

RESEARCH ARTICLE

Molecular Identification, Nutritional Profile and Heavy Metals Content of Edible *Caulerpa* from Binuangeun Coast, Banten

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Abstract

Two species of *Caulerpa*, locally known as “Pedesan” and “Latuh” have been traditionally consumed by coastal communities at Binuangeun, Banten. This study aimed to identify “Pedesan” and “Latuh” using the DNA barcoding method and to evaluate their nutrient and heavy metal contents. Fatty acids were determined by Gas Chromatography Flame Ionization Detector (GC FID), amino acids using Ultra Performance Liquid Chromatography (UPLC), and minerals using Inductively Coupled Plasma Optical Emission Spectrometry (ICP OES). Based on the *tufA* gene sequences, the “Pedesan” was identified as *Caulerpa racemosa* var. *macrophysa* and “Latuh” as *Caulerpa chemnitzia*. Thirteen fatty acids were detected in *C. racemosa* var. *macrophysa* and twelve fatty acids in *C. chemnitzia*. Of the total fatty acid content, *C. racemosa* var. *macrophysa* contained 41.0% unsaturated fatty acids, dominated by linolenic acid and eicosapentaenoic acid. Meanwhile, *C. chemnitzia* contained 47.5%, dominated by oleic acid. Both seaweeds contained fatty acids with the $\omega 6/\omega 3$ ratio lower than 10, which could prevent heart disease risk based on World Health Organization (WHO) recommendation. The primary amino acids content in *C. racemosa* var. *macrophysa* were glutamic acid, alanine, serine and aspartic acid, while those in *C. chemnitzia* were glutamic acid, serine, aspartic acid, and glycine. The high content of glutamic acid in both samples indicated their potential use as food flavor enhancer. The Na/K ratio of *C. racemosa* var. *macrophysa* (40.31) and *C. chemnitzia* (27.48) were higher than those recommended by WHO. Heavy metals were not detected in either “Pedesan” nor “Latuh”, indicating that they are safe for consumption.

Keywords: *Caulerpa*, sea grapes, DNA barcoding, nutritional profile

Introduction

Sea grapes belonging to the genus *Caulerpa*, is an economically important commodity in Asia and Pacific countries. This particular seaweed have been traditionally consumed by the local community (Lako, 2012). In Indonesia, *Caulerpa* is utilized for local consumption and international trade. In Takalar (South Sulawesi) and Buleleng (Bali), sea grapes have been cultured in ponds and the harvested products are exported to Japan. *Caulerpa* is also available almost around the year in Binuangeun Coast, Banten. Among others, only two species of *Caulerpa* in Binuangeun that have been consumed traditionally and traded in the local markets. These *Caulerpa*, locally known as “Pedesan” and “Latuh”, are consumed raw as vegetables or salad. Both *Caulerpa* have a crunchy texture, fresh, and delicious, however “Pedesan” has

a pungent spicy after taste. The higher consumer preference of “Latuh” than “Pedesan” resulting to the higher economic value of the first than the latter.

Some studies on edible *Caulerpa* from the Binuangeun coast have been reported previously, i.e. metabolite (Januar, Wikanta, & Hastarini, 2004; Sihono, Tarman, Madduppa, & Januar, 2018) and nutrition profiles (Putri, Jusadi, Setiawati, & Sunarno, 2017; Suryaningrum & Samsudin, 2019) as well as antioxidant activities (Nursid et al., 2016; Sihono et al., 2018). However, studies on fatty acids, amino acids, heavy metals, and molecular identification of these seaweeds have never been reported yet.

The taste of seaweeds is mostly affected by their nutrient contents. The umami taste in seaweeds is due to amino acid and glutamate contents. Whereas their sourness, sweetness, and saltiness are due to the

contents of acid, sugar, and mineral, respectively (Keast & Contanzo, 2015). Accordingly, it is essential to analyze the nutrient contents of “Pedesan” and “Latuh” from Binuangun. The data will be useful to find the correlation between the taste and the nutrient contents.

Macroalgae are primarily rich in macronutrients (carbohydrates, lipids, and proteins), micronutrients (vitamins and minerals), as well as bioactive compounds such as antioxidants. However, there are some variations on their nutritional content depending on the environmental conditions, harvesting time, species, and their stage of life cycle (Gaillande, Payri, Remoissenet, & Zubia, 2017). *C. racemosa* and *C. lentillifera* contain 60.8% polyunsaturated fatty acids (PUFAs) of the total fatty acids (Gaillande et al., 2017). *Caulerpa* contain high amounts of ω -3 fatty acids, such as α -linolenic acid (18:3n-3), stearidonic acid (18:4n-3), and eicosapentaenoic acids/EPA (20:5n-3) (Nagappan & Vairappan, 2014). They also contain ω -6 fatty acids, i.e. linoleic acid (18:2n-6), arachidonic acid (20:4n-6) (Nagappan & Vairappan, 2014) and docosahexaenoic acid/DHA (Magdugo et al., 2020). Among others, DHA and EPA play a crucial role in the functioning and developing nervous system and brain. They also prevent cardiovascular diseases (Ginneken, Helsper, Visser, Keulen, & Brandenburg, 2011), thus may contribute to the worldwide increase in life expectancy (Lutz, Sanderson, & Scherbov, 2008).

Edible *Caulerpa* contains high amount of mineral as indicated by the high ash content reaching 55% of its dry matter (Gaillande et al., 2017). *Caulerpa* is rich in macro (Ca, K, Mg, and Na) and trace/micro (Cu, Fe, Mo, Mn, Se and Zn) minerals. These constituents are essential for human health. Trace minerals act as cofactor enzymes in metabolism and physiological processes of the body. The symptoms of mineral deficiency and severe physiological as well as metabolic impairment could be resulted from inadequate supply of mineral intake (Santoso, Gunji, Yoshie-Stark, & Suzuki, 2006; Sousa, Moutinho, Vinha, & Matos, 2019).

This research aimed to identify two edible *Caulerpa* species from Binuangun Coast, Banten using the DNA barcoding method. Additionally, we aimed to evaluate their fatty acid, amino acid, mineral, and heavy metal contents.

Material and Methods

Sample Collection

“Pedesan” and “Latuh” were collected on August 2019 during the lowest tide from the intertidal zone (Lat. 6° 50' 5" S, Long. 105° 53' 22" E). Dirt, debris,

and impurities were removed by washing using seawater, while epiphytes attached to the samples were removed by hand. Clean collected samples were placed in a plastic bag and stored in cold storage prior to nutrition and heavy metals analyses. Samples fragments for DNA analysis were placed in a ziplock plastic bag with excess amount of silica gel. The samples were then stored at room temperature prior to analysis. The morphological observation was conducted based on their physical appearance (i.e., the shape and size of ramuli, stolon and their typical branches).

DNA Extraction

DNA extraction was done using modified CTAB method described in Zuccarello et al. (2006). Three dried fragments of each *Caulerpa* sample (approximately 10 mg) were placed in a tube containing 500 μ L of CTAB lysis buffer, 80 μ g Proteinase K (Thermo Fisher Scientific) and 50 μ g RNase A (Qiagen). Samples were ground using a pestle. As much as 50 μ L of 0.1X TE buffer (0.1 Mm EDTA, 1 mM Tris pH 8) was added into the isolated DNA and the samples were stored at -20 °C before use.

Polymerase Chain Reaction (PCR) Amplification

Amplification of *tufA* gene by PCR (Biometra TProfessional Thermocycler) was conducted in a 25 μ L master mix containing 12 μ L PCR buffer KOD (Toyobo), 5 μ L 2 nM Taq polymerase dNTPs (Toyobo), 0.5 μ L KOD FX Neo (Toyobo), 4.5 μ L UPW water and 10 pmol of primers using *tufA* forward: (5'-GGNGCNGCNCAAATGGAYGG-3') and *tufA* reverse (5'-CCTTCNCGAATMGCRAAWCGC-3') (Fama, Wysor, Kooistra, & Zuccarello, 2002).

The touchdown PCR program was run at an initial denaturation process of 94 °C for 4 min; followed by 37 cycles of 94 °C for 30 s, 45 °C for 1 min and 72 °C for 1 min, and final elongation process of 72 °C for 5 min. The quality of PCR products (correct size and adequate concentration) was checked using 2% agarose gel electrophoresis. The PCR product with correct size and adequate concentration of each sample was purified and Sanger sequenced at 1st Base Singapore. The sequences have been submitted into the NCBI database with the accession number as follow: *Caulerpa racemosa* var. *macrophysa*: MZ733614; *Caulerpa chemnitzia*: MZ733615.

Phylogenetic Analysis

DNA sequences from 1st Base Singapore were edited and assembled in both directions using Geneious

Prime (<https://www.geneious.com>) software. Sequence similarity analysis for each sample was compared to the representative *Caulerpa* species from the National Centre for Biotechnology Information (NCBI) using the BLAST (Basic Local Alignment Search Tool) algorithm. The samples and representative species were subjected to Clustal W alignment.

Phylogenetic analysis of alignment sequence was carried out by A neighbor-joining (N-J) Method. The N-J tree was constructed by MEGA 7 with 1,000 bootstrap replications and calculated using the model of Kimura two-parameter. A sequence of *Caulerpa* (*Caulerpella ambigua*, GenBank Accession Number KM 186522.1) was used as an outgroup.

Fatty Acid Content Analysis

Fatty acid analysis was carried out in duplicate. Lipid was extracted using the soxhlet method (AOAC, 2005). Fresh *Caulerpa* was ground and about 2 g sample was put into a thimble. The thimble was then put into the Soxhlet apparatus with a heating mantle temperature of 65 °C. Lipid extraction was carried out with hexane for 6 h. The hexane was then distilled and the obtained extract was dried in an oven at 105 °C. The lipid obtained was used for fatty acid analysis. Conversion of fatty acids to fatty acid methyl ester (FAME) was done using the method of Park & Goins (1994). About 1.5 mL 0.5 M KOH/methanol (Merck) was added into about 0.03-0.04 g lipid heated at 100 °C for 20 min. The solution was then mixed with 1.5 mL of 20% BF₃/methanol (Merck) and reheated at 100 °C for another 20 min. Subsequently, 3 mL of a saturated solution of NaCl and 2 mL hexane (Merck) were added to the solution. The hexane layer containing FAME was separated from the water layer and was added 0.1-0.2 g anhydrous Na₂SO₄ (Merck) for 15 min. The solution was analyzed by GC coupled to FID (Agilent Technologies). The instrument default parameters for determination of fatty acids content were: Column: Supelco SPTM 2560 100 m x 0.25 mm x 0.2 µm; Flow rate: 18.0 cm/sec with column length 100 m; gas carrier: N₂; FID Detector temperature: 240 °C; injector temperature: 225 °C; Split: 1:100. The fatty acid content were calculated in dry weight (DW) (moisture data is not shown).

Amino Acids Content Analysis

Amino acid analysis was carried out in duplicate. The samples were prepared using the method of Waters (2012). As much as 5 mL HCl (Merck) 6N was added onto 0.1 g fresh *Caulerpa*, vortexed, then hydrolyzed at 110 °C for 22 h. After cooling down, the mixture was transferred into a volumetric flask of 50 mL, added with aquabidest to 50 mL. The resulting mixture was

filtered using a 0.45 µm filter. The filtrate (500 µL) was added 460 µL aquabidest and 40 µL AABA. About 10 µL of the solution was added 20 µL reagent fluor A and 70 µL AccQ Fluor Borate then vortexed for 1 min. Before injected into the UPLC system, the solution was incubated at 55 °C for 10 min.

Amino acid content was analyzed using a Waters Acquity UPLC system (Waters, USA). The system was equipped with a tandem quadrupole detector, an autosampler, a binary solvent manager, a PDA detector, and a column heater. The AccQ.Tag Ultra C₁₈ 1.7 µm (2.1 x 100 mm) was used as the separation column. The column heater was set at 49 °C, and the mobile phase flow rate was maintained at 0.5 mL/min. The PDA detector was set at 260 nm, and the injection volume of sample was 1 µL (Tesevic et al., 2014). The amino acid content were calculated in dry weight (DW) (moisture data is not shown).

Mineral and Heavy Metal Content Analyses

Mineral and heavy metal analyses were carried out in duplicate using ICP OES (Perkin Elmer, USA) (Aroyehun, Palaniveloo, Ghazali, Iddid, & Razak, 2019) with slight modification. About 0.5–1.0 g fresh *Caulerpa* was subjected to wet hydrolysis using a vessel containing 5–10 mL HNO₃ (Merck) and digested in Anton Paar microwave. The temperature was raised to 150 °C for 10 min, then hold at 150 °C for 15 min. Digested samples were filtered and then diluted with aquabidest to a volume of 50 mL and injected into ICP OES.

The ICP OES conditions used in this analysis were: RF power (emission intensity): 1300 W; Nebulizer type: concentric glass; Plasma gas flow: 10 L/menit; Auxilliary gas flow: 0.5 L/menit; Nebulizer flow: 0.6 L/menit; Pump speed: 18 RPM; Stabilization Time: 15 s; Flush Time: 15 s; Rinse Time: 5 (manual), 60 (autosampler). The mineral and heavy metal contents were calculated on a dry basis (moisture data is not shown).

Results and Discussion

Species Morphology Observation and Identification

Our current study observed morphological description based on the physical and visual comparison between “Latuh” and “Pedesan” (Figure 1). The morphological observation showed that “Latuh” has a small, trumpet-shaped, and crowded ramuli with radial arrangement as well as unbranched stolon. Whereas, “Pedesan” has a big, spherical-shaped, uncrowded to crowded ramuli with irregular arrangement and

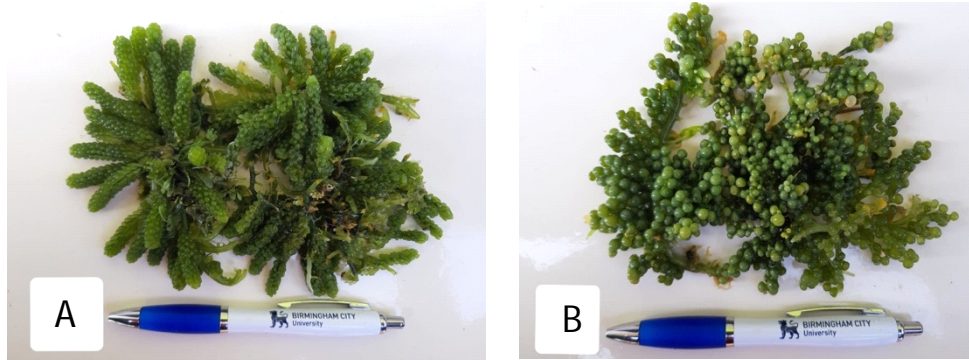


Figure 1. Edible *Caulerpa* from Binuangeun Coast, locally named as: (A) “Latuh”; (B) “Pedesan”.

branched stolon. These morphological variations of *Caulerpa* may be affected by physicochemical factors in their environments, i.e., water temperature, depth, light exposure, and salinity (Estrada, Bautista, & Dionisio-sese, 2020; Garcia, Cortes, Alvarado, & Ruiz, 2011).

Previous morphological identification on *Caulerpa* samples from North Minahasa was carried out by Pulkadang, Keppel, & Gerung (2013). They reported that seven species were identified as *C. racemosa*, *C. racemosa* var. *macrophysa*, *C. sertularioides*, *C. taxifolia*, *C. serrulata*, *C. lentillifera*, and *C. peltata*. The morphological description of *C. racemosa* var. *macrophysa* was similar to that from Binuangeun.

A morphological identification of macroalgae may have complications because multiple morphotypes of macroalgae can be found on the same thallus (Ohba & Enomoto, 1987; Belton, Prud’homme, Huisman, Draisma, & Gurgel, 2014). It remains unclear how ecological factors (e.g., substratum, water motion, temperature, light intensity, or other factors) trigger

the expression of variable morphologies (Coppejans & Prud’homme, 1992).

Species identification lies primarily on the morphological variation of the assimilators, but recent systematic investigations have relied upon DNA sequences to elucidate biodiversity (Fama et al., 2002; Belton et al., 2014). The morphology of *Caulerpa* species can be analyzed based on the thallus structure of their rhizoids, stolons, fronds (the upright assimilators), and branchlets. However, the results were occasionally ambiguous due to the environmentally-induced plasticity of macroalgae phenotypic characters. As a result, classification based on phenotype features produced a large number of species variations with different varieties and subspecies (Calvert, Dawes, & Borowitzka, 1976).

Therefore, ongoing efforts to identify macroalgae are continually improved. Recently, the identification of macroalgae can be carried out using a molecular method to compliment the morphological observation. Previous study has used 18S rRNA genes to identify

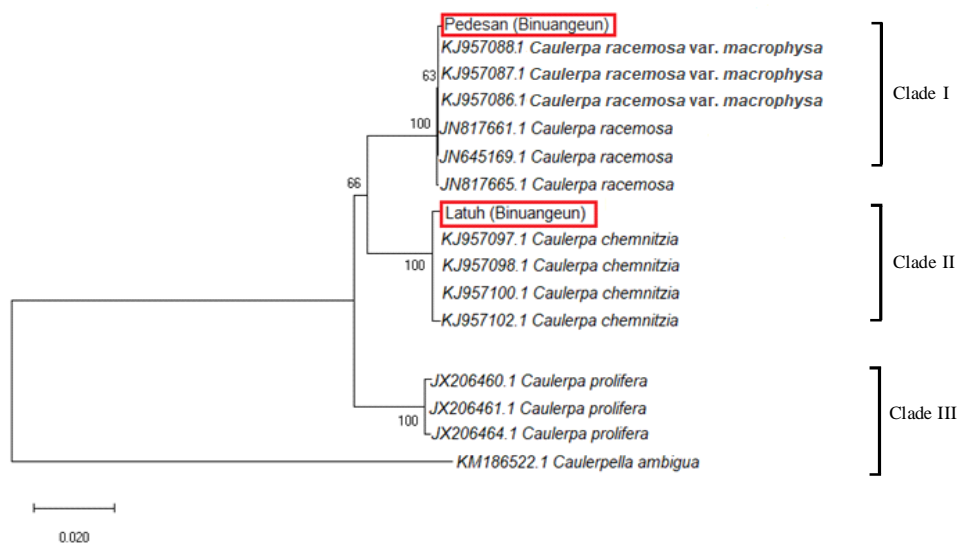


Figure 2. Neighbor-joining tree based on *tufA* sequences of edible *Caulerpa* samples from Binuangeun (“Pedesan” and “Latuh”).

C. sertularioides var. *longipes* and *C. taxifolia* from Mandangin Island, Madura (Zaw et al., 2020).

In this study, the Neighbor-Joining (NJ) tree of *tufA* gene was built with 13 closely related *tufA* sequences of *Caulerpa* from GenBank. The NJ tree (Figure 2) precisely clustered “Pedesan” and “Latuh” into three different clades. “Pedesan” was clustered with *C. racemosa* var. *macrophysa* and *C. racemosa*, whereas “Latuh” was clustered with *C. chemnitzia*. Based on the phylogenetic tree of *tufA* gene, “Pedesan” was identified as *C. racemosa* var. *macrophysa* and “Latuh” as *Caulerpa chemnitzia*. *C. racemosa* var. *macrophysa* in the same clade with *C. racemosa* (Clade I) indicated morphological variations of a single species. The ecological phenotypes considered some of the varieties and forms of *C. racemosa* (Peterson, 1972).

Fatty Acid Composition

In current work, the lipid contents of *C. racemosa* var. *macrophysa* and *C. chemnitzia* were 4.4% (DW) and 12.25% (DW), respectively. Gaillande et al. (2017) reported that *C. cupressoides*, *C. lentillifera*, *C. racemosa*, *C. racemosa* var. *clavifera*, *C. racemosa* var. *laetevirens*, *C. racemosa* var. *turbinata*, *C. sertularioides*, and *C. taxifolia* have low contents of lipid (0.1–7.2% of DW). Other reports stated that *C.*

racemosa contain 1.03–2.21% (DW) (Hao et al., 2019) and 4.20% (DW) of lipid (Aroyehun et al., 2020). However, Rameshkumar, Ramakritinan, Eswaran, & Yokeshbabu (2013) observed that the lipid content of *Caulerpa* was 19.1% (DW). These variation of lipid contents presumably caused by environmental factors, such as season, salinity, habitat, and genetic differences (Sanchez-Machado, Lopez-Cervantes, Lopez-Hernandez, & Paseiro-Losada, 2004).

In this study, thirteen fatty acids (FA) were detected in *C. racemosa* var. *macrophysa*, while only twelve were found in *C. chemnitzia*. The composition of FA in *C. racemosa* var. *macrophysa* and *C. chemnitzia* is presented in Table 1. The *C. racemosa* var. *macrophysa* contained 59.0% saturated fatty acids (SFA), 9.4% monounsaturated fatty acids (MUFA), and 31.6% polyunsaturated fatty acids (PUFA). *C. chemnitzia* contained 52.5% SFA, 26.0% MUFA, and 21.5% PUFA. Palmitic acid (C16:0) was the primary FA in both species, i.e. 41.0±0.1% in *C. racemosa* var. *macrophysa* and 38.6±0.2% in *C. chemnitzia*.

A similar FA profile of *C. racemosa* from Kinabalu, Sabah, Malaysia has been reported. The PUFA content of Malaysian *C. racemosa* was 11.25-31.11 % of total FA, mainly composed of α -linolenic acid (C18:3 ω 3) (Gaillande et al., 2017). Cultivated *C. racemosa* from Malaysia (Aroyehun et al., 2020) and Australia (Paul, Neveux, Magnusson, & de Nys, 2014) as well as a wild species from India (Kumar, Gupta, Kumari, Reddy, & Jha, 2011) had similar FA profiles with our finding. Nagappan and Vairappan (2014) also reported that *C. racemosa* contained 57.83-66.31% SFA, 12.25-12.9% MUFA and 29.68-30.27% PUFA. Both *C. racemosa* var. *macrophysa* and *C. chemnitzia* contained omega-3 FA (EPA and DHA). As shown in Table 1, the first has higher contents of EPA and DHA than the latter.

Furthermore, the ratio of ω 6/ ω 3 is also important to determine the health benefit of FA content in a diet. The low ratio ω 6/ ω 3-diet reduces low-density lipoprotein (LDL) content in blood and prevents atherosclerotic plaque in the arteries leading to cardiovascular disease (Ginneken et al., 2011). In this research, the ω 6/ ω 3 ratio were 1.5 and 0.4 for *C. chemnitzia* and *C. racemosa* var. *macrophysa*, respectively. These ratios met the standard recommended by the World Health Organization (WHO) in the daily diet (< 10) (Nagappan & Vairappan, 2014). Based on these data, *C. chemnitzia* and *C. racemosa* var. *macrophysa* are potential as functional food.

In comparison to other food products, the PUFAs content in *Caulerpa* is higher than those in palm and coconut oils. The fatty acids of palm oil are composed of 48% SFA and 52% UFA. Palmitic acid is accounted

Table 1. Fatty acid composition of *C. racemosa* var. *macrophysa* and *C. chemnitzia* (% of total fatty acids)

Constituent	<i>C. racemosa</i> var. <i>macrophysa</i>	<i>C.</i> <i>chemnitzia</i>
Unsaturated Fatty Acids (UFAs)	41.00	47.5
Monounsaturated Fatty Acids (MUFA)	11.2	26.0
C 16:1 (Palmitoleic acid)	2.3±0.0	1.5±0.0
C 17:1 (Heptadecanoic acid)	4.6±0.0	-
C 18:1 (Oleic acid)	4.3±0.0	24.5±0.0
Polyunsaturated Fatty Acids (PUFA)	29.8	21.5
C 18:2 ω 6 (Linoleic acid)	6.3±0.2	10.2±0.1
C 18:3 ω 3 (Linolenic acid)	13.6±0.4	5.0±0.0
C 20:4 ω 6 (Arachidonic acid)	2.3±0.1	2.1±0.0
C 20:5 ω 3 (Eicosapentaenoic acid/EPA)	5.8±0.3	1.6±0.0
C 22:6 ω 3 (Docosahexaenoic acid/ DHA)	1.8±0.1	1.5±0.1
Saturated Fatty Acids (SFAs)	59.0	52.5
C 12:0 (Lauric acid)	3.6±0.2	3.3±0.0
C 14:0 (Myristic acid)	2.8±0.1	3.2±0.0
C 16:0 (Palmitic acid)	41.0±0.1	38.6±0.2
C 18:0 (Stearic acid)	2.3±0.1	5.3±0.0
C 24:0 (Lignoceric acid)	7.0±0.4	1.2±0.0

Note: Mean values \pm standard deviation

for 85% of total SFAs, while oleic acid composes 88% of total UFAs in palm oil. Only 1.5% of SFAs are composed of lauric and myristic acids (Gesteiro et al., 2019). Coconut oil is predominantly composed of 92.1% SFAs and 7.9% UFAs of total FAs. Short and medium-chain SFAs have been found only in coconut oil in the amount not exceeding 7.6% of FAs. The FA profile of coconut oil is lauric acid (47.7%), myristic acid (19.9%), capric acid (5.5%), caprylic acid (7.6%), MUFA 6,2% and PUFA (ω -6) 1.6% (Orsavova, Misurcova, Ambrozova, Vicha, & Mlcek, 2015). Meanwhile, in coconut oil, the high amount of lauric acid (C12:0) able to decrease the ratio of LDL to HDL cholesterol (Eyres, Eyres, Chisholm, & Brown, 2016).

Amino Acid Composition

Amino acid compositions of *C. racemosa* var. *macrophyssa* and *C. chemnitzia* are shown in Table 2. Both seaweeds exhibited similar content of total amino acids (AAs), essential amino acids (EAAs) and non-essential amino acids (NEAAs). The AAs composition of *C. racemosa* var. *macrophyssa* was AAs: 312.32±2.09 mg/g DW, EAAs: 113.91±0.90 mg/g DW, and NEAAs: 198.41±1.19 mg/g DW. It was similar to *C. chemnitzia*: AAs: 304.03±0.41 mg/g DW, EAAs: 112.55±0.06 mg/g DW, and NEAAs: 191.48±0.35 mg/g DW. Fifteen AAs were detected in both samples, except methionine and thryptophan. The quantity of EAAs in *C. racemosa* var. *macrophyssa* was in the following order: leucine> threonine> phenylalanine> valine> isoleucine>lysine>histidine. Whereas that of *C. chemnitzia* was as follow: lysine> leucine> threonine> valine> histidine> isoleucine>phenylalanine. The EAAs in *C. racemosa* var. *macrophyssa* and *C. chemnitzia* were accounted for 36.5% and 37% of total AAs content, respectively. Similarly, Ratana-arporn and Chirapart (2006) reported that EAAs in *C. lentillifera* composed 37.9% of total AAs. On the contrary, Aroyehun et al. (2020) observed that those of Malaysian *C. racemosa* was only accounted for 4.8% of total AAs. According to Fleurence (1999), the location, habitat, and seasons greatly affect the protein content of seaweed.

The primary amino acids in *C. racemosa* var. *macrophyssa* were glutamic acid, alanine, and serine. Meanwhile, those of *C. chemnitzia* were glutamic acid, serine, and aspartic acid. According to Gaillande et al. (2017), glutamic and aspartic acids are major amino acids in *Caulerpa* species. They constituted 25% of AAs in *C. lentillifera* (Ratana-arporn & Chirapart, 2006) and 24.4% of total AAs in *C. racemosa* (Gaillande et al., 2017). Both amino acids are responsible for

Table 2. Amino acid composition of *C. racemosa* var. *macrophyssa* and *C. chemnitzia* (mg/g DW)

Constituent	<i>C. racemosa</i> var. <i>macrophyssa</i>	<i>C.</i> <i>chemnitzia</i>
Essential Amino Acids (EAAs)		
Histidine	8.39±0.05	12.32±0.08
Isoleucine	11.98±0.21	11.48±0.20
Leucine	21.14±0.15	19.81±0.03
Lysine	11.80±0.19	20.70±0.42
Methionine	ND	ND
Phenylalanine	20.46±0.09	11.48±0.02
Threonine	20.84±0.03	19.20±0.01
Thryptophan	ND	ND
Valine	19.30±0.17	17.56±0.01
ΣEAAs	113.91±0.90	112.55±0.06
Non-Essential Amino Acids (NEAAs)		
Alanine	30.80±0.11	22.82±0.08
Arginine	24.82±0.62	14.89±0.40
Aspartic acid	25.15±0.05	30.77±0.34
Asparagine	ND	ND
Cysteine	ND	ND
Glutamine	ND	ND
Glycine	23.19±0.27	26.55±0.33
Glutamic acid	32.60±0.27	37.58±0.10
Proline	15.47±0.17	12.44±0.01
Serine	30.82±0.07	36.02±0.10
Tyrosine	15.54±0.03	10.41±0.01
ΣNEAAs	198.41±1.19	191.48±0.35
ΣAAs	312.32±2.09	304.03±0.41
Ratio of ΣEAAs / ΣNEAAs	0.57	0.58

Note: Mean values ± standard deviation, ND: Not Detected

developing the particular taste and flavor of seaweeds (Wong & Cheung, 2000). The high content of glutamic acid in “Pedesan” and “Latuh” suggested their potential values as a food seasoning or flavor enhancer.

The quality of protein in seaweeds is determined by the presence and quantity of essential amino acids (Gaillande et al., 2017). Both samples in this study contained EAAs, such as histidine, threonine, phenylalanine, isoleucine, valine, lysine, and leucine. The quality of protein also depends on the ratio of ΣEAAs/ΣNEAAs. As shown in Table 2, those of *C. racemosa* var. *macrophyssa* and *C. chemnitzia* were 0.57 and 0.58, respectively. Based on the recommended ΣEAAs/ΣNEAAs ratio by WHO (0.6) (Aroyehun et al.,

2020), “Pedesan” and “Latuh” may be used as amino acid sources for human intake.

Mineral and Heavy Metal Composition

The mineral compositions of *C. racemosa* var. *macrophysa* and *C. chemnitzia* are shown in Table 3. Both *Caulerpa* species have similar mineral composition in the following order of amount: Na > Ca > K > Mg > Fe > Mn > Zn. A similar result was reported previously (Gaillande et al., 2017), in which *Caulerpa* species showed high levels of Na, K, Ca, and Mg. The noticeable high amount of Na, Mg, Ca, and K in our samples may be contributed from the seawater (Balasubramanian, 2011; Agusman & Wibowo, 2021).

Accumulation of minerals in macroalgae is affected by internal and external factors. The first consists of sulfhydryl ester, amino, hydroxyl, carboxyl, lipids, and/or proteins, while the latter involves seawater, temperature, pH, salinity, and disruptions (Circuncisao, Catarino, Cardoso, & Silva, 2018).

Table 3. Mineral compositions of *C. racemosa* var. *macrophysa* and *C. chemnitzia* (g/100 g DW)

Constituent	<i>C. racemosa</i> var. <i>macrophysa</i>	<i>C.</i> <i>chemnitzia</i>
Mg	1.22±0.03	0.90±0.00
Ca	5.28±0.12	4.14±0.02
K	1.72±0.01	1.78±0.00
Na	40.89±1.54	28.85±0.01
Zn	0.02±0.00	ND
Mn	0.18±0.00	0.08±0.00
Fe	0.29±0.01	0.19±0.00
Na/K molar ratio	40.31	27.48

Note: Mean values ± standard deviation, ND: Not Detected

Table 4. The heavy metal contents of *C. racemosa* var. *macrophysa* and *C. chemnitzia* (mg/kg DW)

Constituen	<i>C. racemosa</i> var. <i>macrophysa</i>	<i>C.</i> <i>chemnitzia</i>	Limit of detection
Hg	ND	ND	0.004
Cd	ND	ND	0.00011
As	ND	ND	0.008
Sn	ND	ND	3.25
Pb	ND	ND	0.009
Cu	ND	ND	0.12

Note: ND: Not Detected

In our current study, the Na/K molar ratio of *C. racemosa* var. *macrophysa* was higher (40.31) than that of *C. chemnitzia* (27.48). A different result was reported by Kumar et al (2011), in which the Na/K molar ratio of *Caulerpa* species was 3.06 to 4.32. Aroyehun et al. (2020) also reported that the Na/K molar ratio of *C. racemosa* (Forsskal) J. Agardh was 2.5. The variation of Na/K molar ratio may have been affected by the different methods of sample preparation (Gaillande et al., 2017). Kumar et al. (2011) and Aroyehun et al. (2020) cleaned the *Caulerpa* samples with fresh water to remove the sediments and dirt. Washing *Caulerpa* with freshwater would create osmotic pressure in the cells that led to the removal of salt. Meanwhile, in the present work, *Caulerpa* samples were cleaned with seawater. Whelton (2014) reported that some macroalgae have high Na/K ratios, which may be regarded as the disadvantage of macroalgae consumption. WHO guideline of sodium intake for children and adults recommends 1:1 Na/K molar ratio to prevent high blood pressure (WHO, 2012). Since the Na/K molar ratios of “Pedesan” and “Latuh” are higher than recommended, their daily intake should be limited. In addition, rinsing the seaweed with fresh water is suggested to reduce the excess of sodium salt on the seaweed thallus surfaces.

The heavy metal contents of “Pedesan” and “Latuh” from Binuangeun are shown in Table 4. Hg, Cd, As, Sn, Pb, and Cu were not detected in both samples, indicating that both samples were safe for human consumption. The absence of heavy metals in our samples may be related to the sampling location that is secluded from the Cilegon industrial area and Merak Port.

Seaweeds are rich in nutrition and bioactive compounds; however, the heavy metals accumulated in them must be observed prior to consumption. This observation is important because of the ability of some macroalgae to accumulate metals (Rodriguez, Huerta-Diaz, Choumiline, Quinones, & Gonzale, 2001). Macroalgae cell walls are composed of sulfate, anionic carboxyl, and phosphate groups responsible for metallic



Figure 3. Geographical location of Binuangeun Coast.

cation binding (Aroyehun et al., 2020). Hg, Cd, As, Pb, and Cu are heavy metals that may be health-threatening when overconsumed (Cardoso et al., 2014). Accumulation of metals in macroalgae depends on their uptake capacity and mechanism of surrounding water gradient concentration (Zbikowski, Szefer, & Latala, 2006).

Conclusion

Edible *Caulerpa* from Binuangun Coast were identified as *C. racemosa* var. *macrophysa* (local name: “Pedesan”) and *C. chemnitzia* (local name: “Latuh”). Based on the fatty acid, amino acid, and heavy metal contents, both edible *Caulerpa* are safe to consume and potentially healthy. However, since the Na/K molar ratio is high, it is suggested that both *Caulerpa* should be rinsed with fresh water to reduce the sodium salt content prior to consumption. This study is the initial step to promote edible *Caulerpa* as an alternative source of functional food. Further studies on the isolation and identification of secondary metabolites and their bioactivities are required to explore these emerging species.

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