c, fu-
coxanthin, and carotenoid) and some PUFAs, such as C18:3n3, C20:3n3, ARA, and EPA. Both PUFA and pigment are integrated parts of the thylakoid membrane in the plastid/chloroplast. The content of plastid constituents will increase with age because this structure is responsible for energy production. In addition, the blade is a vital organ for the photosynthetic process, thus the increase of its proportion in plant structure will increase the amount of pigment, PUFA, and ratio n-3:n-6. Other factors affecting seaweed lipid and fatty acid composition are genetic, hydrodynamic condition, light intensity, temperature, annual cycle, salinity, and mineral content of medium. In S. piluliferum, increasing salinity was associated with increasing levels of C18:4n3, C20:4n6, C20:5n3, total n-3, and total n-6 PUFAs.

Fig. 3 PCA biplot of the lipid-soluble component of dried seaweed S. cristaefolium from four different coastal areas (n per coastal area = 4). Note: SHB = Sayang Heulang Beach, PI = Pari Island, AB = Awur Bay, UGB = Ujung Genteng Beach.

4 Conclusion
In this study, we observed that samples of SHB and UGB had high contents of ash, alginate, and FCSPs yield, and fucose, while AB dan PI samples showed a high amount of total sugar and crude fiber. Different discrimination pattern was found in PCA result on the lipid-soluble component. Blade size seemed to affect the lipid-soluble component profile. The higher amount of pigment content, PUFA, n-3, and n-6 fatty acids, and a lower ratio of n-6 to n-3 fatty acids can be found in SHB and AB samples, which have relatively larger blade size than PI and UGB. These chemical characteristics of tropical brown seaweed S. cristaefolium can be the baseline information for further development of this seaweed as a source of the functional ingredient, especially in food, pharmacy, and nutraceutical.

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Conflict of Interests
Authors declare that there is no conflict of interest.

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