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Phytochemicals Properties and Fatty Acid Profile of Green seaweed *Caulerpa racemosa* from Madura, Indonesia

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Abstract : Three different treatment of *Caulerpa racemosa* - fresh, semi-dried and dried - were assayed for proximate composition, and antioxidant activities (EC50). Fatty acids profile was also investigated by GC-MS. The analysis showed that the water content was significantly different in the three treatments, but showed no difference in protein, fat, carbohydrates, fiber, ash and total phenols. The total flavonoid and antioxidant activity of semi-dried C. racemosa was highest, compared the others. Drying changed the fatty acid composition of *C. racemosa*. Fatty acids from fresh and semi-dried *C. racemosa* followed the same pattern with USFA>SFA, while dried *C. racemosa* SFA>USFA.
Keyword : *Chlorophyta*, fresh, semi-dried, dried, phytochemical properties,fatty acids.

Introduction

Green seaweed from genus of Caulerpa consists of one cell by many nuclei, often found in tropical and sub tropical waters. It is mostly consumed by Asian[1,2]. *Caulerpa racemosa* is a green seaweed that has good contains phytochemicals and fatty acids but it need to be explored. Its phenolic compounds has potent as an antioxidant[3], antibacterial and anti-inflammatory[1], larvicidal[4]. Moreover, its enzymatic antioxidants (superoxide dismutase, catalase) demonstrated high ability to reduce oxidative stress[2].

Fatty acids derived from marine organisms have varies chemical structures which served as a biological marker. It has ability of the biological activity due to characteristic of living environment (Berge and Barnathan, 2005)[5]. Several studies have reported the activity of essential fatty acids from seaweed. ω-3 and ω-6 obtained from *Undaria pinnatifida* could act as anti-inflammatory and pro-inflammatory[6]. Hexadecatetraenoic acid (HDTA) C16:4 ω-3, octadecatetraenoic acid (ODTA) C18: 4 ω-3, linoleic acid (LA) C18: 2 ω-6 and α-linolenic acid (ALA) C18: 3 ω-3 derived from *Ulva pertusa* and *Ulva fasciata* has been known to have activity algicidal[7]. Drying seaweed with heating has a variety of purposes, one of which was to extend the shelf life. Gupta (2010)[8]stated that heating seaweed at various temperatures and times reduced the total number of microbial cells. However, it had been widely known that the drying of fruits and vegetables can alter the nutritional content including phytochemical content material and little information about preparation seaweed extract by drying the green seaweed, especially *C. racemosa*. This study aimed to provide information on the effect of the drying process to the phytochemical content and scavengeing activity of *C. racemosa* from

methanol extract. This study aims to investigating the changes in pyhtochemical properties and fatty acid profiles of *C. racemosa* after drying processing, which has not known yet.

Material and Methods

Sample Preparation

Seaweed *C. racemosa* was obtained from Sumenep, Madura, Indonesia (21.5 Talango Islands ", 05 'latitude and 307 ° 07', 56 '113 ° E) in December 2014. The *C. racemosa* were washed by seawater and continued by fresh water to remove dirt and epiphytes. The C. racemosa was divided into three parts: 1) The fresh was obtained by draining a sample; 2) the semi-dried was obtained by drying at a temperature of 40-50°C for 6 hours; and 3) the dried was obtained by drying the seaweed at temperature of 40-50 ° C for 24 hours.

Proximate analysis

Moisture, crude fiber, ash, protein and fat content were determined according to AOAC (1997)[9]. Carbohydrate was calculated using methods 'by difference'.

Extraction of sample

Each *C. racemosa* was extracted using 300 ml of ethanol per day for three days at room temperature. The resulting extractive solution was evaporated using a rotary evaporation at 40°C to obtain the green seaweed extracts.

Total Phenolics

Total of phenol was analyzed using the Folin-Cociolteau reagent[10]. Green extract (1ml) in 10 ml of deionized water and 1 ml of reagent Folin-Ciocalteau were mixed. 20% (w/v) of sodium carbonate (2ml) was added after 5 min. The solution was followed by incubating for 1 hour at ambient temperature. Absorbance measurements carried out at 750nm. Phenol content deliberated equivalents as mg/g gallic acid (mgGAE/g).

Total Flavonoids

Total of flavonoid was accessed using aluminum chloride as described by Cox et al. (2010)[11]. 250µl of extract in 125ml double destilated water and 75 mL of 5% NaNO2 solution were mixed and followed by incubating for 6 min at room temperature. 150 mL of AlCl3.H2O was added to the solution and followed by incubating 5 minutes. 0.5 ml of NaOH was added and followed by adding 275 mL of up to a volume of 2.5 ml. Total flavonoid standards used are quercetin. The results were shown as mg/g equivalent quercetin (mgQE/g).

DPPH Free-radical Scavenging Assay

DPPH (2,2-Diphenyl-1-picrylhydrazyl) antioxidant activity was done by Ganesan et al. (2011)[12] with approximately modifications. 1 ml of 0.1 mM DPPH in 3 ml of methanol was added to the various concentrations of extract (125-500 mg/L). Absorbance measurements carried out at 517 nm. Calculations performed using the following formula:

$$\text{Activity of antioxidant (\%)} = \mathbf{1} - \left[\frac{Asample - Ablank\ sample}{A\ control}\right] x\ 100$$

A_{sample} = the absorbance of the sample with solution of DPPH , $A_{blank\ sample}$ = the absorbance of the sample without solution of DPPH, $A_{control}$ = the absorbance of DPPH without sample. EC_{50} showed a concentration of sample producing 50% scavenging of the DPPH radical. The lower of EC_{50} value indicates a higher antioxidant activity.

GC-MS

Methanol extract was extracted by hexane for fatty acid analysis. Fatty acids were converted to fatty acid methyl ester (FAME) as described by Park and Goin (1994)[13], samples (100 mg), 100µl methylene chloride, 1 ml of 5 N NaOH were heated at 90°C for 10 min. The solution was then mixed with 14% BF3 methanol and

heated for 10 minutes at the same temperature. 1 ml of hexane and 1 ml of distilled water was added to the solution. The solution was aliquoted and then centrifuged for 5 min at 3000 rpm. Supernatant was analyzed by on an QP2010 GC-MS (Shimadzu) equipped with agilent column DB-1 (length 30 mx 0.25 mm diameter).

Statistical analysis

Completely Randomized Design was used in this study, which each data showed mean of standard error of three replications. Data analysis was performed using Minitab 16 for Windows. Significance was considered by Analysis of variance (ANOVA) between each treatment when $p< 0.05$.

Results and Discussion

Proximate analysis and fiber

Table 1 showed the moisture, ash, protein, fat, carbohydrates, and fiber of three different treatment of *C. racemosa*. The moisture content of fresh *C. racemosa* was significantly higher than <0.05. Drying methods in *C. racemosa* was not obviously significant in the levels of fat, protein, carbohydrate, ash, and fiber.

Table 1. The proximate composition and fiber *C. racemosa* in several treatment

Constituent (%)	Fresh	Semi-dried	Dried
Moisture content	90.00 ± 0.76^a	53.38 ± 1.02^b	11.40 ± 1.51^c
Ash*	42.78 ± 1.96	42.31 ± 2.29	42.29 ± 0.91
Protein*	14.55 ± 0.29	14.03 ± 1.70	16.24 ± 0.09
Fat*	1.13 ± 0.14	1.47 ± 0.99	2.32 ± 0.25
Carbohydrate*	41.54 ± 2.09	42.29 ± 0.58	39.16 ± 0.59
Fiber*	21.74 ± 1.82	21.17 ± 1.20	18.78 ±0.66

(*) Based on the dry based
(a-c) representative significantly difference ($p <0.05$)

Carbohydrates, fats, proteins and minerals in seaweed are bioactive compounds that have the potential as a functional food. Dietary fiber seaweed is a macro-molecules that are not absorbed (Hold and kraan, 2011)[14]. Moisture content and protein of fresh *C. racemosa* were 90% (wb) and 14.03 to 16.24% (db), respectively. It was previously demonstrated by Nagappan and Vairappan (2013)[1] that moisture content and protein of *C. Racemosa* were respectively 87.05 to 93.20% (wb) and 17.28 to 17.36% (db). More over, Murugaiyan and Narashiman (2013)[15], showed *C. racemosa* 15% of protein. Fat content in all treatments seaweed was very low (1.13 to 2.32% db), supported by report of Nagappan and Vairappan (2013)[1]. In addition, *C. racemosa* possessed 39.16 to 42.29% (db) of carbohydrate and 18.78 to 21.74% db of fiber. Results of the analysis showed no difference ($p> 0.05$) in the levels of protein, fat, carbohydrate, ash and fiber. Drying treatment in this study did not have much effect on the nutrient content of *C. racemosa*.

Phytochemical and antioxidant activity (EC50) of *C. racemosa*

Drying did not showed differences ($p> 0.05$) in the total phenol, but it was reverse in total flavonoids. The highest antioxidant activity of *C. racemosa* extract was found in dried seaweed.

Table 2. Content of Total phenol, total flavonoids and antioxidants activitas EC50

Treatment	Fresh	Semi-dried	Dried
Total of fenol (mg GAE/g)	1.00 ± 0.29^a	1.11 ± 0.37^a	1.13 ± 1.51^a
Total of Flavonoid (mg QE/g)	16.66 ± 0.20^a	24.07 ± 1.02^c	20.96 ± 0.66^b
EC_{50} (mg/ml)	0.92 ± 0.05^a	0.58 ± 0.01^b	0.40 ± 0.01^b

(a-c) representative significantly difference ($p <0.05$)

Total Phenol of *C. racemosa* was 1.00 to 1.13 mgGEA/g. This results was similar to Chew et al. (2008)[16] that performed total phenol of *C. racemosa* from Malaysia at 0.965 mgGAE/g based on dried *C.*

racemosa. Total flavonoids in three different treatments was aproximately 16,6624,07 mgQE / g. Highest flavonoid content was found in the semi-dry C. racemosa, and the lowest on fresh C. racemosa. Alteration of phytochemical properties due to hydrothermal processes (Rajauria, et al., 2010)[17].

The highest antioxidant activity of *C. racemosa* was found in dried C. racemosa. Total flavonoid in dried C. racemosa was lower than semi-dried C. racemosa, and total phenols in each sample was no different. Perhaps, it caused by heat treatment that activated phytochemical compounds of *C. racemosa*. Heating could induce deactivation of the oxidative enzyme responsible for breaking down of antioxidant compounds (Rajauria, et al., 2010)[17].

Fatty Acid Profile of *C. racemosa*

The drying process could result different fatty acid composition[19]. It was revealed in compotition of fatty acids of seaweed due to drying. Figure 1, 2 and 3 showed GC-MS chromatogram results from three different treatment of seaweed.

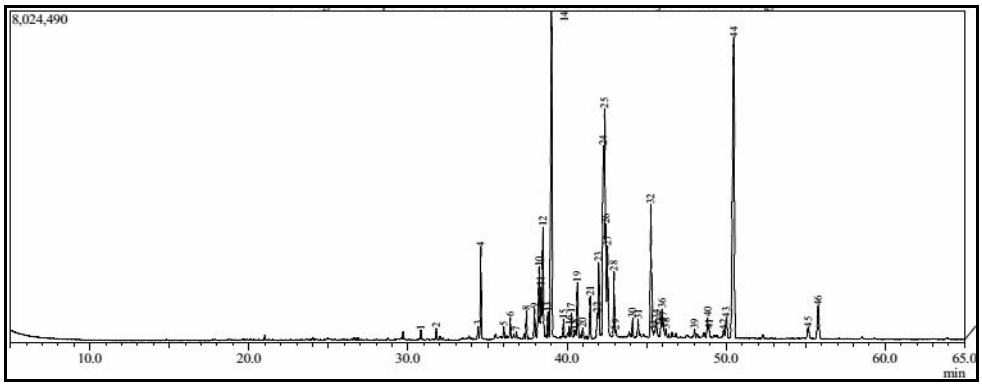

Figure 1. GC-MS chromatograms of Fresh *C. racemosa*

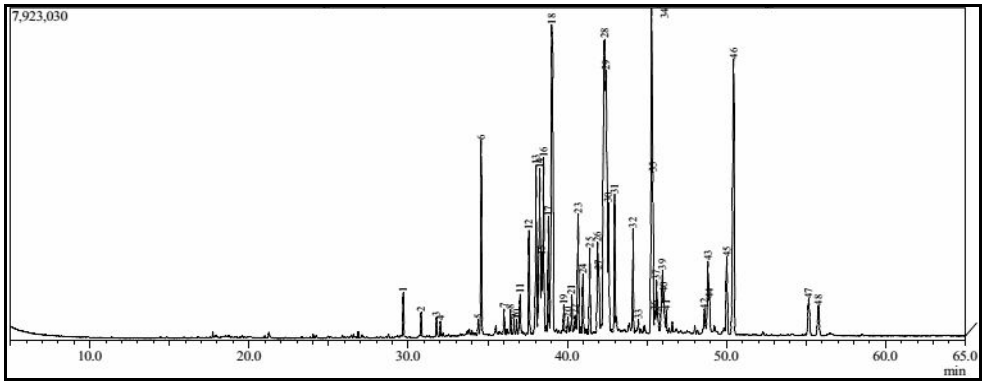

Figure 2. GC-MS chromatograms of semi-dried *C. racemosa*

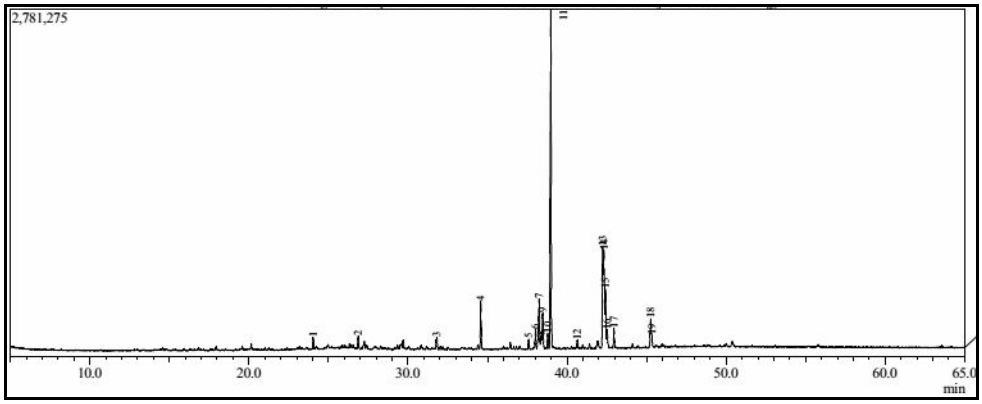

Figure 3. GC-MS chromatograms of dried *C. racemosa*

The composition of fatty acid was calculated from the percentage of peak area to the total area, summarized in Table 2. The dominant saturated fatty acids of among samples was palmitic acid (C 16: 0). The dominant of polyunsaturated fatty acids (PUFA) of dried and fresh *C. racemosa* was α-linolenic acid, meanwhile linoleic acid was dominated in semi-dried *C. racemosa* (Table 3).

Table 3. Fatty Acid Profile *C. racemosa*

Constituents	**Peak area (%)**		
	Fresh	**Semi-dried**	**Dried**
Saturated Fatty Acid			
Dodecanoic acid	nd	0,65	nd
Tetradecanoic acid	2,82	3,48	4,93
Pentadecanoic acid	0,56	0,39	nd
Hexadecanoic acid	15,79	11,53	38,41
Unsaturated Fatty Acid			
9-hexadecenoic acid	4,00	5,54	nd
2-hexadecenoic acid	0,27	0,36	nd
7,10,13-Hexadecatrienoic acid	3,21	4,35	nd
Octadecanoic acid	2,37	2,38	2,01
11-octadecenoic acid	3,22	nd	5,10
10-Octadecenoic acid	nd	2,40	nd
9-octadecanoic acid	3,10	nd	7,58
9,12-Octadecadienoic acid	8,42	12,24	12,39
6,9,12-Octadecatrienoic acid	nd	1,86	nd
9,12,15-octadecatrienoic acid	9,74	8,64	16,76
11-eicosenoic acid	0,32	0,43	nd
11,13-Eicosadienoic acid	0,97	1,77	nd
5,8,11,14-Eicosatetraenoic acid	6,36	10,69	3,31
Methyl eicosa-5,8,11,14,17-pentaenoate	0,20	nd	nd
Methyl-5,11,14,17-eicosatetraenoate	nd	0,41	nd
Methyl eicosa-5,8,11,14,17-pentaenoate	0,20	2,60	0,93
13-docosenoic acid	1,58	2,79	nd
6,9,12,15-docosatetraenoic acid	0,27	0,20	nd
Tetracosanoic acid	1,94	0,60	nd
Total identified amount	65,34	73,31	91,42
SFA	24,24	19,24	47,58
USFA	42,43	54,08	41,83

∗Compound identified by library under the same chromatographic condition, as well as by mass spectra

Fatty acids from fresh and semi-dried *C. racemosa* followed the same pattern with USFA>SFA, while dried *C. racemosa* SFA>USFA . According to Kumari, et al. (2010)[20], while the dominant fatty acids in *C. racemosa* is palmitic acid. Generally, green seaweed possess high fatty acid as taxonomic markers affected by its genetic factors (Van Ginneken et al., 2011)[21]. The dominant monounsaturated fatty acids is oleic acid and the dominant polyunsaturated fatty acids is linoleic acid. Some ω-3 and ω-6 were identified from dried, semi-dried, and fresh C. racemosa. ω-3 has several functions for health including as anticancer (Park, et al. 2013)[22] and prevent cardiovascular disease (Gebauer, et al., 2006)[23]. Polat and Ozogul (2013)[24], reported that some seaweed has a low fat content but high in PUFA. The process of drying seaweed showed some differences in fatty acid composition Alfaia et al. (2010)[25] stated during drying some of the mechanisms occur including decreasing of water content, lipid oxidation, diffusion and exchange, could affect the differences in fatty acid composition.

Some fatty acids also could act as antioxidants. According to Henry et al. (2002)[26] suggested some of saturated and unsaturated fatty acids have antioxidant activities. According to Huang and Wang (2004)[27], antioxidants are classified into two groups, namely the reaction breaking-antioxidant and preventive antioxidants. MUFA and PUFA have one or more double bond which are easily oxidized. Therefore, its ability of antioxidant activity obtained from electron donor ability, thus it could be antioxidant preventive-call as prooxidant.

Conclusion

Drying at 50°C could alter some of component in *C.racemosa*. The highest antioxidant activity obtained from dried seaweed showed by lowest EC50 value. It perhaps influenced by its phytochemical content and fatty acids. Therefore, fatty acids could be as antioxidants which could also be preventive as prooxidant.

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